DEVELOPMENT AND FORMULATION OF AN INTRANASAL DOSAGE FORM FOR CYCLIZINE HYDROCHLORIDE

NTSELISENG SELLOANE BOHLOKO

Licentiate in Pharmaceutical Science (Havana, Cuba)

MPharm (Pharmaceutics) (UDW)

Thesis submitted in fulfillment of the requirements for the degree

PHILOSOPHIAE DOCTOR

In the Faculty of Health Sciences at the Department of Pharmaceutics

At the Potchefstroom University for Christian Higher Education

Promoter: Prof. D G Muller

POTCHEFSTROOM

2003

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

OPSOMMIMG

'n Omvattende oorsig van die nasale toedieningsroete, en veral die nasale geneesmiddel-afleweringstelsel word gegee. Die fisies-chemiese eienskappe, werkingsmeganisme en farmakologie van H1-reseptorantagoniste en veral siklisien word uitgelig. Die tegnieke vir die bepaling van toksisiteit (*in vitro* siliêre slagfrekwensie (SSF) vir menslike nasale biopsies en morfologiestudies van nasale mukosa van die rot), die sintese van siklisienlaktaat, die bepaling van die oplosbaarheid van siklisien.HCl en siklisienlaktaat, die bepaling van die viskositeit van die geformuleerde gel en beoordeling van die neerslag en verdeling van die dispersies in hidroksipropielmetielsellulose (HPMS) in 'n model van die menslike neusholte is gedoen.

In hierdie studie is voorlopige bepalings van die toksisiteit van die verskillende komponente van die formulerings (hulpstowwe en die aktiewe bestanddeel) gedoen. Die resultate van hierdie studies toon dat die pH van sowel die hulpstowwe as die aktiewe bestanddeel die siliêre beweeglikheid beduidend beïnvloed en daarom was alle bepalings van siliêre slagfrekwensie by nasale pH gedoen. Verder is die effek van die konsentrasie (0.0625%m/v, 0.125%m/v, 0.25%m/v, 0.5%mv and 1%m/v) van die hulpstowwe op siliêre beweeglikheid ondersoek. Transmissie-elektronmikroskopie was nuttig om die integriteit van en veranderings in die oppervlakmorfologie van die nasale mukosa van die rot na behandeling met die verskillende hulpstowwe (karboksimetielsellulose, hidroksipropielmetielsellulose, trimetielkitosaan 36.3% KG, Carbopol P934 en polisorbaat-80) teen verskillende konsentrasies te beoordeel.

Van die ondersoekte hulpstowwe toon hidroksipropielmetielsellulose (HPMS) 'n gunstige effek op silia aangesien daar geen ooglopende skade aan die ultrastruktuur waargeneem kon word nie, hoewel daar by die hoogste viskositeit 'n effense afname in siliêre slagfrekwensie (SSF) was. Verder word beweer dat hidroksipropielmetielsellulose (HPMS) 'n biokleefbare hulpstof is wat sy kleefbaarheidseienskappe op die intranasale preparaat sal oordra om die retensietyd tussen die absorberende mukosa en die

geneesmiddel te verbeter en absorpsie van die middel sodoende sal verhoog. Daarom is hierdie hulpstof dus gekies as die ideale een vir gebruik in die formulering van die intranasale preparaat.

Die wateroplosbaarheid van 'n geneesmiddel speel 'n belangrike rol in nasale toediening omdat dit nodig is om die middel in 'n beperkte volume van ongeveer 200 µl toe te dien. Om die wateroplosbaarheid van die swak wateroplosbare siklisien.HCl te verbeter, is 'n laktaatsout gesintetiseer en gekarakteriseer. Dit is gevind dat hierdie verbinding hoogs wateroplosbaar is. Die intranasale preparaat is dus gemaak deur gebruik van die laktaatvorm van siklisien.

'n Enkelblinde studie is gedoen om die farmakokinetiese parameters van sowel Valoid® orale tablette met 100 mg siklisien.HCl (verwysingsmiddel) en die intranasale preparaat met 125 mg/ml siklisienlaktaat (studiemiddel) te bepaal. Die resultate hiervan toon 'n beduidende verbetering in die biobeskikbaarheid van siklisien. Die C_{maks} na orale toediening is 200.79 ng/ml by t_{maks} = 5.57 h en vir die intranasale preparaat is C_{maks} = 5354.22 ng/ml by t_{maks} = 1.59 h.

'n 19.2-voudige toename in die biobeskikbaarheid van die geneesmiddel na intranasale toediening (AOK_{IN} = 122860.70ng/ml/h) vergeleke met orale toediening (AOK_O = 5943.48ng/ml/h) is waargeneem. Hierdie verbetering in biobeskikbaarheid deur nasale toediening toon dat beter nasale absorpsie van die geneesmiddel en dus beter biobeskikbaarheid nie net afhanklik is van gunstige anatomiese en fisiologiese eienskappe van die neusmukosa nie, maar moontlik ook van die inherente fisies-chemiese eienskappe van die geneesmiddelmolekuul en die komponenete van die formulering. Die chemiese modifisering van die swak wateroplosbare siklisien.HCl na die hoogs wateroplosbare siklisienlaktaat bemoontlik dus die inkorporering van meer opgeloste stof in 'n beperkte volume oplosmiddel. Hierdie nuwe eienskap kan dus positief op die transport van siklisien deur die neusmukosa ingewerk het. Verder kon die hidroksi-propielmetielsellulose (HPMS) as komponent van die formulering sy biokleefbaarheid aan die preparaat oorgedra het. Miskien het dit die retensietyd van die doseervorm in die

neuskanale deur binding aan die neusslymvlies verleng en sodoende ook die kontaktyd tussen die absorberende slymvlies en die doseervorm. Hierdie interaksie tussen die slymbinder en die neusslymvlies kon tot die tydelike oopmaak van die digte bindings en uiteindelike toename in die penetrasie/absorpsie van die geneesmiddel gelei het

Sleutelwoorde: intranasaal, siklisien, hidroksipropielmetielsellulose (HPMS), siliêre slagfrekwensie (SSF), biobeskikbaarheid

SUMMARY

A comprehensive review of the nasal route of administration, in particular the nasal drug delivery system has been presented. The physicochemical properties, mode of action and pharmacology of H_1-receptor antagonists, in particular cyclizine HCl, have been highlighted. The techniques for the assessment of toxicity (*in-vitro* ciliary beat frequency (CBF) studies for human nasal explants and morphology studies of the rat nasal mucosa), synthesis of cyclizine lactate, solubility studies of both cyclizine HCl and cyclizine lactate, viscosity determination of the gel formulated and assessment of the deposition and distribution of the hydroxypropylmethyl cellulose (HPMC) dispersions within the human nasal cavity model were conducted.

In this study, preliminary studies on the toxicity of the various formulation components (excipients and active ingredient) were carried out. Results from these studies indicated that for both the excipients and the drug, pH significantly affects the ciliary motility hence all ciliary beat frequency determinations were conducted at nasal pH. Furthermore, effects of the various concentrations (0.0625%(w/v), 0.125%(w/v), 0.25%(w/v), 0.5%(w/v) and 1%(w/v)) of the excipients on ciliary motility were investigated. Transmission electron microscopy (TEM) studies proved useful in evaluating the integrity and changes in the surface morphology of the rat nasal mucosa post treatment with the various excipients (carboxymethyl cellulose, hydroxypropylmethyl cellulose, trimethyl chitosan 36.3% DQ, Carbopol P934 and polysorbate-80) at varying concentrations.

Of the excipients investigated, hydroxypropylmethyl cellulose (HPMC) showed cilio-friendliness since there was no apparent ultrastructural damage, although a slight decrease in ciliary beat frequency (CBF) was observed at the highest viscosity. Moreover, hydroxypropylmethyl cellulose (HPMC) is said to be a bioadhesive excipient, which would therefore confer its bioadhesive properties to the intranasal preparation to enhance the retention time between the absorbing mucosa and the drug and hence increase nasal

drug absorption. This excipient was therefore selected as the ideal for use in the formulation of the intranasal preparation.

The aqueous solubility of a drug plays an important role in nasal administration since it is required that the drug component be applied in a limited volume of about 200µl. To enhance the aqueous solubility of the sparingly water-soluble cyclizine HCl, a lactate salt was synthesised and characterised. This compound was found to be highly soluble in water. The intranasal preparation was therefore manufactured using the lactate form of cyclizine.

A single blind study was conducted to determine and compare the pharmacokinetic parameters for both Valoid® oral tablets containing 100mg cyclizine HCl (reference drug) and cyclizine lactate intranasal preparation 125mg/ml (study drug). The results obtained indicated a significant improvement in the bioavailability of cyclizine. For oral administration C_{max} = 200.79ng/ml at t_{max} = 5.57h and for the intranasal preparation C_{max} = 5354.22ng/ml at t_{max} = 1.59h.

A 19.2-fold increase in drug bioavailability was observed after intranasal administration (AUC_{IN} = 122860.70ng/ml/h) compared with oral administration (AUC_{PO} = 5943.48ng/ml/h). This enhanced bioavailability through nasal administration indicated that enhanced nasal drug absorption and hence increased bioavailability not only depends on the favourable anatomical and physiological characteristics of the nasal mucosa but possibly on the inherent physico-chemical characteristics of the drug molecule and the formulation components. Thus chemical modification of the sparingly water-soluble cyclizine HCl to the highly water-soluble cyclizine lactate facilitated the dissolution of more solute in a limited volume of solvent. This new feature therefore may have impacted positively to the transport of cyclizine across the nasal mucosa. Furthermore, the hydroxypropylmethyl cellulose (HPMC), component of the formulation, could have conferred its mucoadhesive properties to the preparation. Perhaps it increased the retention time of the dosage form within the nasal passages through bond formation with the nasal mucosa thereby increasing the contact time between the absorbing mucosa and

the dosage form. This interaction between the mucoadhesive and the nasal mucosa may have resulted in the modification of tissue permeability (possiblytransient opening of the tight junctions) and eventual increase in the drug penetration/absorption.

Keywords: Intranasal, Cyclizine, Hydroxypropylmethyl cellulose (HPMC), Ciliary beat frequency (CBF), Bioavailability

"Barriers can be built but they are never too high nor too deep for the shepherd to see His flock through". Anonymous

Psalm 35

ACKNOWLEDGEMENTS

To God, the Almighty, You saw your flock through the barriers, You lead me through thick and thin, You never failed me in times of need. It is from thy where our strength and perseverance come from.

The realisation of this thesis would have not been possible without the contribution of the following people.

Mama, Papa, Seitebatso, Nkopane, Liepollo, Lallala and 'Mangoane Papali, your love, encouragement and unfailing support will always be valued.

Prof. Douw Muller, my supervisor, thank you for accepting me into the programme. I came, I saw and I conquered.

The Government of the Kingdom of Lesotho, for the financial assistance offered for the realisation of this endeavour.

The Cuban Government, you opened the way for growth and development to the many third world nations. "Vamos a vencer al enemigo, ya que tenemos las armas". Fidel Castro

Prof.Cassim M Dangor, Director for the School of Pharmacy and Pharmacology at the University of Durban-Westville, a special word of thanks for building the researcher in me. I am now ready for the tough road ahead.

Prof. Antoon Lotter, formulation guru, thank you for all the tips, I learnt a lot from you.

Dr Jan Du Preez and Ms Anita Wessels, HPLC experts, for assisting me with the development of the analytical method.

Dr Cedric Shultz, Lung Unit, Pretoria Academic Hospital, for all the time loaned and assistance for conducting ciliary beat frequency studies at the unit.

Dr Tiedt and Ms Wilma Pretorias, electron microscopy department, for assisting me with the TEM procedure for morphology studies.

Ms Antoniette Fick, for assisting me with the handling of experimental animals.

Mr B Parsons, R&D head Adcock-Ingram, for unconditionally assisting me with the packaging material.

Ms Julie Zeitsman, purchasing manager Schering-Plough, for offering me the packaging material.

Dr Belinda Scrooby, Senior lecturer for anatomy, School of Nursing, for unconditionally allowing me to make use of the model of the lateral cross section through the nose.

Dr Maides M Malan and Ms Freda Hilderbrant, for assisting me with the clinical trials

Mr Andre Joubert, Chemistry Department, for conducting all the NMR analysis for the synthesised compounds.

Mr Naas van Rooyen, for helping me with the procurement of all my study materials.

Prof. Jaco C Breytenbach, Pharmaceutical Chemistry Department, for unconditionally allowing me to use the laboratory and lending your listening ear to all my grieviences and achievements.

Prof. Awie Kotze, Pharmaceutics Department, for all the assistance rendered in times of need.

Mr Kobus Swart, for the IT expertise offered

Dr Suria Ellis, for assisting me with the statistical analysis

Dr Tiaan Brink and Ms Sharlene Nieuwoudt, Pharmacology Department, for allowing me to use the cell culture laboratory unconditionally.

Dr Varsay J Cooper, Head of Internal Medicine Department, Queen Elizabeth II Hospital, Lesotho, I found a friend in you, now and then I had somebody to shout at so as to let the steam out. Thank you for proof reading my thesis.

To all my friends and well wishers, it has been a very bumpy journey, without your encouragement and support I would have never reached the hilltop.

MOTIVATION OF STUDY

The bioavailability of a drug and hence its therapeutic efficacy are influenced by the route of administration. For maximal efficacy of a drug, ease of administration and high absorption rates are prerequisites for the achievement of better patient compliance and greater bioavailability, respectively (Chien et al., 1989).

Direct administration of drugs into the systemic circulation either by rapid intravenous bolus injection or continuous intravenous infusion is superior to other routes of administration with respect to the onset of the therapeutic action. This stems from the lack of a lag phase in drug absorption. Due to this direct access to the general circulation, drug metabolism and degradation both in the liver (first-pass phenomenon) and the gastro-intestinal tract (GIT) is avoided (Ugwoke et al., 2001). Moreover, a constant and prolonged drug absorption period can be achieved, and the blood drug level is programmable to fall within the therapeutic range of the drug in question. The major drawbacks of the intravenous administration include some potential health hazards involved during the administration, which render this route unsuitable for outpatient use in chronic therapy. The pain associated with the drug administration also contributes to low patient compliance. Additionally, the use of both trained personnel and sophisticated equipment further drives up the cost of this method (Ugwoke et al., 2001).

Cheaper alternatives are very attractive, especially if they can duplicate the advantages of the intravenous administration.

The transdermal route can also afford a constant rate of drug delivery however, its major limitations include that delivery is only limited to small lipophilic and active drugs. It also has a long lag phase of absorption due to the low permeability of the highly keratinised stratum corneum (Ugwoke et al., 2001).

On the contrary, with nasal administration, a high and rapid drug concentration comparative to the intravenous route is achievable. The anatomical configuration and

physiological characteristics of the nasal mucosa have rendered the nasal administration route the most feasible alternative route of administration to the parenteral route (Ugwoke et al., 2001). The nasal cavity is well suited for bringing a drug solution into intimate contact with the highly vascularised mucosa. Drug administration through the intranasal route can therefore enhance drug bioavailability by avoidance of the gut wall metabolism thereby allowing achievement of predictable blood drug levels. Furthermore, this route is said to provide a rapid onset of action (Cool et al., 1990). The compliance of patients who require long-term therapy has been shown to improve due to the simplicity and ease of administration when compared to the intravenous route (Quraishi et al., 1997).

The interest in the nasal mucosa as a site of drug administration for both local and systemic drug delivery has prompted the investigation of the nasal absorption and bioavailability of many drug compounds with low bioavailability through the conventional routes of administration. The following are some of the anti-emetic drugs with low oral bioavailability that have been tested in animals and humans via the intranasal route: promethazine HCl AUC_{PO} = 22-25% t_{max} = 50min; AUC_{IN} = 94%; t_{max} = 7.3min (Ramanathan et al., 1998), metoclopramide AUC_{PO} = 33%; t_{max} = 1.26h; AUC_{IN} = 73.31%; t_{max} = 0.12h (Ormrod at al., 1999) and hyoscine AUC_{IV} = 100%; AUC_{IN} = 83%; t_{max} = 0.37h (Klocker et al.,2001). It is evident that there was a marked improvement in the nasal bioavailabilities of these drugs.

Cyclizine hydrochloride is an H_1-receptor antagonist indicated for emesis, motion sickness and nausea and vomiting due to its prominent anticholinergic activity and actions on the vomiting centre. The drug undergoes an extensive first pass effect after oral administration to form an inactive metabolite, norcyclizine, which is 60% protein bound (Clarke, 1986). Thus the bioavailability of cyclizine post oral administration is reported to be low. Little is documented on the pharmacokinetics of cyclizine however; studies indicate a biological half-life of about 13 hours (Walker, 1995).

AIM AND OBJECTIVES OF STUDY

The aim of this study was to investigate the possibility to deliver cyclizine intranasally and to develop and formulate an intranasal dosage form.

The objectives of the study were:

- To evaluate the toxicity of cyclizine and other formulation excipients for intranasal delivery by using the transmission electron microscopy (TEM) and ciliary beat frequency (CBF) techniques.
- To develop a HPLC method for cyclizine in order to determine the drug in biological fluids.
- To determine the bioavailability of cyclizine after oral and intranasal administration using human subjects.
- To try to enhance the intranasal drug delivery of cyclizine by employing various absorption enhancers.
- To develop a formulation in order to optimise the intranasal bioavailability of cyclizine using the intranasal route.

References

Clarke E G C. Isolation and identification of drugs in pharmaceuticals, body fluids and post-mortem materials; 1986 2nd Edition: 497-498

Cool W M, Kurtz N M, Chu G. Transnasal delivery of systemic drugs. *Advances in Pain Research and Therapy*; 1990 14: 241-258

Klocker N, Hanschke W, Tousaint S, Verse T. Scopolamine nasal spray in motion sickness: a randomised, controlled,and crossover study for the comparison of two scopolamine nasal sprays with oral dimenhydrinate. *European Journal of Pharmaceutical Sciences;* 2001 13 (2): 227-232

Ormrod D, Goa K L. Intranasal metoclopramide.*Drugs;* 1999 58 (2): 315-322

Quraishi M S, Jones N S, Mason J D T. The nasal delivery of drugs. *Clinical Otolaryngology*; 1997 22: 289-301

Ramanathan R, Geary R S, Bourne D W A, Putcha L. Bioavailability of intranasal promethazine dosage forms in dogs. *Pharmacological Research;* 1998 38 (1): 35-39

Ugwoke M I, Verbeke N, Kinget R. The biopharmaceutical aspects of nasal mucoadhesive drug delivery. *Journal of Pharmacy and Pharmacology*; 2001 53: 3-22

Walker R and Kanfer I. Sensitive High Performance Liquid Chromatographic determination of cyclizine and its demethylated metabolite, norcyclizine in biological fluids using coulometric detection. *Journal of Chromatography B*; 1995 672: 172-177

CHAPTER ONE

THE NASAL ROUTE OF DRUG ADMINISTRATION

1.1 INTRODUCTION

The nasal route

Our exclusive reliance on traditional routes of drug administration is being currently challenged by the aggressive imaginative thinkers in the pharmaceutical and biotechnological industries. Innovative research targeted at novel sites for administration (mucous membranes, skin) and elimination of discomfort associated with drug administration will ultimately affect critical care practice beyond the now common practice of skin patches (Biddle, 1992). The nasal route has recently drawn a lot of attention as an alternative route of administration for systemically active drugs (Sakane, 1994). Drug absorption through the mucosal surface is generally efficient because of the absence of the stratum corneum epidermis. Among the biopharmaceutical features that distinguish the nasal route from other non-parenteral applications e.g. buccal, peroral, rectal, transdermal and vaginal drug administration, the following have been considered as being potentially relevant:

- A relatively large surface area (epithelium covered with microvilli) available for drug absorption.
- A thin, porous and vascularised epithelium with high total blood flow per cm^3 which ensures rapid absorption and onset of therapeutic action as well as a porous endothelial basement membrane which facilitates the direct transport of substances into the systemic circulation (or even directly into the central nervous system (CNS)).
- Rapid kinetics of absorption and high bioavailability comparable to the parenteral route due to lack of a lag phase in drug absorption.

- Blood is drained directly from the nose into the systemic circulation thereby avoiding both the hepatic extraction effect and gut wall metabolism (Soane, 1999).
- Enhanced bioavailability of polar compounds exhibiting poor oral absorption due to limited permeability.
- Studies have also indicated a lower proteolytic activity in the nasal mucosa than in the gastro-intestinal tract (Kissel, 1998).
- Suitability for administration for long term therapy. Ease of administration of a nasal formulation, which increases the likelihood of patient compliance (Bjorg, 1991).

Considering the large number of problems associated with the following routes of drug administration viz. buccal, oral, parenteral, rectal, transdermal and vaginal, there has been a gradual interest by the pharmaceutical scientists towards exploring the possibilities of intranasal delivery of various drugs (Argawal et al., 1999). These favourable anatomical and physiological characteristics of the nasal mucosa have led to the testing of suitable drug candidates by this route. Thus studies conducted by various researchers (Wyss et al., 1991; Ramanathan et al., 1998; van der Kuy et al., 1999; Linhardt et al., 2000) have indicated that the low bioavailability of certain drugs (e.g. dihydroergotamine, promethazine, buprenorphine) associated to a high 1st pass effect and gut wall degradation, can be enhanced by the employment of the intranasal route. Table 1.1 shows some of the successes of intranasal drug delivery systems.

Table 1.1: Drugs incorporated into the intranasal delivery systems

Drug name	References
17β-estradiol	Studd et al., 1999
Budesonide	Creticos et al., 1998
DDAP	Deitcher et al., 1999
Metoclopramide	Ormond and Goa, 1999
Midazolam	Scheepers et al., 1998
Mupirocin	Davey et al., 1999
Salbutamol	Weksler et al., 1998
Sumatriptan	Felt et al., 1998

The many advantages of the intranasal route has attracted a lot of attention of many researchers and more and more drugs are currently being tested using this route. Table1.2 below shows some of the problem drugs that are still under investigation for future incorporation into the intranasal delivery systems.

Table 1.2: Drugs for future incorporation into intranasal delivery systems

Drug name	References
Benzodiazepines	Hjortkjaer et al., 1999
Desmopressin	Chancellor et al., 1999
Diazepam	Girzurarson et al., 1999
Dihydroergotamine	Logemann et al., 2000
Elactonin	Kohno et al., 1998
Influenza vaccine	Barchfield et al., 1999
Levocabastine	Borum et la., 1998
Recombinant cholera toxin B	Isaka et al., 1999
Vasopressin	Perras et al., 1999

Although there are several advantages associated with the nasal route, it still presents the following limitations:

- The nasal cavity provides smaller absorption area when compared to the gastro intestinal tract.
- The feasibility of application for the delivery of peptides and proteins for systemic use still has the drawback of low bio-availability possibly due to some proteolytic activity occurring at the nasal mucosa and difficulty of permeability due to the big molecular size and the hydrophilic nature of the these molecules (Soane, 1999).
- The histological toxicity of absorption enhancers used in nasal drug delivery is not yet clearly established.
- Potential nasal irritation leads to inconvenience.
- The potential of untoward immunogenic effects from molecules arising with nasal delivery systems.
- The route is adversely affected by local disorders such as rhinitis and pathophysiological changes.
- Large interspecies differences in nasal absorption (Agarwal et al., 1999).
- Lack of adequate aqueous solubility is often a problem for most drugs. The entire drug dose is to be given in a volume of 25-200µl, which requires relatively high aqueous solubility (Behl et al., 1998).

However, this route appears very promising for non-chronic delivery therapy where a rapid effect is desirable e.g. allergic effects, nausea and vomiting, nasal congestion etc (Ascentiis, 1996); and especially for drugs that do undergo an extensive hepatic extraction and/or gut wall degradation.

Current investigation on the exploration of the intranasal route indicates a rapid move and possible replacement of the authentic routes of administration for some problem drugs. For example, Wyss et al., (1991); van der Kuy et al., (1999) and Longemann et al., (2000) found that oral administration of dihydroergotamine was inadequate for the treatment of acute migraine because of the drug's low bioavailability (8%) due to a high first pass effect. In order to circumvent this drawback an intranasal preparation was

prepared. Bioavailability results of the intranasal preparation from this study indicated an improved relative bioavailability of 25%. Lindhardt et al., (2000) also observed a high nasal bioavailability (70%) and a short time for maximal plasma concentration (10 minutes) for buprenorphine compared with the sublingual tablet bioavailability of 15% and t_{max} of higher than 1 hour. In this case the intranasal preparation was found to be desirable since the response is supposed to be immediate. As the time of effect is very important in pain treatment the fast absorption is a major advantage. Furthermore, with this route moderate variations in the bioavailability were observed indicating that the route poses less dosing difficulties compared with the sublingual route. Kagatani et al., (1998) also obtained high absolute nasal bioavailability value (96.9%) for azetirelin, a thyrotropin releasing hormone, with an absorption enhancer (lauroylcarnitine chloride) compared with the poor oral bioavailability of 0.8% in rats.

Development of intranasal formulation has also been exercised in cases where the challenge is the achievement of rapid-onset of absorption to meet the emergency therapeutic purpose of the drug. For example, Li et al., (2002) developed an ethyl laurate -based microemulsion intranasal formulation for diazepam. The new preparation exhibited t_{max} of 2 minutes and bioavailability of 70% compared with the oral preparation with a bioavailability of 50%. Bumetanide which is used in the treatment of oedema associated with congestive cardiac failure, hepatic and renal diseases, is typically prescribed for long-term treatment of oedema when other diuretics have failed. The onset of diuresis occurs within 10 and 30 minutes following intravenous and oral administration respectively and the t_{max} in healthy subjects after oral administration was found to be between 0.5 to 2.2 hours (Ward and Heel, 1984). Yagi et al., (1993) evaluated the pharmacokinetics for bumetanide via the rectal route and obtained a t_{max} of 25 to 50 minutes, which may be considered slow for use in crisis situations. T_{max} following intranasal administration of bumetanide was found to be 15 minutes, which is comparable to that of the intravenous route (Nielson et al., 2000). Intranasal administration of diazepam resulted in a rapid absorption of the drug. Peak concentration was achieved after about 18±11 minutes, compared to serum concentration obtained after 10 minutes post intravenous administration. The rate of absorption was found to be 0.43±0.1 min^{-1}.

Critical time for successful seizure treatment is the first 10 minutes (Gizurarson et al., 1999). Studies on rectal administration of diazepam show a 12 minutes onset time, which is still higher than for the intranasal route although it is said to be sufficient for improving quality of life and effective in controlling seizures (Delgado-Escueta et al., 1982). Other applications of intranasal formulation development include sustained release preparations in cases where although the intravenous preparation may be 100% bioavailable, the drug exhibits a very short duration of therapeutic effect and hence high injection frequencies could be required. For example Sam et al., (1995) reported that apomorphine which reverses the "off" periods in Parkinsonism, has an oral bioavailability of 1.7%, with the intravenous route, the "on-phase" effect is 53±8 minutes and therefore requires high injection frequencies (up to 10 to 15 times a day). Attempts at prolonged delivery by subcutaneous administration with portable pumps caused local ulcerations (Stibe et al., 1988). Sustained intranasal formulation was found to be the ideal solution. Formulation of apomorphine with Carbopol 974P produced mean t_{max} values 2 to3 times higher than the intravenous and subcutaneous routes i.e. 6 to 8 hours as opposed to ±3 hours whiles the bioavailability was found to be equivalent to the subcutaneous preparation (Ugwoke et al., 1999).

1.2 ANATOMY AND PHYSIOLOGY OF THE NASAL CAVITY

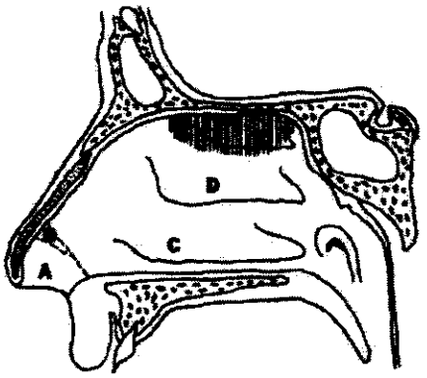


Figure 1.1: General structure of the nasal cavity: A, nasal vestibule; B, internal ostium; C, inferior conchae; D, median conchae; E, superior conchae (Verhoef and Merkus, 1994)

The human skull is composed of two functional sections that protect the delicate structures within them. The neucranium surrounds and protects the brain while the viscerocranium surrounds and protects the eyes, mouth and the nasal cavity (Ridley et al., 1992).

The nasal cavity is divided into two symmetrical halves by the nasal (middle) septum and extends posteriorly to the nasopharynx. The most anterior part of the nasal cavity, the nasal vestibule, opens to the face through the nostril. The antrium is an intermediate region between the vestibule and the respiratory region. The respiratory region, the nasal conchae or turbinates, occupies the major part of the nasal cavity. It possesses lateral walls that divide it into three sections comprising the superior nasal turbinate at the top. Below this is the middle nasal turbinate and the lowest chamber, the inferior turbinate. These folds provide the nasal cavity with a very high surface area compared to its small volume (Ugwoke et al., 2001).

Morphology and physiology of the nose

The basic functioning of the nose are heating and humidification of the inspired air before it reaches the lungs, olfaction, resonance, filtration of particles, mucociliary clearance and antimicrobial, antiviral and immunological activities (Druce, 1986). The anatomy of the nose and the functions of the epithelial cells at different regions of the nasal cavity are such that these functions are performed optimally.

The olfactory region situated above the superior nasal turbinate possesses specialised ciliated olfactory nerve cells for smell perception. The central axons of these nerve cells pass through the cribiform plate of the ethmoid and into the olfactory bulb (Ridley et al., 1992). The total surface area of the olfactory epithelium is 200-400mm^2 (Baroody, 1999).

The nasal vestibule, opening to the outside environment, possesses numerous nasal hairs (vibrissae) that filter large air-borne particles. The epithelial cells in this region are stratified, squamous and keratinised with sebaceous glands. Due to its nature, the nasal vestibule is highly resistant to dehydration and can withstand insults from noxious environmental substances. On the other hand, permeation through this cell lining is very limited. As a result, it is not the preferred site for drug administration and absorption (Ugwoke et al., 2001).

The intermediate region, the atrium, lies between the nasal vestibule and the nasal conchae. This is a transitional epithelial region composed of stratified, squamous cells anteriorly and pseudostratified columnar epithelial cells with microvilli posteriorly. These pseudostratified columnar cells, which are inter-dispersed with goblet cells cover the respiratory region (turbinates). Also present are the seromucus ducts, the openings of subepithelial seromucus glands. Many of these cells have actively beating cilia with microvilli. Each ciliated cell contains approximately 100 cilia. Both ciliated and non-ciliated cells have approximately 300 microvilli. The atrium is also composed of non-ciliated and basal cells. The basal cells differentiate to other epithelial cell types and are believes to aid the columnar cells adhere to the basal membrane (Mygind and Dahl, 1998). A thin sheet of mucus produced by the serous glands and the goblet cells covers

the nasal turbinates and the atrium. Collectively, the epithelium and lamina propia are referred to as the respiratory mucus membrane or respiratory mucosa (Burkitt et al., 1993). The respiratory mucosa comprises the region of optimal drug absorption.

1.2.2 Sensory innervation and nervous system control

Nasal blood supply and secretion are controlled by the autonomous nervous system. Sensory innervation of the nasal cavity is via the ophthalmic and maxillary divisions of the trigeminal nerve (Babin, 1977). The resistance vessels (capillaries), located close to the surface of the nasal mucosa, are muscular vessels with narrow lumen. These vessels are predominantly under adrenergic control but also receive adrenergic innervation, and provide the blood needed to heat and humidify the inspired air. The capacitance vessels (venous sinusoids) are thin-walled and elastic. These are located deeper within the submucosa. They primarily receive adrenergic innervation and are responsible for most of the blood content supply of the nasal mucosa (Ugwoke et al., 2001).

Both the parasympathetic and the sympathetic fibres innervate the nasal secretory glands. The stimulation of the parasympathetic fibres causes an increase in the secretion that is proportional to the frequency of stimulation. It also dilates the capacitance resistance vessels causing an increase in the total nasal blood flow, which effect is not blocked by atropine. Sympathetic stimulation causes a pronounced and rapid contraction of the resistance vessels, decreased capacitance blood flow, decreased nasal air-way resistance and a reduction in total nasal blood flow (Babib, 1977; Baroody, 1999).

From the nostrils the air passes through the nasal vestibule and into the main chamber, which is divided into two roughly symmetrical compartments by the cartilaginous septum. The air stream thus divided; passes through the scroll-like passages (meatus) formed by the maso, maxillo and ethmoturbinates; merges at the septal window; and travels through the nasopharynx (Reed, 1993).

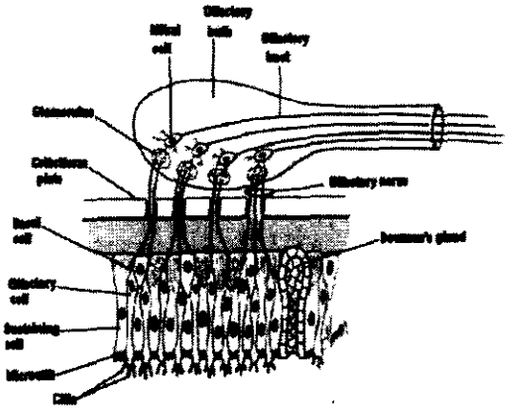


Figure 1.2: Anatomy of the nasal mucosa-cribriform plate interface showing the different cell types of the nasal epithelium. Reproduced from Fundamentals of Otolaryngology, A Textbook of Ear, Nose and Throat Diseases, Saunders, 1989

The surfaces of the nasal cavity are lined with a variety of epithelial types, which tend to facilitate transnasal absorption of drugs. viz.

- The lining of the nasal vestibule and meatus is composed of stratified squamous epithelium containing skin appendages.
- The olfactory mucosa, which lies between the nasal septum and the lateral wall of the nose above the level of the superior turbinate, is of a pseudostratified columnar type with specialised olfactory cells, supporting cells and both mucous and serous glands.
- The respiratory epithelium which is a ciliated and pseudostratified epithelium with an abundance of secretory cells, lines the main cavity of the nasal air way covering the naso and maxilloturbinates, adjacent septum and lateral wall. Each cell in this region has about 120 to 200 cilia and the microvilli between the cilia greatly increases the surface area for absorption. The cilia are covered by a blanket of mucus, which consists of two layers. The outer mucous layer (gel), which is relatively viscous and moves over the surface of the cilia. Between the cilia and below the mucous layer is the serous periciliary fluid (sol layer). In the submucosa is a proliferation of blood vessels including sinusoids forming erectile tissue, which allow the rapid passage of drugs that can cross the epithelium into the blood stream (Quraishi et al., 1997).

1.2.3 Nasal secretion and mucus layer

A blanket of viscoelastic fluid, the mucus, covers the respiratory part of the nasal cavity. A greater quantity of the nasal mucus is secreted from the submucosal glands. These glands are composed of mucus cells (which secrete the mucus gel) and the serous cells, which produce a watery fluid (Lansely, 1993). There are approximately 10^5 seromucus glands in the human nose. Mucus is also produced from the goblet cells as mucus granules (Tos, 1983).

The nasal secretion is a complex mixture, which consists of approximately 95% water, 2% mucin, 1% salts, 1% other proteins such as albumin, immunoglobulins, lysozyme, lactoferrin and < 1% lipids (Kaliner et al., 1984). The production of immunoglobulin A by both the adenoid tissue and the nasal mucosa, plays a very important role in immune protection against bacteria and viruses (Bernstein, 1997).

The mucus glycoproteins consist of a protein core with oligosaccharide side chains crosslinked by disulphide bridges and hydrogen bonds. The heterogeneity exists between the cytochemical characteristics of mucus secretion from seromucus glands and goblet cells (Theate et al., 1981). Approximately 1.5 to 2l of mucus is produced daily (Marom, et al 1984; Chien et al., 1989).

This mucus blanket, which is approximately 5μm thick, is composed of two layers, a lower sol layer and an upper gel layer. The lower layer, which bathes the cilia, is of low viscosity whereas the upper gel layer that rests on the cilia is a high viscosity fluid. Consequently, the viscosity of both layers affects the ciliary beating and the transport of the overlying mucus, the mucociliary clearance (MCC). The mucus viscosity is very sensitive to slight changes in the mucin content. A small increase in the mucin causes a significant increase in the mucus viscosity with resultant prolongation of the mucociliary clearance time (Rice, 1988).

Mucin is a high molecular mass (2×10^6- 4×10^6 Da) glycoprotein crosslinked with disulphide bridges, ionic bonds and physical entanglements. The carbohydrate side

groups attached to the protein backbone include galactose, L-fructose, N-acetylglucoseamine, N-galactoseamine, and N-acetylneuraminic acid (sialic acid). The carbohydrate side chains terminate with a sialic acid or L-fructose group, which convey mucin an anionic polyelectrolyte property at neutral pH. Due to the multiplicity of the hydroxyl groups of the carbohydrate side chains, mucin easily forms hydrogen bonds with other suitable polymers (Kamath and Park, 1994).

The nasal mucus has a number of functions (Chien, 1995):

- covers the mucosa
- forms an enzymatic protection
- acts as an adhesive and transports the particulate matter to the nasopharynx
- has water-holding capacity
- exhibits surface electrical activity
- permits efficient heat transfer

1.2.4 Mucociliary clearance (MCC) system

One of the functions of the upper respiratory tract is to prevent noxious substances (allergens, bacteria viruses, toxins etc.) from reaching the lungs. When such material adhere to, or dissolve in, the mucus lining of the nasal cavity, they are transported towards the nasopharynx for eventual discharge into the gastrointestinal tract (GIT). Clearance of this mucus and the adsorbed/dissolved substances into the gastrointestinal tract (GIT) is called mucociliary clearance (MCC). Effective mucociliary clearance (MCC) has contributions from both the mucus and the cilia. Consequently factors that affect either the mucus or the cilia would influence the MCC (Raphael et al., 1996).

It is of utmost importance that the MCC is not impaired in order to prevent lower respiratory tract infections. Although it has been estimated that the mucus transport rate is 6mm/min (Proctor, 1977), there is a wide variation in MCC between different individuals, but within one subject it is fairly constant. The concept of fast movers and slow movers is well documented. This implies that there are individuals with a very fast

MCC rate and others whose MCC rate is slow (Baroody, 1999). The MCC rate is independent of age and gender (Armengot et al., 1990).

1.2.4.1 Factors that affect the mucociliary clearance (MCC) system

Both temporal, environmental and disease conditions can impact on the MCC rate. All factors that lead to an increase in mucus production, decreased mucus viscosity, increased ciliary beat frequency (CBF) without disrupting the metachronal wave, can increase the MCC rate. The opposite effects, as well as destruction of the viscoelastic properties of the mucus and the disruption of the metachronal wave tend to reduce the MCC rate (Ugwoke et al., 2001).

Environmental conditions such as temperature (23°C) cause a moderate reduction in MCC rate (Ridley et al., 1992). However, Jorissen and Benssen, (1995) reported a linear increase (0.6Hz/°C) with temperature in CBF of nasal biopsy. Sulphur dioxide causes a concentration-dependent and significant reduction in MCC rate (Ridley et al., 1992). Cigarette smoking also decreases the MCC rate due to its influence on the mucus rheology and/or reduction in the number of actively beating cilia (Stanley et al., 1986).

The following pathological conditions of the upper respiratory tract influence MCC rate due to their effect on ciliary beating and/or mucus rheology. These include Kartagener's syndrome, Sjogren's syndrome, asthma, nasal polyps, rhinitis, deviation of the nasal septum, allergic rhinitis, common cold and chronic sinusitis (Ugwoke et al., 2001).

The relevance of disease conditions in nasal drug delivery cannot be over-emphasised. Pathological conditions with increased MCC rate tend to reduce the contact time of the drug with the absorptive nasal mucosal surface whereas decreased MCC rate has the opposite effect. Nasal hyper-secretion dilutes nasally administered drug preparations leading to a reduced concentration gradient, with possible influence on the absorption rate. A change in the pH of the mucus can affect the ionisation of some drugs, and this

can have a significant effect on the overall nasal drug absorption profile (Ugwoke et al., 2001).

1.2.4.1.1 Ciliary beat cycle and mechanism of ciliary beating

For the MCC system to function efficiently as the first line of defence for the lungs, the cilia must beat in a well co-ordinated manner (both in phase and frequency), and this is called the metachronal wave. In this way a co-ordinated clearance towards the nasophanrynx is ensured. In a small part of the anterior nares, the direction of the mucociliary clearance is forward, with clearance of mucus and deposited particles carried out by blowing and wiping the nose (Chien et al., 1989).

A cilium is made of an axoneme surrounded by the ciliary membrane. The axoneme is composed of two central microtubules and nine pairs of peripheral microtubules (A and B microtubules), an arrangement referred to as the "9 + 2" pattern formation of microtubules. The peripheral microtubules are connected to each other by nexin links and the radial spokes connect the central microtubules to the peripheral microtubules. Hence the rigid microtubule structure. Two dynein arms (outer and inner dynein) are attached to the one of each pair of the peripheral microtubules. Due to their ATPase activity, the dynein arms provide the energy required for ciliary beating (Lindberg, 1997).

Ciliary motility generally results from the sliding movement of adjacent axonemal microtubules. The dynein arms provide the mechano-chemistry for the movement as a result of the ATPase activity. One theory which explains the axonemal movement suggests that the dynein A microtubule transiently attaches to, and detaches from the dynein B microtubule after ATP binding and hydrolysis, causing the doublet to move in the opposite direction. While other axonemal structures resist this movement, thereby causing the bending and unidirectional movement (Lee et al., 1991). The switch point theory hypothesises that one set of the doublets is active during the effective stroke and the other set during the recovery phase. Activity therefore switches back and forth between the two sets causing the asynchronous and bending motion (Satir, 1985).

Another theory is that an electrochemical signal over the cell surface may be responsible for synchronising ciliary beating in the metachronal wave, even though this signal is not necessary in initiating the ciliary beating (Guyton, 1981).

Ciliary beating has three identifiable phases, an active/effective phase, the rest phase and recovery phase. During the active phase the cilium maximises its length within the sol layer, reaching out beneath the gel mucus layer and clawing it with the tiny projections on its tip. The active phase is followed by the rest phase when the cilium is bent and almost parallel to the cell surface. The beat cycle is completed by the recovery phase where the cilium recoils back to the initial position, ready for the next cycle. The asymmetric beating enables the propulsion of the mucus in one direction. In one beat cycle each cilium makes an arc of approximately 110°. More time is spent during the rest phase than the active or recovery phases. The CBF varies between 10 to 20 Hz (Sanderson and Dirksen, 1989).

Calcium ion concentration has been strongly linked with ciliary beating. Increased Ca^{2+} influx increases the beat frequency and removal of the extracellular Ca^{2+} leads to a loss of ciliary beating, which is restored by addition of extracellular Ca^{2+} (Satir and Sleigh, 1990).

The cilia are also mechanosensitive appendages. *In-vivo*, this mechanical stimulation is provided by the overlying mucus.

1.3 Factors influencing the nasal pharmacokinetics

The various advantages of the nasal route have made the nasal mucosa a more feasible and desirable site for systemic drug delivery. However, there are factors that should be considered for optimising the intranasal drug administration and these are as follows (Ganderton, 1987):

- Physiological conditions of the nasal vasculature
- Speed of mucus flow
- Drug loss (crying, swallowing, drainage)

- Head position
- Venous drainage of the mucosal tissue
- pH of the absorption site and dosage form.
- Presence of infection
- Atmospheric conditions
- Dosage form factors
- Drug concentration and volume of dosage form.
- Physicochemical properties of the drug (molecular size, degree of ionisation of the drug, relative liposolubility)
- Density and viscosity of the dosage form (liquid preparations)
- pH and tonicity of dosage form (liquid preparations)
- Excipients especially the vehicle
- Techniques and devices for administration
- Droplet or solid particle size
- Site of deposition
- Rate of clearance

In general it is clear that the advantages of delivering a drug through the nasal route outweigh the disadvantages. Nonetheless, the many advantages of the intranasal drug delivery system do not simply justify the incorporation of any drug into such a delivery system. It is imperative that the physical, chemical and biological properties of the drug candidate be evaluated to prevent its unwarranted incorporation into this drug delivery system (Quraishi et al., 1997).

1.3.1 Factors to be considered for the selection of the candidate drug for intranasal delivery

The following are some of the rate–limiting physico-chemical properties to transmucosal permeation of a drug, which have to be considered prior to formulation of a drug into the intranasal delivery system.

Partition coefficient (log P)

Log P is a measure of the degree of partition of a compound from an oily phase to an aqueous phase. Thus it characterises the lipophilic/hydrophilic nature of a compound. The higher the log P the more lipophilic is the drug. The nasal mucosa is said to be a very thin membrane with a very high lipid content, thus making it more permeable to lipophilic compounds. Thus drugs with high log P will exhibit greater kinetics of transmucosal permeation in the nasal mucosa. For hydrophilic compounds, thus those with low log P, the rate of permeation through the nasal mucosa will be slower (Shao et al., 1994; Argawal et al., 1999).

Dissociation constant

Biological membranes in general constitute a substantial barrier to drug transport prior to absorption in the blood stream. During the transcellular transport process, a drug has to pass through the cellular membrane via a concentration gradient-dependent diffusion process. While many factors can affect this pathway the most important factors include pH, pK_a and partition coefficient. It is known that only the undissociated (neutral) species of a penetrant molecule can only partition into the lipophilic biological membrane as defined by the pH-partition hypothesis. For weak bases (those with high pKa) an increase in the pH of the solution increases the fraction of the unionised species and hence increases the extent of nasal mucosa permeation and absorption. For weak acids (those with low pKa) a decrease in the pH of the solution increases the fraction of the unionised species and hence increases the extent of mucosal permeation and absorption, implying that the mode of transport of the molecules/species across the nasal mucosa is transcellular. Since transport of the molecules/species across the nasal mucosa also occurs via the paracellular pathway, ionised species (those with low pKa) will also permeate through. Thus the absorption of drugs is partially dependent on the pH-partition theory (Agarwal et al., 1999).

Findings by various researchers on the effects of pH, pK_a and partition coefficients of drugs on their nasal absorption indicated that the pH of the formulation and the surface membrane have a direct effect on the rate of absorption. The nasal absorption of weak

electrolytes such as salicylic acid and aminipyrine in rats is highly dependent on the degree of ionisation (Corbo et al., 1989; Corbo et al., 1990; Gonda et al., 1990; Vora et al., 1993). These findings were also supported by the pH-partition theory.

Molecular weight

The nasal absorption of drugs decreases exponentially with an increase in the molecular weight of penetrant when the molecular weight is greater than 1000 Dalton. Thus, there is an inverse relationship between molecular weight and absorption rate. Furthermore, the effect of molecular weight on the absorption of drugs is related to the effective size of the molecule, e.g. cyclic peptides are better absorbed than linear ones. The aqueous partitioning which is sensitive to variation in molecular weight/volume of the penetrant plays a greater rate-limiting role in the absorption across the oral epithelia than the nasal epithelia (Quraishi et al., 1997; Agarwal et al., 1999). A limited amount of work has been done to evaluate the effects of molecular weight and size on their nasal absorption.

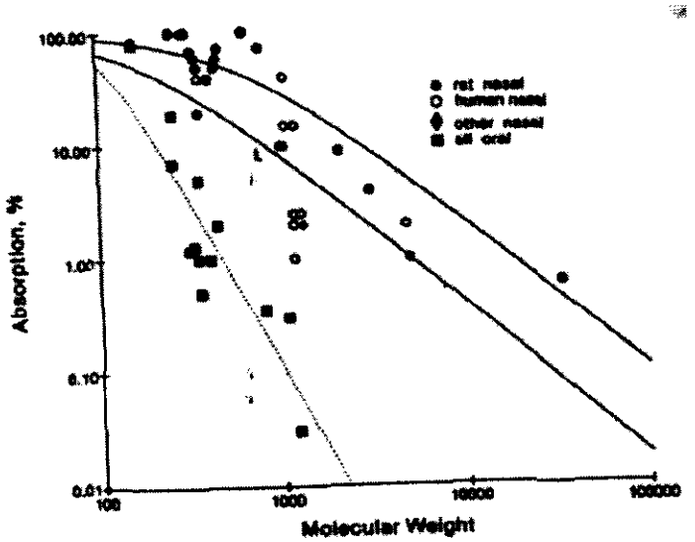


Figure 1.3: Log-log plot of the absorption after nasal and oral administration of various compounds versus molecular weight (McMartin et al., 1987).

Studies by various researchers (Fisher et al., 1987, McMartin et al., 1987) on the effect of molecular weight on the nasal absorption in rats using water soluble compounds with weights varying from 160 to 34000 indicated an inverse relationship between nasal absorption and molecular weight (figure 1.3). Wherever possible, the authors also

collected data on the oral absorption of the same compounds. A log-log plot of these results showed that the rate of oral absorption decreases faster than that of the nasal absorption, as a function of molecular weight. Thus, from their results it can be deduced that the nasal absorption results are consistent with non-specific diffusion channels through the aqueous channels between the cells, which impose a size restriction on permeability (Behl et al., 1998).

Particle size and morphology

Particle size and morphology of drug particles constitute important properties for particulate nasal preparations. These factors are related to the rate of drug dissolution and hence should be controlled to obtain suitable drug dissolution properties in the nasal passages. *In-vitro* dissolution rates in suitable simulated fluids should be considered. Particle size and morphology of drug particles/nasal preparation determine the grittiness and possible irritation to the nasal cavity. Too fine particles, below 5 microns should be avoided for nasal administration as they are inhaled directly into the lungs. The ideal particle size for nasal preparations for deposition in the nasal cavity is in the range 5-10 microns (Behl et al., 1998).

The nasal permeation/absorption and bioavailability of drugs do not only depend on the physico-chemical properties inherent to the drug, the following factors also play important roles in determining the extent of nasal permeation/absorption:

Biochemical and physiological characteristics

The nasal mucosa possesses the metabolic capacity to protect the lower airway from toxicants. Thus, it is an enzymatic barrier to nasally administered drugs consisting of different proteolytic/hydrolytic enzymes. These enzymes are both oxidative and conjunctive. The following are some of the enzymes found in the nasal cavity:

Cytochrome P-450:

Nasal cytochrome P450 is a family of hemoproteins, which play a central role in the metabolism of xenobiotics and are also involved in both the synthesis and catabolism of endogenous compounds. Nasal cytochrome P450 is preferentially located in the olfactory epithelium and in many species the concentration in this area is second only to that of the liver (Voigt et al., 1985).

In the olfactory epithelium, cytochrome P450 is found in the sustentacular cells, basal cells and Bowman's glands. There is also evidence that the cilia of the olfactory neurones also contain cytochrome P450. The relevance of localisation of nasal cytochrome P450 to specific cell types in specific regions of the nasal cavity is difficult to predict. Individual nasal cells may have higher levels of cytochrome P450 than the individual hepatocytes. Much of the cytochrome P450 in the olfactory epithelium are concentrated in the Bowman's glands and this may be particularly relevant to the metabolism of inhaled and instilled xenobiotics that undergo extensive first pass metabolism during passage through the epithelial cells lining the airway (Reznik-Schuller, 1982).

Although the concentration of nasal cytochrome P450 is low, its metabolic activity surpasses that of the liver. Thus the olfactory cytochrome P450 is said to be catalytically active due to the abundance of NADPH- cytochrome P450 reductase in the olfactory epithelium than in the liver hence the high drug metabolising capacity of this tissue (Ding et al., 1986; Reed et al., 1986).

Importance of cytochrome P450

Nasal cytochrome P450 acts as a first line of defence for the lungs and/or maintain acuity of olfaction. They also activate certain xenobiotics, which induce a toxic or carcinogenic response in the nasal cavity (Reed, 1993).

Carboxylesterases

Carboxylesterases catalyse the hydrolysis of carboxylesters, carboxylamides, and carboxylthioesters. A wide range of drugs, insecticides, herbicides are substrates for these

enzymes. Although hydrolysis of an ester or an amide results in detoxification, it can occasionally be an activating process especially in the metabolism of acetate and acrylate esters (Reed, 1993).

Studies have indicated that esterase activity is generally higher in the olfactory epithelium than in the respiratory epithelium and that the nasal mucosa has similar esterase activity as the liver. These enzymes are more prevalent in the Bowman's glands and their ducts and in the sustentacular cells. No esterase activity has been detected in the neuronal cells, basal cells or nerve fibres (Bogdanffy et al., 1987; Dahl et al., 1987; Bogdanffy et al 1991).

Glutathione S-Transferases

The glutathione S-transferases (GSTs) are a family of iso-enzymes, which catalyse the conjugation of glutathione with a wide variety of electrophilic compounds. Such conjugation can be classified as a detoxification reaction and the enzymes play a crucial role in protecting the body against many of the reactive species formed during cytochrome P450-dependent metabolism (Reed, 1993).

Like cytochrome P450, the glutathione S-transferases (GSTs) are located in the olfactory epithelium. Immunohistochemistry has demonstrated the presence of the glutathione S-transferases (GSTs) in the Bowman's glands and sustentacular cells of the olfactory epithelium. The location of these detoxifying enzymes in the same cell types as the cytochrome P450 affords some degree of protection to the nasal mucosa; however their low activity is of concern (Bogdanffy, 1990). In addition to their role in detoxification, the glutathione S-transferases (GSTs) are also capable of activating certain compounds to carcinogenic and mutagenic species (Reznik et al., 1980).

Aldehyde and Formaldehyde Dehydrogenases

The isoenzymes of aldehyde hydrogenase catalyse the oxidation of a broad spectrum of aldehydes to acids using NAD(P) as a cofactor. Formaldehyde hydrogenase is more

specific, oxidising only formaldehyde complexed with glutathione. In both cases the oxidation is considered as a detoxifying reaction (Reed, 1993).

Studies have demonstrated the presence of both aldehyde and formaldehyde dehydrogenase in the nasal mucosa (Cassanova-Schmidtz, 1984). Localisation of formaldehyde dehydrogenase is more prevalent in the olfactory epithelium than in the respiratory epithelium and its activity is similar to that of the liver. Aldehyde dehydrogenase activity is greater in the respiratory epithelium in particular the ciliated and non-ciliated epithelial cells, than in the olfactory epithelium (basal and Bowman's glands) (Bogdanffy, 1986).

The significance of the distribution of these enzymes is apparent when the primary site of nasal injury following exposure to aldehyde is considered. Acetaldehyde does not induce lesions in the respiratory epithelium-lined lateral meatus (aldehyde dehydrogenase rich region) but rather in the olfactory epithelium, which has relatively little aldehyde dehydrogenase activity. Similarly, formaldehyde-induced lesions are both frequent and severe in the lateral meatus, a region of low formaldehyde dehydrogenase activity (Morgan, 1990).

Other xenobiotic transformation enzymes

High concentrations of the mitochondrial cyanide-metabolising enzymes have been demonstrated in the nasal tissue. The enzyme is more active in the olfactory epithelium than in either the respiratory mucosa or liver, and plays an important role in the detoxification of inhaled hydrogen cyanide and cyanide released in the nose due to cytochrome P450-dependent metabolism of inhaled organic nitriles (Dahl, 1989).

Nasal mucociliary clearance (MCC)

This is an important defence mechanism of the nose, which protects the body against the inhalation of foreign substances. Inhaled/instilled particles are cleared from the nasal cavity by mucociliary clearance (MCC). The mucociliary system provides the pulmonary mechanism in the upper and lower respiratory tracts. Normal ultraciliary structure is a

presumed prerequisite for effective function. Furthermore, protein structures (nexun, dyneis) provide support for the system and are important in the ciliary beat cycle (Robson, 1993).

Cilia

These are small densely packed organelles that are 5 to7 mm long protruding from the cell surface. Their main action is biological transport of a layer of mucus over the cells. To fulfil this task, the cilia beat synchronously creating a wave-like pattern on the cell surface called metachronal wave. Ciliary systems are dynamic and respond to changes in their beating parameter to a variety of stimuli e.g. mechanical, electrical and hormonal. These changes of ciliary beat patterns can reveal a lot of information of the events taking place within the cell. Therefore cilia can perform the function of an indicator for activities going on within inside the cell without the need of probing into the delicate cell environment (Korngreen, 1994). One of the cellular events that is directly related to a change in the ciliary beating is the calcium concentration. Studies have shown that there is a correlation between calcium concentration and ciliary beat parameters. Calcium was shown to activate directly the beating of isolated cilia (Naitoh and Kaneko, 1972; Tamm, 1988; Tamm, 1989). The calcium second messenger signalling system is a highly dynamic one. Over the last decade a paucity of data was revealed regarding the functional role of the cystolic calcium in the living cell. Intracellular free calcium is an important link of chain signal transduction from stimuli on the cell surface to the cellular response. The simplicity that was at first attributed to the calcium messenger system has gradually been replaced by the realisation that this system has complex behaviour in both time and space (Rasmussen, 1990). It has been argued that after the initial rise in cytosolic free calcium concentration, usually caused by the release of calcium from the internal stores, there is a calcium influx resulting in a rise of calcium ions in small submembrenous domains thus prolonging the duration of cellular response without the risk of cell toxification by a high concentration of calcium. These submembranous domains are by nature hard to detect due to the inability of the present technique to visualise the calcium concentration (Rasmussen, 1990).

Ciliary cells are inherently suitable for detecting changes in the calcium concentration near the cell membrane. Because the cilia are closely covered by plasma membranes, any change in the calcium concentration that occurs will affect the ciliary beating. Another aspect of both ciliary beating and intracellular calcium is their ability to produce oscillations both in calcium and frequency as has been shown on sheep trachea (Salathe and Bookman, 1993). Since the changes in ciliary beating and in calcium concentration have a great variability much averaging is needed to discern trends in each one of them. This averaging is not enough to measure the response to a given stimulus, ciliary beat frequency and the change in intracellular calcium concentration separately in nasal MCC, should not be affected by nasally administered medications and excipients. Furthermore, the rheological properties of the mucus layer can change after contact with the pharmaceutical formulations. This will affect the clearance and exposure of the drug to the mucosa (Agarwal et al., 1999).

Significance of mucociliary clearance (MCC)

Mucocilliary clearance (MCC) imposes a time constraint upon contact between the applied formulation and the absorptive surface. Drug absorption can only occur while the drug is retained at the site of absorption and the time allowed for this is dictated by the rate of mucus transport. MCC operates from the nasal cavity to the terminal bronchioles with cilia transporting the mucus in the direction of the orophanrynx. Mucocilliary transport depends on a successful interaction between the three components: cilia, mucus and periciliary fluid. Changes in any one of these components may alter the characteristics of transport (Lansley, 1993).

Periciliary fluid

The periciliary fluid is composed of a low viscosity fluid whose depth is less than the length of an extended cilium when mucus is present. It originates from the epithelium due to the balance between Cl^- secretion and Na^+ absorption. This, it is formed by the epithelial cell exudate. This fluid contains soluble proteins (lacto ferrin, lysozyme, neutral aminopeptidase and endopeptidase, secretory IgA) many of which are derived from the serous cells of the submucosal glands. This layer is considered to be relatively stable in

contrast to the mucus, which is replaced every 10 to 20 minutes, and may provide many of the protective functions attributed to the mucus. The periciliary layer is covered by a more viscous upper layer of 0.5 to 5μm deep (Kaliner, 1991).

Mucus

This is a viscoelastic gel layer that is essential for the transport of particulate matter by ciliary activity. Mucus has often been considered to exist as a continuous blanket of 5 to 10μm. Mucus is secreted by the goblet cells. It is composed of mucins, which, are glycoproteins of variable molecular weight from a few hundred Daltons to more than ten million Daltons. Approximately 80% of the weight of the mucin molecule consists of carbohydrates. About 3% of the mucus layer consists of mucins while 90 to 95% consists of water with the electrolytes, serum, immunoglobulins and lipids (King et al., 1974).

The mucus is expelled from the goblet cells as highly condensed granules by exocytosis. Upon exocytosis the secreted granules undergo massive swelling and the mucins are mixed and annealed to form a visco-elastic gel that is transportable by the cilia. When the mucus glycoproteins are dissolved in water, an entangled network is formed. This mucus gel is stabilised by non-covalent interactions between the mucin molecules, and collapses when the disulphide bonds are reduced. Mucus exhibits a non-Newtonian behaviour: it possesses both viscous (fluid) and elastic (solid) properties hence it is described as viscoelastic. The viscoelastic properties of the respiratory mucus enable it to sufficiently relax to be propelled by the cilia. The elasticity of the mucus means that it can accept efficient energy transfer from the cilia whiles its viscous properties enable it to relax sufficiently to permit propulsion. Elasticity appears to be the more important parameter to efficient transport and has an optimum range of 1 to $2Nm^{-2}$ (Lansley, 1993).

Common cold or pathological conditions

The common cold or any pathological conditions involving mucociliary dysfunction can greatly affect the rate of nasal clearance and subsequently the therapeutic efficacy of the drug administered intranasally. Impaired MCC causes longer contact times of the airway

mucosa with the bacteria and viruses, which could lead to infections of the upper respiratory tract. The efficiency of the nasal MCC depends on three factors namely:

- The amount of ciliary input, which is determined by the length, and density of the cilia and the CBF.
- The amount of mucus and the depth of the periciliary fluid.
- The viscoelastic properties of the mucus

Changes in any of these three parameters may cause an impairment of the mucociliary system. Several pathological conditions exist in which the MCC does not function properly:

- In primary ciliary dyskinesia, infections of the respiratory system occur frequently.
- In cystic fibrosis where there is an impairment of the MCC the mucus has a reduced water content and the transport rate is delayed.
- In the case of viral and bacterial infection the MCC system is compromised due to cilia loss and a change in the rheological properties of the mucus.
- Nasal obstruction as a result of extensive polyposis would reduce the capacity of nasal absorption. In addition, atrophic rhinitis or severe vasomotor rhinitis could reduce the usefulness of the nose to absorb a drug. In certain cases, an excessive response of the secretory system to some irritants could drain away any intranasally administered medication prematurely (Agarwal et al., 1999).

Assessment of the ciliary beat frequency (CBF)

Several methods have been described to measure the CBF from the upper airways *in-vitro* (Wanner, 1977; Van de Donk et al., 1980). Some of these are based on cinematographic or video detection. In this method a video was recorded and evaluated at low speed to determine the CBF (Gilain et al., 1993; Ganbo et al., 1995). Video recordings of beating cilia have also been digitalised and the resulting images were used for computer analysis (Salathe and Bookman, 1995; Sisson, 1995). Placing a pinhole photodiode on a video, which records beating cilia, a photoelectric signal is obtained which can further be analysed by the computer. High-speed digital video camera analyses

the CBF and metachrony of cultured cells or epithelial sheets with a high temporal and spatial resolution (Yoshitsugu et al., 1993; Sanderson and Dirksen, 1995). The video camera can also be connected to a fluorescence imaging system making it possible to study the physiology of ciliary activity by simultaneously measuring the CBF and the intracellular ions or messengers (Sanderson et al., 1993).

The ciliary activity can also be assessed by photoelectric detection, in which either reflected or transmitted light is employed. Measuring variations in light reflected is possible both *in-vitro* and *in-vivo*. These reflections can originate from the cilia and /or from the mucus layer, and these may contribute to the reflected image. A new laser scattering system has been developed to measure CBF and determine the metachronal wave period and direction. This method is based on a heterodyne mode correlation analysis light scattering system with two spatially separated focal spots. The back-scattered signals from the adjacent groups of cilia are cross-correlated to give the phase distribution of the ciliary activity (Wong et al., 1988).

The most commonly applied photoelectric method for ciliary activity measurement is the transmitted light technique. This is the most convenient means of quantifying ciliary beating. The light is transmitted through the ciliated epithelium and the changes in the light intensity due to ciliary movements are detected by a photosensitive cell. The transmitted light technique allows a precise and direct measurement of CBF and an average of a group of cilia beating is obtained. The only limitation of this method is that only CBF of the cilia at the edges of the piece of tissue investigated, can be measured nonetheless, in cell cultures the light transmission through the cell mono-layer is possible (Dalhamn and Raylander, 1962).

The measurement of CBF *in-vitro* is a very accurate and reproducible technique to determine the effects on ciliated epithelium. Therefore CBF measurements are a good *in-vitro* screening method to establish the potential toxicity of the drugs and excipients, and to compare the nasal drug formulations. However, the careful interpretation of the ciliary CBF data is vital since the effects of nasal formulations in vitro are usually more pronounced than the *in-vivo* effects. The cilia are protected by the mucus layer *in-vivo*

whereas *in-vitro* the cilia are in direct contact with the investigated substance. Furthermore, *in-vivo* the respiratory nasal epithelium can be expected to recover from damage. Thus, it is not possible to make predictions regarding the effects of chronic use of a formulation on the MCC *in-vivo* based on *in-vitro* effects of CBF. These factors therefore have to be taken into account when determining the cilio-toxicity of drug formulations (Ingels et al., 1981; Van de Donk et al., 1982; Batts et al., 1990; Rusnak et al., 1994)

In order to make preliminary predictions of the effects of substances on the nasal MCC, not only *in-vitro* CBF studies need to be performed but *in-vivo* studies on animals as well. These studies can include the following (Ennis et al., 1990; Donovan and Zhou, 1995; Marttin et al., 1995; Marttin et al., 1996):

- Nasal mucociliary transport rate studies
- Morphological studies of the nasal epithelia/cavity
- Determination of the release of marker compounds in the nasal cavity after administration.

To predict the safety of nasal formulations for human use, it is vital to investigate its safety at biopharmaceutically and therapeutically relevant concentrations. Both *in-vitro* and *in-vivo* studies should be performed and comparison between the formulation under study and the one already marketed should be established. For accurate and reliable evaluations of the potential side effects of the new nasal formulation, the effects of its long-term use in animals and in humans should be determined. This is of importance since most nasal formulations are designed for sub-chronic and chronic use (Marttin, 1997).

1.4 Nasal mucociliary clearance (MCC) system tolerance to drugs and excipients

1.4.1 Rationale for the determination of the effects of drugs and excipients on the nasal mucociliary clearance (MCC) system.

Many substances can influence the MCC system of the airways, either by stimulation or by inhibition. The stimulatory effect of drugs on the nasal MCC is of clinical importance, because these substances can be used to improve the pathological conditions of the mucociliary system. For nasal drug delivery, however, possible inhibitory effects are most relevant, because they can result in undesired side effects on the MCC system. When the MCC system is impaired by components of the nasal formulation, this can be prohibitive of their therapeutic use. Investigating the effects of drugs and excipients on the nasal mucociliary functioning is an important issue, because of the growing number of nasal drug formulations currently investigated for pharmacotherapy. Some of these preparations are meant for long term treatment. Any adverse effects on the ciliated epithelium may limit the patient's acceptance of the nasal formulation and thus use in chronic nasal drug delivery. In view of the important functions carried out by the cilia, it is obviously desirable that drugs in general and excipients should be as "cilio-friendly" as possible (Merkus, 1992).

1.4.2 Effects of drugs and excipients on nasal mucociliary clearance (MCC)

A great deal of effort has been invested in the design of formulations for intranasal delivery of a wide variety of systemic drugs. A thorough assessment of the components of the absorbing site viz. proteolytic enzymes, mucociliary system, the monocellular nasal epithelium; and the possible routes of transport across the nasal epithelium viz. paracellular transport and passive diffusion, suggest the incorporation of the following excipients to the nasal formulation:

- Enzyme inhibitor
- Permeation enhancer to mediate drug transport across the nasal epithelium especially for hydrophilic drugs

- Mucoadhesive agent to prolong the contact time between the absorbing surface and the dosage form so as to enhance drug absorption at the site of absorption.

Literature indicates that the rate of MCC is every 20 minutes which implies that the time of residence of the administered drug/formulation at the site of absorption will only be 15 to 20 minutes which time is insufficient for the complete release of the drug from the delivery system. This limited contact time between the formulation and the absorbing surface results in low concentration of drug absorbed and hence low bioavailability of the drug (Cornaz and Buri, 1994).

Screening of pharmacologically active moieties for use in nasal application is of utmost importance since these species can impair the mucociliary system due to their inherent physicochemical properties. Adjustments as regards such properties would therefore be deemed necessary in cases where intolerance is observed. Most drugs are either weak bases or weak acids thus, the pH of these compounds can have a pronounced effect on the ciliary movement. Studies by Pujara et al., (1995) on the effect of solution pH on the rat nasal epithelium using 0.07M phosphate buffer solutions ranging in pH from 2 to 12 indicated that at pH 2 to pH 5.9 and pH 9 to pH 12, 80% to 90% cilio-inhibition and protein release (cell lysis) were observed. At pH 7 to pH 8.8 very little decrease in ciliary movement and protein release were observed. These results suggest that pH tolerance for nasal preparations is within the range 6.6 to 8.8 (physiologic pH for the nose).

1.5 Nasal Pharmacokinetics

1.5.1 Basic mechanisms of transport across the cell membrane

Mammalian cell membranes separate cells from their environment and from one another. Many pharmacologically active moieties cannot readily move across the cell membranes. Under physiological conditions, there are several mechanisms governing the transport of molecules across cell membranes. Methods for facilitating the transport of molecules across the epithelial cells can be categorised into two major groups. Thus, the paracellular and transcellular transport (Lee, 1991).

1.5.1.1 Paracellular transport

This is the transport of molecules around or between the cells. Tight junctions or similar situations exit between the cells. At these tight junctions, the cell membranes are brought into extremely close opposition, but are not fused, so as to occlude the extra-cellular space. Consequently, ions or molecules may not be able to pass through the intercellular spaces (Lee, 1991).

1.5.1.2 Transcellular transport

Transcellular transport can be divided into the following subgroups:

Passive transport:

This refers to the movement of a solute along its concentration and electrical gradient. Small and non-ionic molecules usually cross-cell monolayers by passive transport. The rate at which a molecule diffuses across the lipid bilayer of the cell membrane depends on the size of the molecule and its relative solubility. Generally the smaller and more hydrophobic the molecule the more rapidly it will diffuse across the bilayer. Cell membranes are also permeable to some small water-soluble molecules such as ions, sugars and amino acids (Hseih, 1994).

Active transport:

This differs from passive transport in that the transport process is mediated by membrane transport proteins coupled to an energy source. Furthermore, the solutes can be transported against a concentration gradient. Many active transport systems are driven by the energy stored in ion gradients. Na^+ is the usual co-transported ion whose electrochemical gradient provides the driving force for the active transport of a second molecule such as glucose, amino acid and dipeptide. Hydrolysis of ATP or other high energy compounds (Na^+ K^+ ATPase which pumps Na^+ out and K^+ into the cell against their electrochemical gradients) on the surface of the protein also provides the energy for active transport (Sweadner et al., 1990).

Endocytosis:

Transport of macromolecules is mediated by both endocytosis and transcytosis. The macromolecules are enclosed by cell membranes and migrate within the membrane-bound vesicle to the perinuclear region of the cell where the vesicles coalesce with the lysosomes in which intracellular digestion occurs (Rodman et al., 1990).

Transcytosis:

In transcytosis, the macromolecules bind to the receptors on the surface of the epithelial cells, then the receptor-macromolecule complex is incorporated into the vesicle and carried into the cell. The complex remains intact in endosomes and is retrieved in transport vehicle that fuse with the membranes of the opposite side of the cell monolayer (Warshaw et al., 1974; Mostov et al., 1985).

1.6 Biological barriers to drug permeability

Cell membranes are barriers to macromolecules and to most polar compounds but are relatively permeable to water and small hydrophobic molecules. These barriers which are also common sites of drug administration, may function in a physical, chemical or biological fashion or a combination thereof depending on the specific mode and site of application of a given drug delivery system. Generally, biological barriers are site specific and are related to the histological organisation of the specific site (Wheater et al., 1979).

1.6.1 Permeability enhancement: overcoming transport thresholds across biological barriers

Regardless of the means of enhancement the common goal of permeability enhancement is to reduce the "threshold" of the biological barriers. For example, the input of the electric energy in iontophoresis provides the driving force (energy) for facilitating the transport of ions across the skin. The incorporation of chemical enhancers in a formulation is intended to lower the "activation energy" of drug transport.

There are various methods of enhancing drug transport across the biological barriers depending on the site of drug administration (Hseih, 1994).

Permeation enhancement technologies include the following (Hseih, 1994):

Physical means:

- Iontophoresis
- Phonophoresis
- Thermal modulation
- Mechanical modulation

Chemical means:

- Permeation enhancers
- Prodrug design

Biological means

- Receptors
- Combination of the above

In nasal administration, the most commonly employed method of permeation enhancement is the use of chemical enhancers thus the chemical means.

1.6.2 Absorption enhancers

1.6.2.1 Introduction

From the onset of drug delivery research, scientists have investigated the enhancement of drug absorption to improve the efficacy of treatment and to reduce the toxicities associated with systemic therapy. Actually there is a growing need for suitable compounds to enhance drug absorption across the biological barriers of the skin, mucosa, cornea and the blood-brain barrier. The failure in the search for compounds to reach commercialisation is primarily due to the concerns about their related toxicities. The understanding of permeation enhancement of drugs is still very uneven and limited (Kim, 1987; Hsieh, 1988).

Definition of permeation enhancers

These are compounds that can increase the absorption of co-administered drugs by increasing the mucosal permeability and hence the absorption of poorly permeable drugs (Hirai et al., 1981).

1.6.2.2 Mechanisms of action of permeation enhancers

The mechanisms that lead to increased nasal drug absorption under the influence of enhancers are quite diverse and only partly understood (Martin, 1995). The influence of the enhancers on the absorption of drugs across the nasal membrane is related to a direct effect on the drug and/or influence on the mucus layer and/ or the nasal mucous epithelium. For some drugs the solubilising or stabilising effect is of importance. With respect to the mucus and the mucosal membrane, enhancers may act by altering the properties of the mucus layer, by opening the tight junctions between the epithelial cells, creating disorders in the phospholipid domain in the membrane or by facilitating the leaching of proteins and lipids from the membrane (Merkus, 1993).

Several mechanisms of action of transepithelial permeation enhancers have been proposed. These can be of two major categories.

1.6.2.2.1 Change in the permeability of membranes

A permeation enhancer can influence the mucosa in five ways:

Action on the mucus layer

The mucus layer covering the cell surface of the mucosa can be seen as an unstirred layer acting as a barrier to the diffusion of drug molecules. Ionic surfactants are capable of reducing the mucus viscosity elasticity. Consequently the barrier function of the layer is reduced and an increase in the permeability of the mucosa can be achieved (Martin et al., 1978; Marriott et al., 1983).

Action on tight junctions

The intercellular tight junction is one of the major barriers to paracellular transport of macromolecules and polar compounds. It has been found that tight junction structure and permeability can be regulated by many potential physiological factors including the cyclic AMP (Duffey et al., 1981), intercellular calcium concentration (Palant et al., 1983; Pitelka et al., 1983) and transient mucosal osmotic loads (Brightman et al., 1973; Madara, 1985). Several studies have shown that the possible mechanism of permeation enhancers is due to their effect on the tight junctions of the epithelial membranes (Murakami et al., 1982; Murakami et al., 1984). The effects of these compounds result in the loosening of the tight junctions and an increase in paracellular transport of poorly absorbed drugs. As with chelators, the removal of endogenous calcium from the epithelial cells by the formation of Ca-EDTA complexes loosens the intercellular tight junction and hence increases the drug absorption. Thus chelating-type penetration enhancers increase absorption by interacting with the calcium ions in the epithelial surface causing a temporary change in the integrity of the membrane and an increase in paracellular transport (Murakami et al., 1982).

Action on membrane components

The epithelial cell membranes contain proteins and phospholipids. The hydrophobic interactions between the acyl chains of lipid molecules result in the formation of a well-organised phospholipid bilayer. These ordered bilayers are poorly permeable to both macromolecules and highly polar compounds. Due to insufficient lipophilicity and the large molecular size, many highly polar molecules and macromolecules cannot cross the bilayers (Shen and Lin, 1994). Numerous studies have shown that permeation enhancers (detergents) can increase the permeability of the membrane by interacting with the membrane components (lipids and proteins). Thus, these compounds solubilise the phospholipids and membrane proteins. The extraction of the membrane components causes an increase in membrane permeability. Other compounds (fatty acids and monoglycerides) induce a disordering effect on the interior and the interaction of polar regions of the membrane (Muranushi et al., 1981 Muranushi, 1985). This interaction of

the fatty acids with the membrane may trigger a transient "corn" shaped lipid complex in which the polar head group region is smaller than the one subtended towards the end of the acyl chain, as a result the bilayer configuration is destabilised.

Action on vesicular transport

Limited transport of macromolecules across the epithelial cells can be accomplished by the vesicular transport process. In this process, pinocytotic vesicles derived from the apical surface of the epithelial cells are transported to the lateral cell surface where they deposit their content into the intercellular space. An increase in pinocytosis results in an enhanced transport of the drug across the epithelial cells (Cooper et al., 1978).

Action on the enzymatic activity

Peptides and proteins are prone to enzymatic degradation during their passage through the mucosal membrane. This degradation process has limited the development of non-parenteral administration of proteins and peptide drugs. Significant inhibition of the degrading effect of the proteolytic enzymes would enhance the absorption of protein and peptide drugs (Hirai et al., 1981; Lee et al., 1987). Compounds such as surfactants especially bile salts, inhibit enzymatic activity by 80%. Although the inhibition efficacy is high, the safety for use on the mucosal epithelia is still questionable (Hirai et al., 1981).

1.6.2.2.2 Change in physicochemical properties of drugs

Permeation enhancers can also promote/facilitate drug absorption by interacting with the drug itself and thus modifying its physicochemical properties in the following ways:

Action on drug solubility and dissolution rate

For water insoluble drugs, the extent of absorption is usually controlled by the drug solubility and dissolution rate. A permeation enhancer may therefore exert its effects by increasing the solubility and dissolution of the drug. Examples of such include sodium salicylate and bile salts (Touitou et al., 1986; Touitou et al., 1987).

Dispersion of protein aggregation

A high proportion of certain drugs exists as micro-crystals or polymers in commercial preparations. Enhancers such as bile salts are said to disperse these micro-crystals and aggregates and completely solubilise them into monomers thereby reducing the molecular size of the drug to facilitate drug absorption at the absorptive surface (Blundell et al., 1972; Gordon et al., 1985).

Formation of micelles

A significant change in the ability of a drug to permeate a biological membrane may result from an interaction with a permeation enhancer. The properties of drug-enhancer complexes i.e. solubility, molecular size, diffusiveness and lipid-water partition, can differ significantly from the properties of the free drug resulting in an increased absorption of the problem drug (Gordon et al., 1985).

1.6.2.3 Factors influencing the efficacy of permeation enhancers

Studies have indicated that increased absorption by permeation enhancers can be influenced by numerous factors i.e. route of administration, lipophilicity of the enhancer, physicochemical properties of the problem compounds and the ability of the permeation enhancers to increase membrane permeability.

Thus the absorption efficacy of poorly permeating drugs across the nasal mucosa and poorly water-soluble lipophilic drugs can be improved by the use of absorption enhancers (Martin, 1995).

Ideally these absorption enhancers should have the following properties:

- Immediate and predictable duration of action
- Reversible effect on the barrier properties of the nasal mucosa following administration of the preparation
- Not permit the entry of potentially dangerous environmental material
- Compatibility with drugs and other excipients

- Temporarily fluidise the membrane bilayer structure to facilitate drug transport (Agu et al., 2000)

Many agents have been suggested as intranasal permeation enhancers. Enhancers such as chelating agents (EDTA and salicylates), fatty acids (oleic acid and capric acid), glycyrrhetinic acid derivatives (cabenoxolone), cyclodextrins (dimethyl-cyclodextrin), surface-active agents (saponin and laurenth-9) and bile salts (deoxy-cholate, glyco-cholate, taurocholate, deoxy fusidate and glyco fusidate) have been used in various intranasal formulations. Unfortunately the commonly used enhancers appear to be ciliotoxic and have undesirable effects on the nasal components. What is needed is an effective enhancer for the transport of the pharmacologically active moiety across the nasal membrane, which is free of undesirable effects as experienced with the current nasal formulations (Merkus et al., 1993; Marttin et al., 1995).

1.6.2.3.1 Effects of absorption enhancers on the nasal tissue morphology and mucociliary clearance

In recent years *in-vitro* and *in-vivo* histopathological studies have demonstrated the effects of some enhancers on the mucociliary system (mucociliary transport rate, nasal morphology and ciliary beat frequency (CBF)).

1.6.2.3.1.1 Mucociliary transport rate

The potential toxicity of some absorption enhancers has been tested with the frog palate by measuring the mucociliary transport rate before and after application of the excipient. Some enhancers showed a complete and irreversible arrest of the transport while others had no effect on mucociliary transport rate. Gizurarson et al., (1990) observed a complete and irreversible arrest of the mucociliary transport post frog palate treatment with 1% L-α-lysophosphatidylcholine (LPC), 1 % deoxycholate (DC), 1% laureth-9 and Na tauro-24, 25-dihydrofusidate (STDHF). Similar results were described by Braga et al., (1990) for taurocholate (TC). 1% Glycocholate (GC) and 1% didecanoyl-

lysophosphatidylcholine had no damaging effects on the mucociliary transport rate (Gizurarson et al., 1990).

1.6.2.3.1.2 Nasal morphology

The histological effects of the enhancers studied at different contact times with the nasal epithelium characterised the gross structural and cellular changes of the nasal mucosa *in-vivo* using the scanning/transmission electron microscope. The study revealed that following 5 to 15 minutes of exposure to selected enhancers (bile salts and laureth-9) severe erosion of the nasal mucosa occurred. This was characterised by total loss of cellular and ciliary identity as well as prevalence of extracellular debris. Studies by Ennis et al., (1990) on the effects of permeation enhancers on the surface morphology of the rat nasal mucosa indicated that the order of increasing morphological damage resulting from a 5 minute exposure of the nasal mucosa to each enhancer was as follows: 0.5% solulan C-24 $\approx$ 0.5% solulan C-24/0.5% Na tauro-24, 25-dihydrofusidate (STDHF) <.5% Na tauro-24, 25-dihydrofusidate (STDHF) < 1% .5% Na tauro-24, 25-dihydrofusidate (STDHF) < 1% laureth-9 < 1% Na tauro deoxycholate $\approx$ 1% Na deoxycholate. In this study the scanning electron micrographs for the nasal membranes were evaluated for damage on the surface morphology integrity, ciliary morphology and extracellular/mucus debris. Krishnamoorthy et al., (1995) also studied the damaging effects of selected cyclodextrins to the rat nasal mucosa and found that the damage trend was as follows: sulfobutyl ether-β-cyclodextrin (SBEβCD) $\leq$ hydroxy ethyl-β-cyclodextrin (HEβCD) <monomethyl-β-cyclodextrin (MβCD) < dimethyl-β-cyclodextrin (DMβCD). These results indicated that the degree and nature of chemical substitution on the cyclodextrin ring caused a significant effect on the membrane damage and hence on the potential absorption enhancement. Agu et al., (2000) observed that cilio-inhibition caused by selected cyclodextrins on the nasal mucosa was lower for the human model than reported for the chicken trachea model.

1.6.2.3.1.3 Ciliary beat frequency (CBF)

Ciliary beat frequency (CBF) determines the mucociliary transport rate and hence is the most important parameter in the mucociliary system. Under normal circumstances, CBF

is in the range 15 to 20 Hz/min and any deviation from this range indicates stimulation or inhibition of the ciliary movement. Through research, some enhancers were found to halt ciliary movement, indicating their toxicity to the nasal mucosa whiles others had no apparent effect, indicating "cilio-friendliness". However, the degree of cilio-toxicity is said to be concentration dependent. Cyclodextrins for example, at concentrations between 5% and higher, induce ciliostasis within an hour but below this range they show moderate effect on the ciliary movement. Furthermore, the inhibitory effect on the CBF is reversible after rinsing the tissue with an isotonic solution (Locke-Ringer solution). Studies by Merkus et al., (1993) indicated that 5 % hydroxy propyl-β-cyclodextrin (HPβCD), 1.8% β-cyclodextrin (βCD) and 5% γ-cyclodextrin (γ-CD) exert a minor effect on the ciliary beat frequency (CBF). While both 5% α-cyclodextrin (α-CD) and 5% dimethyl-β-cyclodextrin (DMβCD) induced ciliostasis within an hour. These effects were found to be less severe than those obtained with 0.5% Na tauro-24, 25-dihydrofusidate (STDHF), 0.3% laureth-9. 0.3% deoxycholate, 1.5% glycocholate and 1.5% taurodeoxycholate.

Tolerance studies of cellulose derivatives on the mucociliary system have been well documented. Studies by Ugwoke et al., (2000) on the effect of carboxymethyl cellulose (CMC) on ciliary movement of human explants and on the morphology of the rabbit nasal mucosa showed that carboxymethyl cellulose (CMC) exhibited both concentration and time dependent inhibitory effects on the ciliary movement. Only mild to moderate cilio-inhibition was recorded with the different concentrations (0.1%(w/v), 0.25%(w/v) and 1%(w/v)) used. No gross necrosis, squamous metaplasia nor ciliary degeneration was observed, mild to moderate inflammation was observed after four weeks of polymer treatment. Carbopol 971P (polyacrylic acid) was found to have similar effects on ciliary movement and on nasal morphology as carboxymethyl cellulose. Effects were concentration and time dependent. Ciliary movement inhibition increased with the increase in the polymer concentration (0.1%(w/v)-1%(w/v)) (Ugwoke et al., 2000).

It is therefore clear that enhancers with a mild effect on the nasal morphology also show minor effects on the nasal CBF. Moreover, severe tissue damaging compounds also exhibit irreversible ciliotoxicity potency.

1.7 Absorption enhancers

The following are some of the commonly employed absorption enhancers for nasally administered preparations:

Chitosan

Chitosan is a linear polysaccharide comprised of two monosaccharides: N-acetyl-D-glucosamine and D-glucosamine linked together by B (1-4)glucosidic bonds (Mathur, 1990). Most chitosan salts are positively charged and soluble in water. The term chitosan refers to a family of polymers individually characterised by their molecular weight and ratio of acetylated to deacetylated units (Soane, 1999).

Mechanism of action

Chitosan salts are said to enhance drug absorption via the paracellular route through neutralisation of fixed anionic sites in the tight junctions between the mucosal cells (Artusson, 1994; Borchard, 1996).

Toxicity evaluations of chitosan revealed no significant morphological and histological changes in the nasal epithelia of rats under study (Illum, 1994).

Cyclodextrins

This group of compounds is said to offer the following advantages:

- Improved drug solubilisation
- Protection against physicochemical and enzymatic degradation
- Potential for improved absorption

Results of the effects of the various cyclodextrins on ciliary beat frequency, revealed no significant reduction in ciliary beat frequency indicating that cyclodextrins are "cilio-friendly". Cilio–inhibition was only observed in cases of high concentrations of the

cyclodextrins but this was found to be reversible. Thus, the degree of cilio-inhibition was found to be concentration dependent. Therefore no ciliostasis was generally observed with the cyclodextrins (Krishmamoorthy, 1995).

Mucoadhesives

Nasal mucociliary-clearance is one of the most important limiting factors to nasal drug delivery. It severely limits the time allowed for drug absorption to occur and effectively rules out sustained nasal drug administration. Mucus transport times, as measured for indigo carmine and saccharine, range from 3 to 20 minutes in men (Duchateau, 1985). The nasal route of administration commonly provides fast peak levels of drugs in circulation. The rapid nasal mucociliary clearance may be disadvantageous in obtaining reproducible absorption profiles. However, certain polymers (mucoadhesive polymers) can adhere onto the nasal mucosa for reasonably prolonged periods, thus preventing nasal clearance. The use of these polymers is therefore an avenue for improving nasal drug absorption as well as prolonging the duration of action of the nasally administered drugs (Ugwoke, 2000).

Mechanism of action

Bioadhesion is defined as the attachment of synthetic or biological macromolecules to a biological tissue (Peppas and Burri, 1985). When applied to a mucosal epithelium, bioadhesive interaction occurs primarily with the mucus layer and this phenomenon is referred to as muco-adhesion. Mucoadhesives have received considerable attention recently due to their ability to prolong the residence time of a dosage form on to the mucosal surface to enhance drug bioavailability. Muco-adhesion can therefore be regarded as a practical method of drug immobilisation or localisation (Mortazavi, 1992; Mortazavi, 1993).

1.7.1 Mucoadhesion theories

Muco-adhesion can be explained by a number of theories. No individual theory has been universally accepted as the singular mechanism by which bio-adhesion occurs, a combination of these theories may be used to describe the phenomenon. The adhesion

theories include: the electronic theory, wetting theory, adsorption theory and diffusion theory.

Electronic theory

According to this theory there is a double layer of electrical charge at the interface between the bio-adhesive polymer and the tissue, due to a transfer of electrons upon contact. This electron transfer is due to the difference in structure between the bio-adhesive and the glyco-protein chains in the mucus. Bio-adhesion in this case is due to an attraction across the electrical double layer (Derjaguin et al., 1977; Madsen, 1998).

Adsorption theory

This suggests that the bioadhesion is due to secondary forces such as van de Waals forces and hydrogen bonding. The fracture theory of bio-adhesion relates the force necessary to separate two surfaces to the adhesive bond strength (Kinloch, 1980).

Wetting theory

This mainly applies to liquid bio-adhesive systems. It analyses the ability of a paste to spread over a biological surface. This theory uses the analysis of the spreading coefficient of a liquid bio-adhesive material over a tissue by displacement of the surrounding gastric fluid. The calculation of the interfacial tension between the bio-adhesive liquid and tissue, as per Helfand and Tagami in 1972, indicates that the interfacial tension is proportional to 0.5 where is the Flory polymer-polymer interaction parameter. Low values of this parameter correspond to structural similarities between polymers and an increased miscibility (Kaelbe, 1977).

Diffusion theory

This involves the inter-penetration of the polymer chains in the interfacial region. In bio-adhesion, the polymer is first brought into intimate contact with the mucus, and over time, the concentration gradient across the interface causes the diffusion of the chains of the bio-adhesive polymer into the mucus layer and also the diffusion of the glyco-protein chains of the mucus into the bio-adhesive polymer. The rate of diffusion is dependent on

the chemical potential and the diffusion coefficient of a macromolecule through a cross-linked network. The chains that have diffused across the interface serve as anchors to aid in securing the bio-adhesive device in place semi-permanently. The inter-penetration distance necessary for good bio-adhesion is approximately equal to the end-to-end distance of the macro-molecular chains (Voyutskii, 1963; Mortazavi, 1992; Mortazavi, 1993; Madsen, 1998).

Thus for mucosal absorption to occur the mucoadhesive polymer interacts with the tight junction through a combination of the above theories to facilitate drug absorption by transiently widening the tight junctions (Hochman and Artusson, 1994).

Mucoadhesives therefore, have the potential to offer the following advantages:

- Prolong the residence time of the dosage form at the site of absorption
- Intensify the contact between the underlying epithelial barrier
- Modulate the permeability of the epithelial tissue by loosening the tight junctions
- Act as proteolytic enzyme inhibitors (Lehr, 2000).

Limitations

In spite of these excipients' multi-functionality, muco-adhesives still have the disadvantage of adhering to their substrate by non-specific interactions. Therefore such muco-adhesives cannot distinguish between adherent, shed-off mucus or the surfaces of other gut contents. This implicates that non-specific muco-adhesives may adhere to surfaces they are not intended for. Thus premature interaction with other substrates may prevent the muco-adhesive from binding to the mucosal tissue surface for which they were intended.

Furthermore, mucoadhesives use the mucus gel layer covering the mucosal tissue surfaces as a connecting link to the epithelium rather than adhering to the cell membrane itself. The maximum duration of time for adherence may therefore be limited by the turnover time of the mucus gel layer.

Although increasing the overall membrane permeability by opening the tight junction enhances drug absorption, this bears a certain risk since other noxious compounds may also be absorbed (Lehr, 2000).

Examples of musoadhesives

The following are some of the muco-adhesives employed in drug formulation:

Starch

Mechanism of action

A conceivable hypothesis for the mechanism of action of starch is that, due to water absorption in the gelling process, starch is able to dehydrate the epithelial mucosa causing a reversible shrinkage of the epithelial cells. This shrinkage could lead to the physical separation of the intracellular junctions and thus enhance para-cellular absorption (Bjork, 1990).

Toxicological evaluation of starch on the nasal epithelia after an eight-week study, revealed no effect on the mucociliary clearance and no evidence of inflammation or toxicity (Edman, 1992).

Chitosan

Mechanism of action

The mucoadhesive properties of chitosan are mostly due to an ionic interaction of the positively charged amino groups of the D-glucosamine units of chitosan with the negatively charged sialic acid groups of mucin or other negatively charged groups of the mucosal membrane (Lehr et al., 1992; Fiebrig et al., 1994). Studies conducted by Fiebrig et al., (1994) indicated that the interactions between chitosan and mucin were highly pH dependent with the strongest interaction at pH values where both the sialic units and chitosan amino groups were well ionised.

Determination of bioadhesion

Quantification of the bioadhesive forces between the polymer and the mucosal tissue is a useful indicator for screening systems designed to increase residence time in the nasal cavity. To quantify bioadhesion many researchers have devised different methods. A single universal method has not been developed and it has been difficult to compare results from individual researchers.

The most commonly employed techniques to measure bioadhesion are described below:

***In-vitro* techniques:**

These techniques are used to test polymers against a variety of synthetic and biological tissue samples (synthetic mucus, natural mucus, frozen tissue, and freshly excised tissue). In vitro technique can be categorised into two:

Those based on Wilhelmy plate technique:

This is traditionally used to measure dynamic contact angles and involves the use of microtensiometer or a microbalance. In this technique, a glass slide is coated with the polymer being examined and then dipped into the container of mucus. Measurement for surface tension, contact angle and adhesive forces are made with available software (Santos, 1999).

Shear method:

With this method, two glass slides are coated with a polymer being examined and mucus such that the mucus forms a thin film between the two polymer-coated slides. The force required to separate the two surfaces is measured (Santos, 1999).

The main setback with these methods is the failure to mimic physiological conditions

1.8 Pharmaceutical considerations for the development of an intranasal drug delivery system

1.8.1 Micromeretic properties

Particle size

This is the most important parameter since it determines both the location for deposition of the dosage form and the rate of release of the drug from the dosage form. For nasal delivery whereby drug deposition has to be in the highly ciliated region, a particle size in the range of 40 to 60μm would be acceptable since any size below this range would be carried with the air stream down the tracheobronchial region while larger particles will be

mainly deposited in the anterior unciliated portion of the nose (Proctor, 1992). Thus for effective delivery aerodynamic particle size is recommended. Furthermore, larger particles tend to be cleared faster than smaller ones since larger ones cause more discomfort in the nasal cavity and hence trigger the sneeze response.

Droplet size

The mean flow rate of the mucociliary system in healthy subjects is about 5 to 20mm/min thus administered drugs are cleared within 15 to 20 minutes depending on the particle size and the site of deposition. Particles with an aerodynamic size above 10 to20μm are all deposited in the nasal cavity whereas those with particle size smaller than 1μm pass with the inspired air into the lungs (Agarwal et al., 1999).

Drug distribution

Drug distribution in the nasal cavity determines the covered surface area of the mucosa for absorption thus the area covered by the formulation for intimate contact with the absorbing mucosa. This is affected by (Agarwal et al., 1999):

Area of nasal membrane exposed.

Volume of solution applied. Thus, application of a large volume of solution from a nasal drop gives a good distribution over the nasal cavity whereas a small volume gives unsatisfactory results.

Types of nasal drug delivery systems.

Studies have indicated that various delivery systems showed significant differences of drug distribution in human nose (Agarwal et al., 1999). Newman, (1997) in his study on the influence of different delivery devices on drug distribution within the nasal cavity, observed that a mean of 97% of the emitted dose from both the pressurised aerosol and the pump spray was deposited in the nose. Analysis of the deposition within the nose showed a trend towards a larger deposition area for the pump spray compared with the pressurised aerosol. Thus with the pump spray the site of impaction extended from the nasal valve into the turbinates which implies a wider drug distribution.

Site of deposition

The degree of absorption also depends on the site of deposition of nasally administered drug, which also depends on the delivery system, and the technique of administration. Studies indicate that particles delivered by a nasal spray are deposited mainly in the anterior part of the nose and slowly cleared whereas those delivered by nasal drops are dispersed more extensively in the nasal cavity and cleared rapidly (Agarwal et al., 1999).

Delivery systems

Developing an appropriate formulation for a given drug is vitally important. The choice of an inappropriate formulation can render the drug useless, ineffective or mediocre. The specific formulation properties, which affect drug absorption, depend on the route of administration and the dosage type selected. A considerable amount of work has been reported on nasal drug products and dosage forms (Behl et al., 1998).

Specific types of dosage forms, which are used to deliver formulations into the nose, are important in determining the nasal absorption profiles of drugs. Choice of a certain dosage form generally depends on the drug being developed, indication being pursued, patient population and marketing aspects (Behl et al., 1998). With the aim to prolong the nasal residence time and to improve absorption efficiency remarkable approach has been reported using the formulation approach. Various delivery systems are currently used for the administration of the drugs through the nasal cavity i.e. nasal drops, spray, cotton pledget, insufflator, insert and jelly. The delivery efficiency of these systems affects the site of deposition, intranasal drug distribution and the degree of absorption. Thus the method of intranasal administration impacts on the therapeutic efficacy and the toxicity of the formulation (Agarwal et al., 1999).

1.8.2 Examples of some intranasal drug delivery systems

1.8.2.1 Nasal drops

This type of delivery system is perhaps the simplest and most convenient form of administering drug formulations nasally. Solutions are most suited for it. The only disadvantage is that an exact amount of the formulation cannot be delivered. For most prescription type drugs, this may not be the desired dosage form (Behl et al., 1998).

1.8.2.2 Solution/Suspension sprays

As more sophisticated drug delivery systems became available, especially, the metered dose nasal actuators, solution formulations were packaged in such delivery systems. Aerosol type systems using propellants were utilised in the early days. As the harms of the propellants became known, mechanical actuators/pumps were developed. Presently spray solutions are delivered through metered dose nasal actuator systems, which deliver precisely volumes as low as 25μl (Behl et al., 1998).

Suspension forms are also administered using the metered dose nasal actuator systems. These unlike for the solution formulations, are designed according to the specific physical properties of the preparation thus particle size and morphology of the drug particles (Behl et al., 1998).

1.8.2.2.1 Sprays Vs Drops

The site of deposition in the nose is highly dependent on the dosage form. Nasal sprays deposit more anteriorly than drops, resulting in slower clearance (Hardy, 1985; Washington, 1992). Studies have indicated that sprays distributed smaller volumes throughout the nasal cavity better than drops. The clearance of nasal formulation can also be affected by the viscosity of the preparation resulting in decreased clearance and delayed absorption due to the inherent diffusion network formed by the excipients, which consequently controls the rate of release of the drug from the network.

1.8.2.3 Powders

When a suitable solution or suspension dosage form is not feasible for formulation development, dry powder dosage is normally the alternative. This type of dosage form is most suited for locally acting drugs. Powders are not highly favoured dosage forms for they can cause irritation of the nasal mucosa and confer the nasal tissue a gritty feeling. Furthermore, such dosage forms are more difficult and costly to manufacture due to the particle size and morphology. For this dosage form, specialised delivery devices can be used to deliver metered doses nasally (Behl et al., 1998).

1.8.2.4 Gels

Gels are thickened/gelled solution or suspensions of drugs. Until recent development of metered dose gel devices, there was no precise method of delivery. Gels can offer the following advantages over other dosage forms:

- reduction of postnasal drip back into the throat and therefore minimisation of any bad taste and drug loss from the nasal cavity.
- reduction of anterior leakage of the drug out of the nasal cavity.
- for certain drugs, the irritation potential from the drug itself and/or excipients is minimised, for gels afford the use of certain soothing agents which are not incorporated in other dosage forms.
- gels can be developed for both systemic as well as local drug delivery (Behl et al., 1998).

1.8.2.5 Emulsions and ointments

Only a limited amount of work has been reported on the development of emulsions and ointments for nasal drug delivery. The full potential of these dosage forms still needs to be illustrated. By their physical properties, they would seem to be more appropriate for locally acting drugs while systemic activity needs careful consideration thus the delivery device should be designed to administer the precise dose. The major disadvantages of these dosage forms include poor patient acceptance, development efforts needed are high, problems in delivering the precise dose from metered dose nasal actuators (Behl et al., 1998).

1.8.2.6 Specialised systems e.g. Microspheres with adhesion properties, liposomes

As the state of the art advances in formulation development, many researchers are using exotic formulation systems for nasal drug delivery. Recently, microsphere technology has been applied in designing formulations for nasal drug delivery. The primary rationale for such work is to provide a better chance for the drug to be absorbed by allowing a more intimate and prolonged contact between the drug and the nasal mucosa. It is well known that solutions, suspensions and powders are rapidly cleared from the nasal cavity. Drugs, which are not absorbed from such dosage forms, stand a better chance for absorption when formulated in gelling microspheres made by compacting material such as starch, gelatine, albumin and dextran (Behl et al., 1998).

A number of literature reports have appeared on this general approach of optimising nasal drug absorption. Some notable examples of improved nasal drug delivery by use of the microsphere approach will be reviewed.

Illum et al., (1996) reported an improvement in the nasal delivery of gentamycin in rat and sheep from 1% absolute bioavailability to 50% absolute bioavailability by the incorporation of the drug into microspheres.

The controversy regarding the safety and efficacy of nasal absorption enhancer systems on the nasal membrane and especially, about the long term toxicological consequences has led to the exploration of an alternative strategy for enhancing nasal absorption. Researchers are currently looking into the means of preventing the rapid clearance rate of the delivery systems from the nasal cavity thereby prolonging the contact time between the drug and the nasal mucosa. Due to mucociliary functions, material applied intranasally, will normally be cleared from the nasal cavity into the nasopharynx with an average speed of 5mm/min. Nasal delivery systems in the form of bioadhesive microspheres that are capable of forming gel-like structures when in contact with the mucus, should be cleared slowly from the nasal cavity thereby prolonging the time of contact between the delivery system and the absorbing mucosa. Such systems should

have the potential of releasing the drug in a controlled manner thereby possibly increasing the absorption efficiency of the drug (Illum, 1987).

1.9 Administration devices

Drug therapy requires that administration of the dosage form be accurate and reproducible. This therefore places stringent demands on the device for nasal drug delivery. The major mechanism of nasal deposition of particles is by inertial impaction that occurs following a change in the direction of air-flow. Other contributory mechanisms are gravitational sedimentation and Brownian diffusion. Particle deposition by interception and electrostatic precipitation are of no importance in nasal deposition (Kublik and Vidgren, 1998).

Depending on the type of formulation, a variety of devices have been used to administer drugs intranasally especially in experimental studies and the choice or type of device depends on the physical properties inherent to the final formulation. Devices for liquid formulations include the following:

- instillation catheters
- droppers
- unit dose containers
- squeeze bottles
- pump spray
- airless and preservative free sprays
- compressed air nebulisers
- and metered dose inhalers (MDI)

Devices for powder dosage forms include:

- insufflators
- mono dose and multi dose powder inhalers
- pressurised MDIs

Of the sited devices used for nasal administration, metered dose nebulisers and metered dose aerosols are superior in terms of accuracy and reproducibility (Dondetti. 1996). However, the duration and condition of storage, as well as the physicochemical properties of the formulation such as viscosity, surface tension and homogeniety (e.g. of suspensions) affect the metering accuracy. Those with manual actuation are much cheaper and may be preferred due to environmental concerns. However, they require thorough and regular cleaning since they are prone to bacterial contamination, and require frequent incorporation of preservatives, which could be cilio-toxic (Batts et al., 1989).

1.10 Parameters used to calculate bioavailability

Different bioavailability parameters can be calculated if the relationship between the drug plasma concentration and time required to reach this concentration is known.
The following are some of the most commonly employed parameters:

Area under the plasma drug concentration vs time curve (AUC)

Area under the plasma drug concentration versus time curve is a measurement of the extent of the bioavailability of the drug under study. This area reflects the total amount of active drug that reaches the systemic circulation. The unit of AUC is concentration multiplied by time (e.g.,μg.hr/ml) (Shargel and Yu, 1993).

Area under the curve of first moment of plasma drug concentration vs time curve (AUMC)

Area under the first moment of plasma drug concentration versus time curve. This area also reflects the total amount of active drug that reaches the systemic circulation. The unit of AUC is concentration multiplied by time^2 (e.g.,μg.hr^2/ml) (Shargel and Yu, 1993).

Peak drug plasma concnentration (C_{max})

The peak plasma concentration represents the maximum drug plasma concentration reached after drug administration (Shargel and Yu, 1993).

Time of peak drug plasma concentration (T_{max})

The time of peak drug plasma concentration corresponds to the time required to reach maximum drug plasma concentration post drug administration (Shargel and Yu, 1993).

These parameters can be read off directly from the concentration-time curve or calculated by using the compartmental models. i.e. When the data fits into an open one-compartmental model, C_{max} and t_{max} can be calculated using equations 1.1 (Ritchel, 1991) and 1.2 (Irwin, 1990).

$$T_{max} = \frac{\ln \frac{k_a}{k_e}}{k_a - k_e} \qquad \text{Equation 1.1}$$

$$C_{max} = Ao\left[\frac{k_e}{k_a}\right]^{\frac{k_e}{k_e - k_a}} \qquad \text{Equation 1.2}$$

Where:

K_a = absorption rate

K_e = elimination rate

Ao = intercept (x = 0) of the descending linear part of the graph of ln C as a function of time

Mean residence time (MRT)

The mean residence time is the average time (hours) spent by a single drug molecule in the body before excretion via elimination processes, regardless of the route of administration. When a drug disappearance curve exhibits a monophasic decline after intravenous administration on a semilog scale, its MRT_{iv} the time required for 63.2% of the dose to be eliminated from the body. MRT values after administration by routes other than intravenous are always greater than MRT_{iv} (Kwon, 2001).

$$MRT = \frac{AUMC}{AUC} \qquad \text{Equation 1.3}$$

Where:

AUMC = Area under the first moment-curve

Mean Absorption time (MAT)

The mean absorption time indicates the rate of drug absorption post extravascular administration and is based on the differences in MRT after different modes of administration (Kwon, 2001).

$$MAT = MRT_{in} - MRT_{iv} \qquad \text{Equation 1.4}$$

Where:

MRT_{in} = Mean residence time of drug post administration in a non-instantaneous (oral, intramuscular etc.) manner.

MRT_{iv} = Mean residence time of drug post intravenous bolus administration.

Biological half-life ($T_{½}$)

The biological half-life ($T_{½}$) is the period of time in hours over which the drug concentration in plasma decreases by one-half. A drug's half-life is often related to its duration of action and may also indicate the time for the next dosing (DiPiro et al., 2001).

$$T_{½} = \frac{0.693}{k_{el}} \qquad \text{Equation 1.5}$$

Where:

k_{el} is the slope during the terminal phase of the concentration-time plot on a semilog scale.

Both absolute and relative bioavailabilities can be calculated using these parameters

Absolute bioavailability is the fraction of a drug reaching systemic circulation upon extravascular administration compared with the dose size of the drug administered intravenously (Kwon, 2001).

$$F = \frac{AUC_{ex,0-\infty}.D_{iv}}{AUC_{iv,0-\infty}.D_{ex}} \qquad \text{Equation 1.6}$$

Where:

$AUC_{ex,0-\infty}$ and $AUC_{iv,0-\infty}$ are the AUC from time 0 to ∞ after extravascular and intravenous administration respectively.

D_{ex} and D_{iv} are extravascular and intravenous drug doses respectively.

Relative bioavailability gives the proportion of drug reaching the systemic circulation upon extravascular administration compared with that of a standard dose of the drug administered via the same route (Kwon, 2001).

$$F = \frac{AUC_{0-\infty,A}.D_B}{AUC_{0-\infty,B}.D_A} \qquad \text{Equation 1.7}$$

Where:

$AUC_{0-\infty,}A$ and $AUC_{0-\infty,}B$ are AUC from time zero to infinity

References

Agarwal V, Mishra B. Recent trends in drug delivery systems: Intranasal drug delivery. *Indian Journal of Experimental Biology*; 1999 37 : 6-16

Agu R U, Jorissen M, Wilems T, Van Den Mooter G, Kinget R, Verbeke N, Augustijns P. Safety assessment of selected cyclodextrins: effect on ciliary activity using human cell suspension culture model exhibiting *in-vitro* ciliogenesis. *International Journal of Pharmaceutics;* 2000 193: 219-226

Armengot M, Basterra J, Marco J. Nasal mucociliary function during the menstrual cycle in healthy women. *Review of Laryngology Ortolaryngoscope and Rhinology;*1990 111:107-109

Artusson P, Lindmark T, Davis S S, Illum L. Effect of chitosan on the permeability of monolayer of intestinal epithelial cells (caco-2). *Pharmaceutical Research;* 1994 11: 1358-1361

Ascentiis A D, Bettini R, Camponetti G, Catellani P L, Peracchia M T, Santi P, Colombo P. delivery of nasal powders of (-cyclodextrins by insufflation. *Pharmaceutical Research*; 1996 3 (5) : 734

Babin R W. A review of the autonomic control of nasal function. *Ear Nose Throat Journal;* 1977 56:36-46

Barchfeld G L, Hessler A L, Chen M, Pizza M, Rappouli R van Nest G A. The adjuvants MF59 and LT-K63 enhance the mucosal and systemic immunogenicity of subunit influenza vaccine administered intranasally in mice. *Vaccine*; 1999 17 (7-8) : 695-704

Baroody F M. Anatomy and physiology. In: *Rhinitis. Mechanisms and management*; 1999: 1-22

Batts A H, Marriott C, Martin G P, Wood C F, Bond S W. The effect of some preservatives used in nasal preparations on the mucus and ciliary components of mucociliary clearance. *Journal of Pharmacy and Pharmacology;*1990 42: 145-151

Batts A, Marriott C, Martin G P, Bond S W. The effect of some preservatives used nasal preparations on mucociliary clearance. *Journal of Pharmacy and Pharmacology*;1989 41: 156-159

Behl C R, Pimplaskar A P, Sileno A P, deMeirless J, Romeo V D. Effects of physicochemical properties and other factors on systemic nasal drug delivery. *Advanced Drug Delivery Reviews*; 1998 29 : 89-116

Bernstein J M, Reddy M S, Scannapieco F A, Faden H S, Ballow M. The microbial ecology and immunology of the adenoid: implications for otitis media. *Ann. NY Academy Science*; 1997 830: 19-31

Bjorg E, Edman P. Characterisation of degradable starch microspheres as a nasal delivery system for drugs. *International Journal of Pharmaceutics*; 1990 62 : 187-192

Bjork E, Bjurstran S, Edman P. Morphological examination of rabbit nasal mucosa after administration of degradable starch microspheres., *International Journal of Pharmaceutics*; 1991 75 : 73-80

Blundell T L, Cutfield J M, Cutfield S M, Dodson E J, Dodson G G, Hodgkin D C, Mercola D A. *Diabetes;*1972 21:492

Bodganffy M S, Randall H W, Morgan K T.Histochemical localisation of aldehyde dehydrogenase in the respiratory tract of the Fshv-344 rat. *Toxicology Appl. Pharmacology*; 1986 82: 560-567

Bogdanffy M S, Kee C R, Hinchman C A, Trela B A. *Drug Metabolism Disposition;* 1991 19:124-129

Bogdanffy M S, Randall H W, Morgan K T. *Toxicological Applications in Pharmacology;* 1987 88:183-194

Bogdanffy M S. Biotransformation enzymes in the rodent nasal mucosa: the value of histochemical approach. *Environmental Health Perspective;* 1990 85: 177-186

Borchard G, LeuBen H L, de Boer A G, Verhoef J C, Lehr C M, Juninger H E. The potential of mucoadhesive polymers in enhancing intestinal peptide drug absorption. III: Effects of chitosan-glutamate and carbomer on epithelial tight junctions in vitro. *Journal of Controlled Release;* 1996 39: 131-138

Borum S, Nielsen K, Bisgaard H, Mygind N. Experimentally induced nasal hyper-secretion does not reduce the efficacy of intranasal levocabatine. *Rhinology*; 1998 36 (4) : 153-155

Braga P C, Allegra L, Rampoldi C, Beghi G, Ornaghi A, Carminitis G, Zheng Y R, Bartucci F. Topical tolerability of calcitonin assessed by mucociliary transport velocity investigation. *Drug Research;* 1990 40: 938-941

Brightman M W, Hori M, Rapoport S I, Reese T S, Westergaard E.*Journal of Comp. Neurology;*1973 152:317

Burkitt H G, Young B, Thomas N W. Wheater's functional histology. *A text and colour atlas;* 1993 Longman

Casanova-Schimdtz M, David R M, Heck H d'A. Oxidation of formaldehyde and acetylaldehyde by NAD^+ dependent dehydrogenase in the rat nasal mucosal homogenates. *Biochemical Pharmacology;* 1984 33: 1137-1143

Chancellor M B, Atan A, Rivas D A, Watanabe T tai H L, Kumon H. Beneficial effect of intranasal desmopressin for men with benign prostatic hyperplasia and nocturia: preliminary results. *Technology Urology;* 1999 5 (4) : 191-194

Chien Y W, Su K S E, Chang S. Nasal systemic drug delivery. *In Drugs and the Pharmaceutical Sciences*; 1989 1-26 Marcel Dekker New York

Chien Y W. Biopharmaceutics basis for transmucosal delivery. *S.T.P Pharma. Sci;* 1995 5:257-275

Cooper M, Teichberg S, Lifshitz. *Lab Invest;*1978 38:447

Corbo D C, Liu J C, Chien Y W. Characterisation of the barrier properties of mucosal membrane. *Journal of Pharmaceutical Sciences;* 1990 (79) 202-206

Corbo D C, Liu J C, Chien Y W. Drug absorption through mucosal membranes: Effect of mucosal route and penetrant hydrophilicity. *Pharmaceutical Research;* 1989 (6) 848-852

Creticos P, Fireman P, Settipane G, Bernstien D, Casale T, Schawrtz H. Intranasal budesonide aqueous pump spray (Rhinocort Aqua) for the treatment of seasonal allergic rhinitis. Rhinicort Aqua study group. *Allergy Asthma Proc*; 1998 19 (5) : 285-294

Dahl A R, Miller S C, Petridou-Fischer. *Toxicological Letters;* 1987 36:129-136

Dahl A R. *Toxicological letters;* 1989 45: 199-205

Dalhamn T, Rylander R. Frequency of ciliary beat measured with a photo-sensitive cell. *Nature;* 1962 196:592-593

Davey P, Crag A M, Hau C, Malek M. Cost-effectiveness of prophylactic nasal mupirocin in patients undergoing peritoneal dialysis based on randomised, placebo-controlled trial. *Journal of Antimicrobial Chemotherapy*; 1999 43 (1) : 105-112

Deitcher S R, Tuller J Johnson J A. Intranasal DDAVP induced increases in plasma von Willebrand factor alter the pharmacokinetics of high purity factor VIII concentrates in severe haemophiliac patients. *Haemophilia*; 1999 5 (2) : 88-95

Delgado-Escueta A V, Westerlain C G, Treiman D M, Porter R J. *N Engl. Journal of Medicine;* 1982 306: 1337-1340

Derjaquin B V, Toporov YP, Mueler V M, Aleinikova I N. On the relationship between the electrostatic particles and the molecular component of adhesion of elastic particles to a solid surface. *Journal of Colloid Interface Science;* 1977 58: 528-533

Ding X, Koop D R, Crump B L, Coon M J. *Molecular Pharmacology;* 1986 30: 370-378

DiPiro J T, Blouin R A, Pruemer J M, Spruill W J. Half-life In:*Concepts in Clinical Pharmacokinetics, 2nd Ed.* 2001: 35

Donda I, Gipps E. Model of disposition of drugs administered into the human nasal cavity. *Pharmaceutical Research*; 1990 7: 69-75

Dondetti P, Zia H, Needham T E. Bioadhesive and formulation parameters affecting nasal absorption. *International Journal of Pharmaceutics*; 1996 127: 115-133

Donovan M D, Zhou M. Drug effects on *in-vivo* nasal clearance in rats. *International Journal of Pharmaceutics;*1995 116:77-86

Druce H M. Nasal physiology. *Ear, Nose and Throat Journal*; 1986 65: 201-205

Duchateau G S M J E, Graamans K, Zuidema J, Merkus F W H M. Correlation between ciliary beat frequency and mucus transport rate in volunteers. *Laryngoscope*; 1985 95: 854-859

Duffey M E, Hainau B, Ho S, Bentzel C J. *Nature (London);*1981 204:451

Edman P, Bjork E. Nasal delivery of peptide drugs. *Advanced Drug Delivery Review*; 1992 8: 165-177

Ennis R D, Borden L, Lee W A. The effects of permeation enhancers on the surface morphology of the rat nasal mucosa: A scanning electron microscopy study. *Pharmaceutical Research;* 1990 7 (5): 468-475

Fiebrig I, Harding S E, Davis S S. Sedimentation analysis of potential interactions between mucus and putative bioadhesive polymers. *Prog. Coll. Polymer Science;* 1994 94: 66-73

Fisher A N. Brown K, Davis S, Parr G, Smith D A. The effect of molecular size on the nasal absorption of water soluble compounds in the albino rat. *Journal of Pharmacy and Pharmacology*; 1987 (39) 357-362

Ganbo T, Hisamatsu K, Mizukoshi A, Inoue H, Kikushima K, Kou J, Kozuka Y, Murakami Y. Effect of ibudilast on ciliary activity of human paranasal sinus mucosa *in-vitro. Drug Research;*1995 45:883-886

Ganderton D, Jones T. *Drug Delivery to the Respiratory Tract*; 1987 : 133-134

Gibaldi M. *Biopharmaceutics and clinical pharmacokinetics*.4th ed. Philadephia: Lea and Febiger; 1991:20

Gilain L, Zahm J M, Pierrot D, Fuchey C, Peynegre R, Puchelle E. The nasal epithelial cell culture as a tool in evaluating ciliary dysfunction. *Acta Laryngology;*1993 113:772-776

Gizurarson S, Gudbrandsson F K, Jonsson H, Bechgaard E. Intranasal administration of diazepam aiming at the treatment of acute seizures: clinical trials in healthy volunteers. *Biological and Pharmaceutical Bulletin*; 1999 22 (4) : 425-427

Gizurarson S, Marriott C, Martin G P,, Bechgaard E. The influence of insulin and some excipients used in the nasal insulin preparations on mucociliary clearance. *International Journal of Pharmaceutics;*1990 65: 243-247

Gonda I, Gipps E. Model of disposition of drugs administered into the human nasal cavity. *Pharmaceutical Research*; 1990 7: 69-75

Gordon G S, Moses A C, Silver R D, Flier J S, Carey M C. *Proc.Natl. Acad.Sci USA;* 1985 82:7419

Guyton A C. Pulmonary ventilation In: *Textbook of medical physiology;* 1981:476-490 Saunders Philadelphia

Hardy J G, Lee S W, Wilson C G. Intranasal drug delivery by spray and drops. *Journal of Pharmacy and Pharmacology;* 1985 (37) 294-297

Helfand E, Tagami Y. Theory of the interface between immiscible polymers. Journal of *Chemical Physics*;1972 56: 3592-3601

Hirai S, Yashiki T, Mima H. Mechanisms for the enhancement of the nasal absorption of insulin by surfactants. *International Journal of Pharmaceutics;* 1981 9 (2):173-184

Hjortkjaer R K, Bechgaard E, Gizurarson S, Sutdak C, McDonald P, Greenough R J. Single and repeated dose local toxicity in the nasal cavity of rabbits after intranasal administration of different glycol formulations containing benzodiazepines. *Journal of Pharmacy and Pharmacology;* 1999 51 : 377-383

Hochman K, Artusson P. Mechanism of absorption enhancement and tight junction regulation. *Journal of Controlled Release;* 1994 29: 253-267

Illum L. Nasal delivery .The use of animal model to predict the performance in man. *Journal of Drug Targetting;* 1996 3: 427-442

Illum L, Ferry N F, Davis S S. Chitosan as a novel nasal drug delivery system for peptide drugs. *Pharmaceutical Research*; 1994 11: 1186-1189

Illum L, Jogernsen H, Bisgaard H, Krogsgaard O, Rossing N. Bioadhesive microspheres as a potential nasal drug delivery system. *International Journal of Pharmaceutical Sciences*; 1987 39:189-199

Ingels K J A O, Kortmann M J W, Nijziel M R, Graamans K, Huizing E H. Factors influencing ciliary beat measurements. *Rhinology;* 1991 29: 17-26

Irwin W J. Kinetics of drug decomposition: basic computer solutions. Oxford: Elsevier; 1990:85

Jorissen M, Bessen A. Influence of culture duration and ciliogenesis on the relationship between ciliary beat frequency and temperature in nasal epithelial cells. European Arch. *Ortorhinolaryngology;* 1995 252: 451-454

Kaelbe D H, Moacanin J. A surface energy analysis of bioadhesion. *Polymer*; 1977 18: 475-481

Kagatani S, Inabba N, Fukui M, Sonobe T. Nasal absorption kinetic behaviour of azetirelin and its enhancement by acylcarnitines in rats. *Pharmaceutical Research;* 1998 15 (1): 77-81

Kaliner M A. Human nasal respiratory secretions and host defense. *American Review of Respiratory Disease;* 1991 144:s52-s56

Kaliner M, Marom Z, Patow C, Shelhamer J. Human respiratory mucus. *Journal of Allergy and Clinical Immunology;*1984 73: 318-323

Kamath K R, Park K. Mucoadhesive preparations .In: *Encyclopaedia of Pharmaceutical Technology;* 1994: 133-163 Marcel Dekker, New York

King M, Gilboa A, Meyer F A, Silberberg A. On the transport of mucus and its rheologic simulants in ciliated systems. *American Review of Respiratory Disease;* 1974 110:740-745

Kinloch A J. The science of adhesion I: Surface and interfacial aspects. *Journal of Material Science*; 1980 15: 2141-2166

Kissel T, Werner U. Nasal delivery of peptides: An in-vitro cell culture model for the investigation of transport and metabolism in human nasal epithelium. *Journal of controlled release*; 1998 53 : 195-203

Kohno T, Murasugi N, Sakurai H, Watanabe K, Nakamuta H, Koida M, Ogouchi T, Inoue T, Yanaka M, Nomura M Yanagawa A. determination of the bioavailability of intranasal elcatonin in humans: development of a sandwich transfer enzyme immunoassay for elcatonin. *Journal of Clinical Laboratory Analysis*; 1998 12 (6) : 356-362

Korngreen A, Priel Z. Simultaneous measurement of ciliary beating and intracellular calcium. *Biophysical Journal;*1994 67:377-380

Krishnamoorthy R, Volka A M, Shao Z Z, Mitra A K. Cyclodextrins as mucosal absorption promoters for evaluation of nasal mucotoxicity. *International Journal of Pharmaceutical Sciences;* 1995 41:296-301

Krishnamoorthy R, Wolka A M, Shao Z, Mitra A K.Cyclodextrins as mucosal absorption promoters IV. Evaluation of nasal mucotoxicity. *European Journal of Pharmacy and Pharmacology;*1995 41 (5): 296-301

Kublik H, Vidgren M T. Nasal delivery systems and their effect on deposition and absorption. *Advanced Drug Delivery Reviews*; 1998 29: 157-177

Kwon Y. Pharmacokinetic study design and data interpretation. In. *Handbook of Essential Pharmacokinetics, Pharmacodynamics and Drug Metabolism for Industrial Scientists*, Kluwer Academic Publishers; 2001:8-23

Lansley A B. Mucociliary clearance and drug delivery via the respiratory tract. *Advanced Drug Delivery Review;*1993 29: 299-327

Lee V H L. *Peptide and Protein Drug Delivery;* Marcel Dekker New York 1991: 13-16

Lee V, Yamamoto A, Kompella U. Mucosal penetration enhancers for facilitation of peptide and protein drug absorption. *Critical Review of Therapeutic Drug Carrier System;* 1991 8: 91-192

Lehr C M, Bouwastre J A, Bodde H E, Juninger H E. A structure surface energy analysis of microadhesive contact angle measurement on polycarbophil and pig intestinal mucosa in physiological relevant fluids. *Pharmaceutical Research*; 1992 9:70

Lehr C M. Lectin-mediated delivery of the second generation of bioadhesives. *Journal of Controlled Release*; 2000 65: 19-29

Li L, Nandi I, Kim K H. Development of an ethyl laurate-based microemulsion for rapid -onset intranasal delivery of diazepam. *International Journal of Pharmaceutics;* 2002 237 (1-2): 77-85

Lindberg S. Mucociliary transport. *In: Rhinologic Diagnosis and Treatment*; 1997: 155-174 Thieme, New York

Lindhardt K, Ravn C, Gizurarson S, Bechgaard E. Intranasal absorption of buprenorphine-*in-vivo* bioavailability study in sheep. *International Journal of Pharmaceutics;* 2000 205: 159-163

Logemann C D, Rankin L M. Newer intranasal migraine medications. *American Family Physician*; 2000 61 (1) : 180-186

Madara J L. *Journal of Cell Biology*;1983 97:125

Madsen F, Eberth K, Smart J D. A rheological examination of the mucoadhesive/mucus interaction: the effect of mucoadhesive type and concentration. *Journal of Controlled Release*; 1998 50:167-178

Marom Z, Shelhamer J, Kaliner M. Nasal mucus secretion. *Ear, Nose and Throat Journal*; 1984 63: 36-44

Marriott C, Brown D T, Beeson M F.*Biorheology;* 1983 20:71

Martin G P, Marriott C, Kellaway I W. *Gut*; 1978 19:103

Marttin E, Verhoef J C, Romeijn S G, Merkus F W H M. Effects of absorption enhancers on rat nasal epithelium *in-vivo:* release of marker compounds in the nasal cavity. *Pharmaceutical Research;* 1995 12:1151-1157

Marttin E, Verhoef J C, Romeijn S G, Zwart P, Merkus F W H M. Acute histopathological effects of benzalkonium chloride and absorption enhancers on rat nasal epithelium *in-vivo. International Journal of Pharmaceutics;*1996 141:151-160

Marttin E. Nasal mucociliary clearance In: Mechanistic studies in nasal drug delivery and absorption enhancement;1997:33

McMartin C, Hutchinson L E F, Hyde R, Peters G C. Analysis of structural requirements for the absorption of drugs and macromolecules from the nasal cavity. *Journal of Pharmaceutical Sciences;* 1987 76: 535-540

Merkus F H W M, Shuster van Hees M T. Influence of levocarbastine suspension on ciliary beat frequency and mucociliary clearance. *Allergy*; 1992 47:230-233

Merkus F W H M, Schipper N G M, Hermens W A J J, Romeijn S G, Verhoef J C. Absorption enhancers in nasal drug delivery: efficacy and safety. *Journal of Controlled Release;*1993 24: 201-208

Morgan K T. Montecello Environmental Health Perspective; 1990 85: 209-218

Mortazavi S A, Carpenter B G, Smart J D. An investigation into the rheological behaviour of mucoadhesive mucosal interface. *International Journal of Pharmaceutics*; 1992 83 : 221-225

Mortazavi S A, Smart J D. An investigation into the role of water movement and mucus gel dehydration in mucoadhesion. *Journal of Controlled Release;* 1993 (25) 197-203

Murakami T, Sasaki Y, Yamajo R, Yata N. *Chemical Pharmaceutical Bulletin;*1984 32:1948

Murakami T, Yata N, Tamauchi H, Kamada A. *Chemical Pharmaceutical Bulletin;*1982 30:659

Muranushi N, Takagi N, Muranushi S, Sezaki H. Effect of fatty acids and monoglycerifdes on permeability of lipid bilayer. *Chemistry and Physics of Lipids;* 1981 28 (3):269-279

Muranushi S. *Pharmaceutical Research;* 1985 108:2

Mygind N, Dahl R. Anatomy, physiology and functions of the nasal cavities in health and disease. *Advanced Drug Delivery Review;*1998 29: 3-12

Naitoh Y, Kanebo H. Reactivated Triton-extracted models of Paramecium: modification of ciliary movement by calcium ions. *Science Washington D C;* 1972 176: 523-524

Nielson H W, Bechgaard E, Twile B, Didriksen E, Sorenson H. Intranasal administration of different liquid formulations of bumetanide to rabbits. *International Journal* of *Pharmaceutics;* 2000 204: 35-41

Ormrod D, Goa L K. Intranasal metoclopramide. *Drugs;* 1999 58 (2) : 315-322

Palant C E, Duffey M D, Mookerjee B K, Ho S, Bentzel C J. *American Journal of Physiology;* 1983 245:C203

Peppas N A, Buri P A. Surface, interfacial and molecular aspects of polymer bioadhesion on soft tissue. *Journal of Controlled Release;* 1985 2: 257-275

Perras B, Pannenborg H, Marshali L, Pietrowsky R, Born J, Fehn H L. Beneficial treatment of age related sleep disturbances with prolonged intranasal vasopressin. *Journal of Clinical Psychopharmacology*;1999 19 (1) : 28-36

Pitelka D R, Taggart B N, Hamamoto S T. *Journal of Cell Biology;* 1983 96:613

Proctor D F. The upper airways I: Nasal physiology and defence of the lungs. *American Review of Respiratory Diseases*; 1977 165: 97-129

Pujara C P, Shao Z, Duncan M R, Mitra A k. Effects of formulation variables on the nasal epithelial cell integrity: biochemical evaluations. *International Journal of Pharmaceutics;* 1995 114:197-203

Quraishi M S, Jones m S, Mason J D T. The nasal delivery of drugs. *Clinical Ortolaryngology;* 1997 22: 289-301

Ramanathan R, Geary R S, Bourne D W A, Putcha L. Bioavailability of intranasal promethazine dosage forms in dogs. *Pharmacological Research;*1998 38 (1): 35-39

Raphael J H, SelwynD A, Mottram S D, Langton J A, O'Callghan C. Effects of MAC halothane, enflurane and isoflurane on the ciliary beat frequency of human nasal epithelium *in-vitro.British Journal of anaesthesia;* 1996 76:116-121

Rasmussen H, Rasmussen J E. Calcium as intracellular messenger: From simplicity to complexity. *Curr. Trp. Cell Regul*; 1990 31:1-109

Reed C J. Drug metabolism in the nasal cavity: relevance to toxicology. *Drug Metabolism Review*; 1993 25: 173-205

Reed J C, Lock E A, De Matteis F. *Biochemical Journal;* 1986 240: 585-592

Reznik G, Stinson S F, Ward J M. *Arch.Toxicology;* 1980 46:233-240

Reznik-Schuller H M. *Cancer Letters;* 1982 16: 109-114

Ridley D, Washington N, Wilson C G. Drug delivery to the buccal and nasal cavities, anatomical and physiological considerations. *In: Buccal and Nasal administration as an Alternative Parenteral Administration*; 1992 : 29-39 Editions de Sante, Paris

Ritchel W A. *Handbook of basic pharmacokinetics* 4th ed. Hamilton: Drug Intelligence Publications;1993:6-12

Robson A M, Smallman M M, Wanner A. Protein kinase C- dependent phosphorylation of a ciliary membrane protein and inhibition of ciliary beating. *Journal of Cell Science*;1993 106: 1211-1220

Rodman J S, Mercer R W, Stahl P D. *Current Opinion Cell Biology;* 1990 2:664

RusznakC, Devalia J L, Lozewicz S, Davies R J. The assessment of nasal mucociliary clearance and the effect of drugs. *Respiratory Medicine;* 1994 88:89-101

Safety assessment of selected cyclodextrins –effect on ciliary activity using human cell suspension culture model exhibiting *in-vitro* ciliogenesis. *International Journal of Pharmaceutics;* 2000 193 (2): 219-226

Sakane T, Akizuki M, Yamashita S, Sezaki H, Nadai T. Direct transport from the rat nasal cavity to the cerebrospinal fluid: The relation to the dissociation of the drug. *Journal of Pharmacy and Pharmacology*; 1994 46 : 378-379

Salathe M, Bookman R J. Coupling of Ca^{2+} and ciliary beating in cultured tracheal epiuthelial cells. *Journal of Cell Science*; 1995 108: 431-440

Salathe M, Bookman R J. Single cell measurement of ciliary beat frequency and intracellular calcium in tracheal epithelial cells. *Biophysical Journal*; 1993 64: 264 (Abstract)

Sam E, Jeanjean A P, Maloteaux J M, Verbeke N. Apormorphine pharmacokinetics after intranasal and subcutaneous application. *European Journal of Drug Metabolism and Pharmacokinetics;* 1995 20: 27-33

Sanderson M J, CharlesA C Dirksen E R. Measurement of the temporospatial dynamics of intercellular calcium signalling with digital fluorescence microscopy. *American Laboratory;*1993 25:29-36

Sanderson M J, Dirksen E R. Mechanosensitive and beta-adrenergic control of the ciliary beat frequency of mammalian respiratory tract cells in culture. *American Review of Respiratory Disease*; 1989 139: 432-440

Sweadner K J, Goldin S M. *N. Engl. Journal of Medicine;* 1980 302:777

Sweadner K J, Goldin S M. *North England Journal of Medicine;* 1980 302:777

Tamm S L. Calcium activation of microcilia in ctenophore Boroe. *Journal of Comp.Physiology (A);* 1988 163: 100-112

Tamm S L. Control of reactivation and microtubule sliding by calcium, stronium and barium in detergent-extracted microcilia of Boroe. *Cell Motility Cytoskeletal;* 1989 12: 104-112

Theate L G, Spicer G S, Spock A. Histology, ultrastructure and carbohydrate cytochemistry of surface and glandular epithelium of human nasal mucosa. *American Journal of Anatomy*; 1981 162: 243-263

Tos M. Distribution of mucus producing elements in the respiratory tract. Difference between upper and lower airways. *European Journal of Respiratory Disease;* 1983 64 (Suppl. 128):269-279

Touitou E, Alhaique F, Fisher P, Memoli A, Riccieri M, Santucci E. *Journal of Pharmaceutical Sciences;*1987 76:791

Touitou E, Fisher P. Journal of Pharmaceutical Sciences;1986 75:384

Ugwoke M I, Agu R U, Jorissen M, Augustjins P, Sciot R, Verbeke N, Kingnet R. Nasal toxicological investigation of Carbopol 971P formulation of apomorphine: Effects of ciliary beat frequency of human nasal primary cell culture and *in-vivo* on rabbit nasal mucosa. *European Journal of Pharmaceutical Sciences;* 2000; 9: 387-396

Ugwoke M I, Agu R U, Jorissen M, Augustjins P, Sciot R, Verbeke N, Kingnet R. Toxicological investigations on the effects of carboxymethyl cellulose on ciliary beat frequency of human nasal epithelial cells in primary suspension culture and *in-vivo* on rabbit nasal mucosa. *International Journal of Pharmaceutics;* 2000 205: 43-51

Ugwoke M I, Sam E, Mooter G V, Verbeke N, Kinget R. Bioavailability of apomorphine following intranasal administration of mucoadhesive drug delivery systems in rabbits. *European Journal of Pharmaceutical Sciences;* 1999 9:213-219

Ugwoke M I, Verbeke N, Kinget R. The biopharmaceutical aspects of nasal mucoadhesive drug delivery. *Journal of Pharmacy and Pharmacology;* 2001 53: 3-22

Van de Donk H J M, Zuidema J, Merkus F W H M. Correlation between the sensitivity of the ciliary beat frequency of human adenoid tissue and chicken embryo tracheas for some drugs. *Rhinology;* 1982 20: 81-87

Van de Donk H J M, Zuidema J, Merkus F W H M. The influence of pH and osmotic pressure upon tracheal ciliary beat frequency as determined with a new photoelectric registration device. *Rhinology;*1980 18:93-104

Van der Kuy P H M, Lohman J J H M, Hooymans P M, Ter Berg J W M, Merkus F W H M. Bioavailability of intranasal formulations of dihydroergotamine. *European Journal of Pharmacology;* 1999 55: 677-680

Verhoef J C, Merkus F W H M. Nasal absorption enhancement: Relevance to nasal drug delivery. In de Boer A G ed *Drug absorption enhancement. Concepts, possibilities, limitations and trends;* 1994: 119-153

Voigt J M, Guengerich F P, Baron J. *Cancer Letters;* 1985 27: 241-247

Vora J, Christensen G, Reginato M, Maciz R, Guchoco C, Oh C. Influence of formulation variables on plasma growth hormone releasing peptide anaesthetised rats. *Journal of Controlled Release*; 1993 24:193-200

Voyutskii S S. Autoadhesion and adhesion of high polymers. *Interscience;* 1963 NewYork

Wanner A. Clinical aspects of mucociliary transport. . *American Review of Respiratory Disease;* 1977 116:73-125

Ward A, Heel R C. Bumetanide, a review of its pharmacodynamic and pharmacokinetic properties and therapeutic use. *Drugs;* 1984 28: 426-464

Warshaw A L, Walker W A, Isselbacher K J. *Gastroenterology;* 1974 66:987

Weksler N, Brill S, Tarnapolski A, Gurman G M. Intranasal salbutamol instillation in asthma attack. *American Journal of emergency Medicine*; 1999 17 (7) : 686-688

Wheater P R, Burkitt H G, Daniels V G. *Functional Histology: A Text and Colour Atlas,* Churchill Livingstone, New York, 1979

Wong L B, Miller I F, Yeates D B. Stimulation of ciliary beat frequency by automatic agonists: *in-vivo. Journal of Applied Physiology;*1988 65:971-981

Wyss P A, Rosenthaler J, Nuesch E, Aellig W H. Pharmacokinetic investigation of oral and intravenous dihydroergotamine in healthy subjects. *European Journal of Pharmacology;* 1991 41: 597-602

Yagi N, Kenmotsu H, Shimode Y, Oda K, Sekikawa H, Takada M. Bioavailability and diuretic effect of bumetanide following rectal administration of suppositories containing weak acids in human subjects. *Biological and Pharmaceutical Bulletin;* 1993 16:1124-1129

Yoshitsugu M, Rautiainen M, Matsune S, Nuutinen J, Ohyama M. Effect of exogenous ATP on ciliary beat of human ciliated cells studied with differential interference microscope equipped with high speed video. *Acta Otolaryngology;*1993 113:655-659

CHAPTER TWO

INTRODUCTION TO ANTIHISTAMINES AND CYCLIZINE

2.1 INTRODUCTION

2.1.1 Chemistry of antihistamines

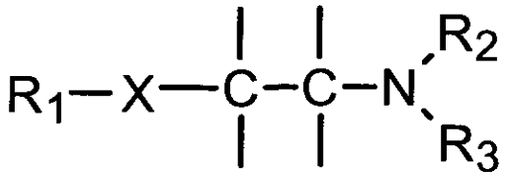


Figure 2.1: General structure of antihistamines

The general structure of antihistamines can be depicted by the structure above.

Most antihistamines are substituted ethylamines. Generally these molecules consist of 3 portions, namely the nucleus R_1, that is composed of an aromatic group and /or heterolytic groups, which may be separated from the linkage (X) by a methylene group. The linkage consists of atoms such as N, O, or C and the ethylamine group (Drug Info, 1988_a)

2.1.2 Classification of antihistamines

Antihistamines are classified on the basis of the "X" linkage group substitution as indicated in table 2.1:

Table 2.1: Classification of antihistamines (Drug Info, 1988_b)

Linkage "X"	Classification	Examples
N	Ethylenediamine derivative	Antazoline
O	Ethanolamine (aminoalkyl ether) derivative	Diphenylhydramine
C	Propylamine derivative	Chlorpheniramine
N phenothiazide nucleus	Phenothiazide derivative	Promethazine
N piperazine nucleus	Piperazine derivative	Cyclizine

2.1.3 Structure activity relation

The hydrogenation of rings in the R_1 portion of the molecule reduces the antihistaminic activity of the compound. Usually the antihistaminic activity is increased by the substitution of a halogen atom in the para position of the phenyl or benzyl group of the R_1 portion. For maximum activity, the terminal nitrogen of the ethylamine group should be a tertiary amine with methyl groups or a cyclic moiety in R_2 and R_3. In optically active compounds, the dextro isomer is usually more active than the levo isomer (Drug Info, 1988_c).

The basic ethylamine group common to all antihistamines is also common to anticholinergics, ganglionics, anaesthetics, adrenergic blocking agents and antispasmodics, hence antihistamines may be expected to exhibit some of the activities of these other classes of drugs and hence their use as anti-emetics (Drug Info, 1988_d).

Most antihistamines are lipophilic enough to cross the blood brain barrier and interact with the H_1-receptors and hence their sedative effects. This effect is sometimes used therapeutically.

2.2 General pharmacology of antihistamines

Historically, the term antihistamine has been used to describe drugs that act as H_1-receptor antagonists. Although the drugs that antagonise H_2-receptors are also commercially available (cimetidine, ranitidine and famoditine), these are generally not referred to as antihistamines but H_2-receptor antagonists (Drugs Info, 1988_e).

The anti-emetic and anti-motion sickness of some antihistamines appear to be from their anticholinergic and central nervous system depressant properties. The diphenylhydramine effects on Parkinsonism syndrome and drug induced extrapyramidal reactions are also due to its anti-emetic effects. Some antihistamines also demonstrate a quinidine-like effect on myocardial conduction and may therefore enhance the pressor action of norepinephrine.

2.2.1 Mechanism of action

Antihistamines competitively antagonise most of the smooth muscle stimulating actions of histamine on the H_1-receptors of the gastro intestinal tract, uterus, large blood vessels and bronchial muscles. The antagonistic actions of the antihistamines are as follows:

- Contraction of the sphincter of Oddi, biliary induced contraction of the smooth muscles and the bile ducts are partially mediated.
- Feeble antagonistic action to the bronchospasm induced by the antigen-antibody reactions.
- Histaminic action that results in increased capillary permeability and the formation of oedema.
- Suppression of flare and pruritis which accompany the endogenous release of histamine (Drug Info, 1988_f).

2.3 CYCLIZINE

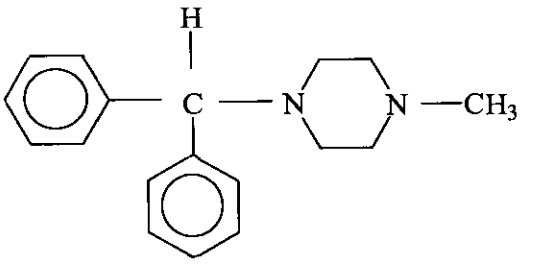


Figure 2.2: Structure of cyclizine

2.3.1 Chemical and Physical properties of cyclizine

Chemical names	: 1 -(diphenylmethyl)-4-methyl piperazine
Empirical formula	: C_{18} H_{22} N_2
Molecular weight	: 266.40
Description	: It is a creamy white, almost odourless crystalline powder or small crystals
Solubility	: It is slightly soluble in water and alcohol (1 in 115 parts), soluble in chloroform (1 in 75 parts) and insoluble in ether
Acidity	: A 2% solution in alcohol: water (2:3) has a pH of 4.5 to 5.5
Melting point	: Ranges from 106°C to 109°C
Dissociation constant	: $pK_a = 8.32$
Log P	: 3.97
Absorbencies in the Ultra Violet region	: Aqueous acid : 275nm, 262nm, A (1%, 1cm) = 28_a, 268nm Aqueous alkali 260nm A (1%, 1cm) = 16_b

Storage	: Should be stored in airtight containers and protected from light.

Cyclizine is a piperazine derivative with actions of the histamine H_1-receptor antagonist used mainly as an anti-emetic and an anti-motion sickness drug (Clarke, 1986; USP 26 NF 21, 2003).

2.3.2 Pharmacological properties

2.3.2.1 Mechanism of action

The mechanism by which cyclizine exerts its anti-emetic and anti-motion sickness is not precisely known but may be related to its central anticholinergic actions, thus it tends to diminish vestibular stimulation and depresses the labyrinth function. An action on the medullary chemoreceptor trigger zone may be involved in the anti-emetic effects (USP DI, 1998).

Like all antihistamines, cyclizine competitively inhibits the H_1-receptors of the smooth muscles to histamine stimulation. Within the vascular tree, it also inhibits both vasoconstrictor effects of histamine and to a degree the more rapid vasodilator effects that are mediated by H_1-receptors on the endothelial cells (Hardma, 1996).

Effects on capillary permeability: It strongly blocks the action of histamine that results in the increase in capillary permeability and the formation of oedema and wheal (Hardma, 1996).

Effects on exocrine glands: Cyclizine does not inhibit the secretion of gastric fluids but suppresses histamine evoked salivary, lachrymal and other exocrimal secretions with variable responses (Hardma, 1996).

2.3.2.2 Clinical applications and dosage

Cyclizine is an H_1-receptor antagonist with central nervous system depressant, anticholinergic, anti-emetic, antispasmodic and local anaesthesia effects. It is mainly used as an anti-emetic and for anti-motion sickness due to its prominent anticholinergic activity and actions on the vomiting centre. It is therefore indicated for the following:

- Prevention and treatment of motion sickness
- Treatment of irradiation sickness
- Control of post-operative and drug induced vomiting
- Treatment of nausea and vomiting
- Symptomatic treatment of vertigo caused by Mernier's disease and other labyrinth disturbances (Martindale, 1992).

The different clinical uses and doses for each disease state are presented in table 2.2 below. The dose largely depends on the indication presented. Usually the intramuscular/intravenous and oral dose range between 25mg to 300mg and the rectal dose is between 100mg and 300mg.

Table 2.2: Clinical presentations and recommended dose

Indication	**IV/ Oral/rectal dose Adult**	**IV/ Oral/rectal dose Paediatric**
Motion sickness	100mg tid supp, 50mg tid po/im	50mg tid supp 12.5mg tid po/im
Post–operative nausea	50mg im before end of operation	12.5mg im before end of operation
Nausea and Vomiting/Labyrinth disorders	100mg tid supp, 50mg tid po/im	50mg tid supp 12.5mg tid po/im

2.3.2.3 Pharmacokinetics

There is limited information on the pharmacokinetics of antihistamines and cyclizine in particular.

2.3.2.3.1 Half-life

There is no documented information on the pharmacokinetics of cyclizine however, studies indicate a biological half-life of about 13 hours (Walker, 1995).

2.3.2.3.2 Absorption

Cyclizine is well absorbed following oral or parenteral administration. Peak plasma concentrations after a single oral dosing of 50mg occurs after 2 to 3 hours and last for 4 to 6 hours following oral administration. Usually symptomatic relief begins after 15 to 30 minutes post oral administration (Drug Info, 1988_g).

2.3.2.3.3 Metabolism

The drug is extensively metabolised by N-demethylation in the liver to form an inactive metabolite, norcyclizine that is widely distributed throughout the tissues especially the kidneys, liver, lungs, and spleen. In plasma, norcyclizine is 60% protein bound. Due mainly to the drug's extensive hepatic extraction, its bioavailability after oral administration is reported to be low (Clarke, 1986).

2.3.2.3.4 Distribution

The distribution of cyclizine has not been well characterised but available literature indicate that high concentrations of both the drug and the inactive metabolite (norcyclizine) are found in the kidneys, liver, lungs and spleen (European Pharmacopoeia, 2000).

2.3.2.3.5 Elimination

The metabolic fate of cyclizine is not clearly stated however, reports indicate that the drug undergoes an extensive hepatic metabolism and is excreted as an inactive metabolite (norcyclizine) in urine (Walker, 1995).

2.3.2.4 Pharmacodynamics

2.3.2.4.1 Side effects

Due to its anticholinergic effects an overdose of cyclizine may induce the following side effects (USP DI, 1998):

- Blurred vision
- Constipation
- Difficult or painful urination (urinary retention)
- Dryness of mouth, nose and throat
- Dizziness
- Tachycardia
- Loss of appetite
- Nervousness
- Restlessness
- Trouble in sleep/skin rash

2.3.2.4.2 Contraindications

The use of cyclizine is contraindicated in patients with the following diseases or disorders:

- Acute asthma
- Glaucoma
- Urinary retention
- Prostrate hypertrophy
- Chronic pulmonary disease
- Shortness/difficulty of breathing

For geriatrics, no documented information is available on the relationship of age to effects of cyclizine but caution should be taken as regards usage by geriatrics. This group of patients tends to exhibit sensitivity to anticholinergics, which are pharmacologically related to cyclizine and therefore constipation, dryness of mouth, and urinary retention may occur (USP DI, 1998).

2.3.2.4.4 Drug Interaction

Concurrent use of cyclizine with:

- Anticholinergics and other drugs with anticholinergic activity potentiates the anticholinergic side effects.
- Central nervous system depressants potentiates the sedative effect of these medications.
- Prior administration of cyclizine may reduce the anti-emetic response to apomorphine in the treatment of poisoning (USP DI, 1998).

References

AHFS *Drug Information*; 1988_a : 2

AHFS *Drug Information*; 1988_b : 2

AHFS *Drug Information*; 1988_c : 2

AHFS *Drug Information*; 1988_d : 2

AHFS *Drug Information*; 1988e : 2

AHFS *Drug Information*; 1988_f : 2

AHFS *Drug Information*; 1988_g : 2

Clarke E G C. *Isolation and identification of drugs in pharmaceuticals, body fluids and post-mortem materials*; 1986 2nd Edition: 497-498

European Pharmacopeia, 2000:1646-1647

Hardma J G, Limbird L E. *Goodman and Gillman's Pharmacological basis of therapeutics* 9th Edition; 1996: 584, 585, 586-7

The Extra Martindale Pharmacopoeia;1992 30th Edition : 1279

USP 26 NF 21; The Official Compendia Standards; 2003 : 526

USP DI Drug Information for Health Care Professionals 18th Edition; 1998 : 1118

Walker R and Kanfer I. Sensitive High Performance Liquid Chromatographic determination of cyclizine and its demethylated metabolite, norcyclizine in biological fluids using coulometric detection. *Journal of Chromatography;* 1995 672 : 172-177

CHAPTER THREE

CHEMICAL IDENTIFICATION OF CYCLIZINE HCl AND SYNTHESIS OF CYCLIZINE LACTATE

3.1 Quality control

3.1.1 Identification of Cyclizine HCl powder

Cyclizine HCl powder was subjected to identification by determination of the melting point, infra-red (IR) spectrum and ultraviolet (UV) spectrum for the pure drug as stipulated in the official monographs, USP 26 NF 21.

3.1.1.1 Melting point determination

3.1.1.1.1 Materials and Method

Cyclizine HCl powder was donated by the Institute of Industrial Pharmacy (Potchefstroom University for CHE, RSA) and the melting point was determined by using the Shimadzu Differential Scanning Calorimetry (DSC-50) (Japan) instrument.

The differential scanning calorimetry (DSC) thermograms were recorded with a Shimadzu Differential Scanning Calorimetry (DSC-50) instrument. 2.716mg of cyclizine HCl was placed in an aluminium crimp cell on a sample holder. The sample was heated under a stream of nitrogen gas at a heating rate of 10°C per minute and the gas flow was maintained at 45ml/min. Three melting points readings were recorded and a mean was calculated.

3.1.1.1.2 Results and Discussion

The melting point of cyclizine HCl as depicted in the DSC thermogram in Figure 3.1 was found to be 108.54°C. This melting point falls within the specified limits as stipulated in the USP26 NF 21.

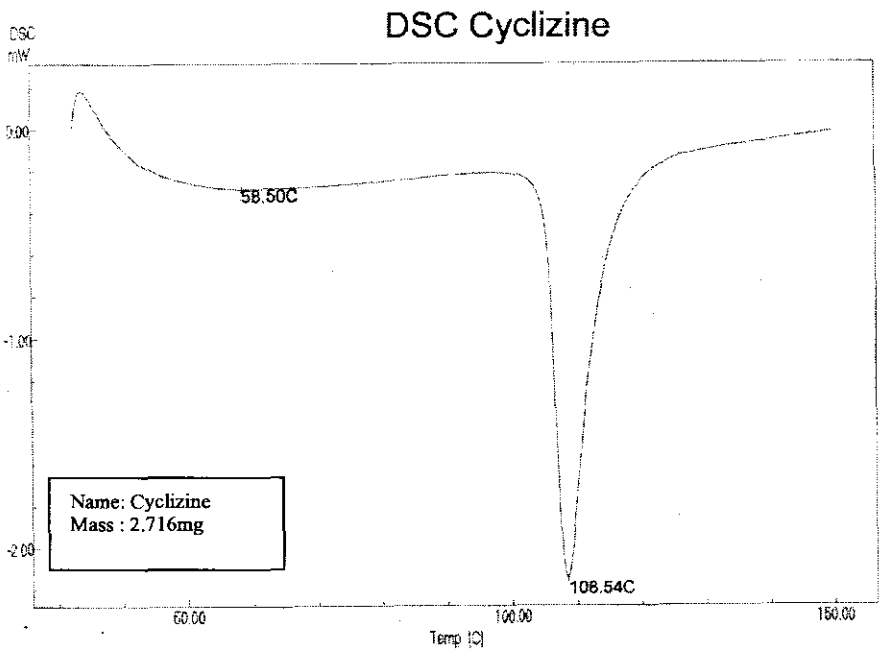


Figure 3.1: DSC profile of cyclizine HCl raw material

3.1.2 Ultra-violet Absorption

3.1.2.1 Materials and method

Cyclizine HCl was donated by the Institute of Industrial Pharmacy (Potchefstroom University for CHE, RSA) and the Shimadzu UV-Vis 160A spectrophotometer (Japan) was utilised to determine the absorbencies and corresponding wavelengths of the cyclizine solution.

The ultraviolet absorbance of cyclizine HCl was determined by employing the Shimadzu UV-Vis 160A spectrophotometer using 1mm quartz cuvettes. The method of analysis employed (USP 26 NF 21:197U) was similar to that used by Clarke (1986). The cyclizine HCl solution with a concentration of 0.1mg/ml was examined spectrophotometrically over a spectral range of 200nm to 400nm.

3.1.2.2 Results and Discussion

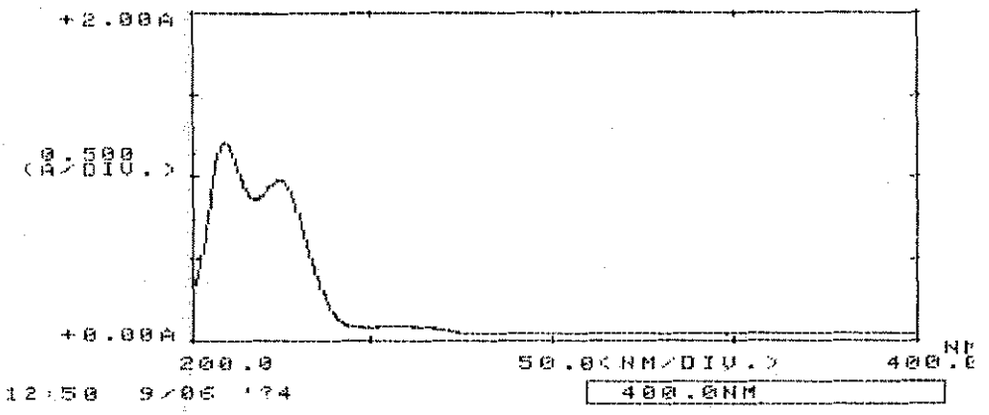


Figure 3.2: UV spectrum of cyclizine raw material

Figure 3.2 depicts the UV spectrum of cyclizine HCl solution. The solution exhibited maximum absorption at 208nm and 225nm. These wavelengths of maximum absorption exhibited by the solution were similar to those found by Clarke (1986) (see Appendix #2). For this study the 208nm wavelength was chosen as the most suitable for all drug analyses due to the high absorbance of the drug at this wavelength (table 3.1).

Table 3.1: UV Absorption of cyclizine HCl

Reading	Absorbance	Wavelength (nm)
1	1.999	208
	1.528	225
2	1.997	208
	1.531	225
3	1.991	208
	1.531	225

3.1.3 Infra-red Absorption

3.1.3.1 Materials and method

Cyclizine HCl was donated by the Institute of Industrial Pharmacy (Potchefstroom University for CHE, RSA) and potassium bromide was purchased from BDH, RSA. The Magna Violet Fourier-transform infra-red (FTIR) 550 spectrophotometer (Japan) was used for infra-red (IR) analysis.

The USP 26 NF 21 method for the infrared (IR) absorption spectrophotometric identification test (197k) was employed. 1mg of cyclizine HCl powder was intimately mixed and ground with 10mg of potassium bromide powder. The powder mixture was then inserted onto a die and subjected to pressure under vacuum. The disc formed was then mounted directly onto the sample beam path of the Fourier-transform infra-red (FTIR) spectrophotometer and the infra-red (IR) spectrum of this test compound was recorded.

3.1.3.2 Results and Discussion

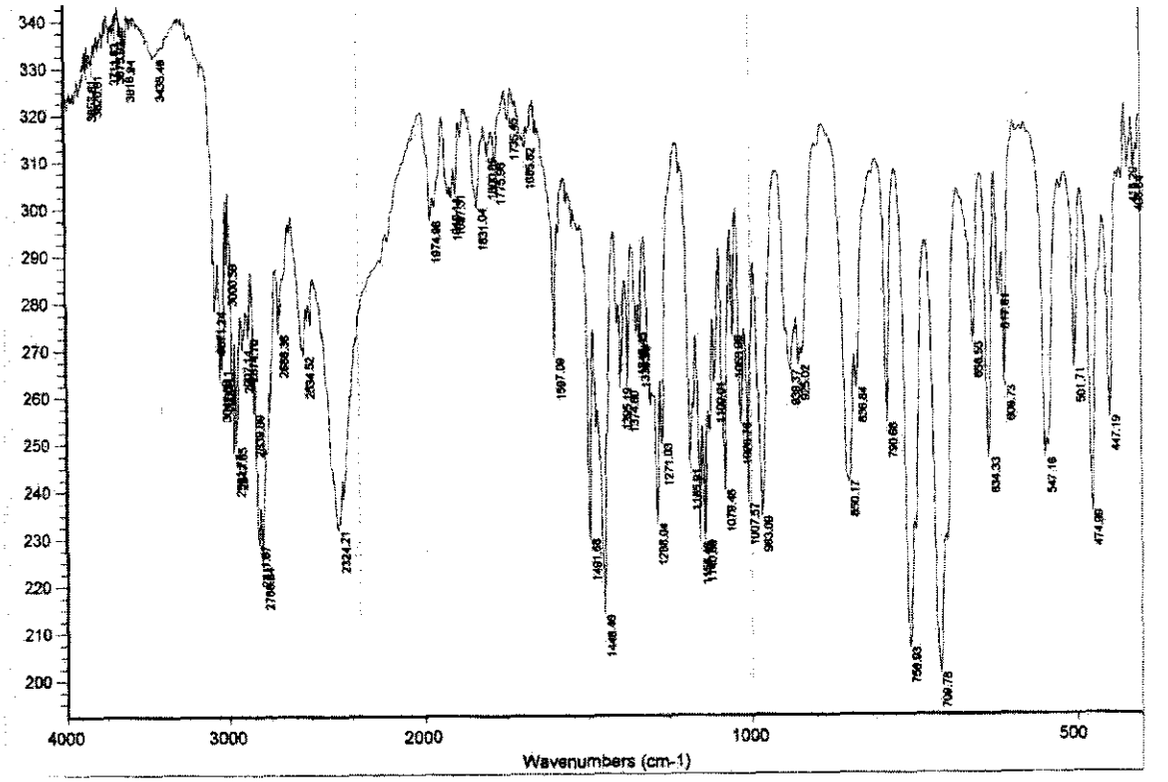


Figure 3.3: Infra-red spectrum of cyclizine HCl raw material

Figure 3.3 depicts the infra-red (IR) spectrum obtained for cyclizine HCl raw material. The test compound exhibited principal peaks at the following wavenumbers: 706.78cm^{-1}, 756.93cm^{-1}, 658.55cm^{-1}, 983.09cm^{-1}, 114.69cm^{-1}, 1597.09cm^{-1} and 2947.65cm^{-1}. These principal peaks observed are similar to those obtained by Clarke (1986) (see Appendix 3). Furthermore, the infra-red (IR) spectrum for cyclizine HCl obtained was concordant with the BP 2002 reference spectrum of cyclizine HCl (see Appendix # 2).

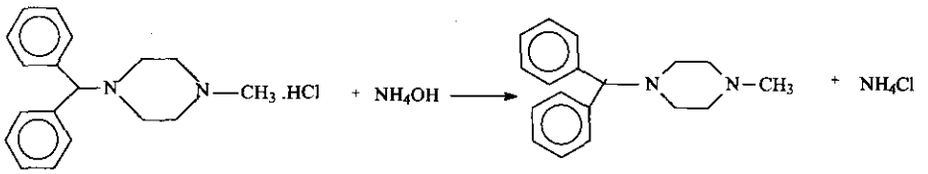
3.2 Chemical synthesis of cyclizine lactate

3.2.1 Rationale for the synthesis of cyclizine lactate

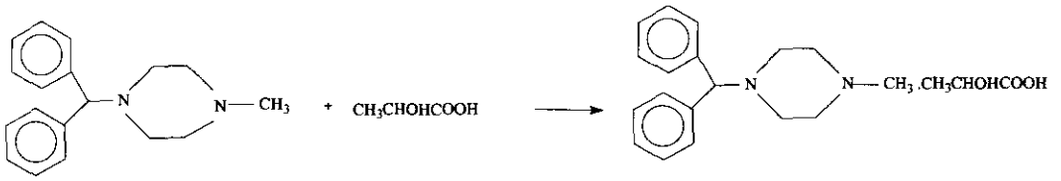
One of the disadvantages of the nasal delivery of drugs is that the size of the dose should be small and it should be delivered in an aqueous vehicle that will be in harmony with the nasal environment. Thus for the dose to be delivered nasally, the drug must exhibit sufficient aqueous solubility. Lack of aqueous solubility is often a problem. The optimum volume required for nasal administration is in the range 25-200µl (Behl et al., 1998). Solubility not only limits the drug absorption rate per se, it can also limit the formulator's ability to formulate a product if the drug is not sufficiently soluble in the desired vehicle. Furthermore, Chien et al., (1989) indicated that the enhancement of nasal drug absorption can be achieved by formation of a salt or ester with increased solubility in the nasal fluid.

Cyclizine HCl, as reported in the USP 26 NF 21, is slightly soluble in water (1 part in 115). This solubility is too low to deliver a therapeutic dose intranasally. An alternative salt (cyclizine lactate), which shows high aqueous solubility was therefore synthesised for inclusion into the intranasal formulation.

3.2.1.1 Materials and method



1 mole cyclizine HCl 1 mole cyclizine



1 mole cyclizine 1 mole lactic acid 1 mole cyclizine lactate

(Vogel, 1989)

Cyclizine HCl powder and lactic acid were of analytical grade were donated by the Institute of Industrial Pharmacy (Potchefstroom University for CHE, RSA). 32%(v/v) ammonium hydroxide solution and methanol (analytical grade) were purchased from Merck RSA. Gemini 300 Nuclear Magnetic Resonance (NMR) spectrophotometer was used for ^{1}H NMR analysis of the synthesised compounds.

1.512g of cyclizine HCl powder were dissolved in 10ml methanol. 5.47ml of 32%(v/v) ammonium hydroxide solution was added and the mixture was stirred continuously with the aid of a magnetic stirrer for an hour. The resulting suspension was then filtered and the residue retained and dried in a dessicator overnight. About 10mg of the new powdery product was analysed using the nuclear magnetic resonance ^{1}H NMR spectroscopy for structural identification. Once the structure was confirmed that the product obtained was the cyclizine base, it was then dissolved in 10ml methanol and treated with 0.365ml of

lactic acid. The mixture was likewise stirred continuously with the aid of a magnetic stirrer for an hour after which the solvent was evaporated off using a rotary vacuum. The new product was analysed again using nuclear magnetic resonance ^{1}H NMR spectroscopy for structural identification. After confirmation of the structure for the new compound solubility studies were then performed.

3.2.1.2 Results and Discussion

Identification of the precursors and the primary products was verified by use of the nuclear magnetic resonance ^{1}H NMR technique. The spectra of cyclizine HCl (precursor), cyclizine base (intermediary) and cyclizine lactate (end-product) are presented in Figures. 3.4, 3.5, and 3.6 respectively.

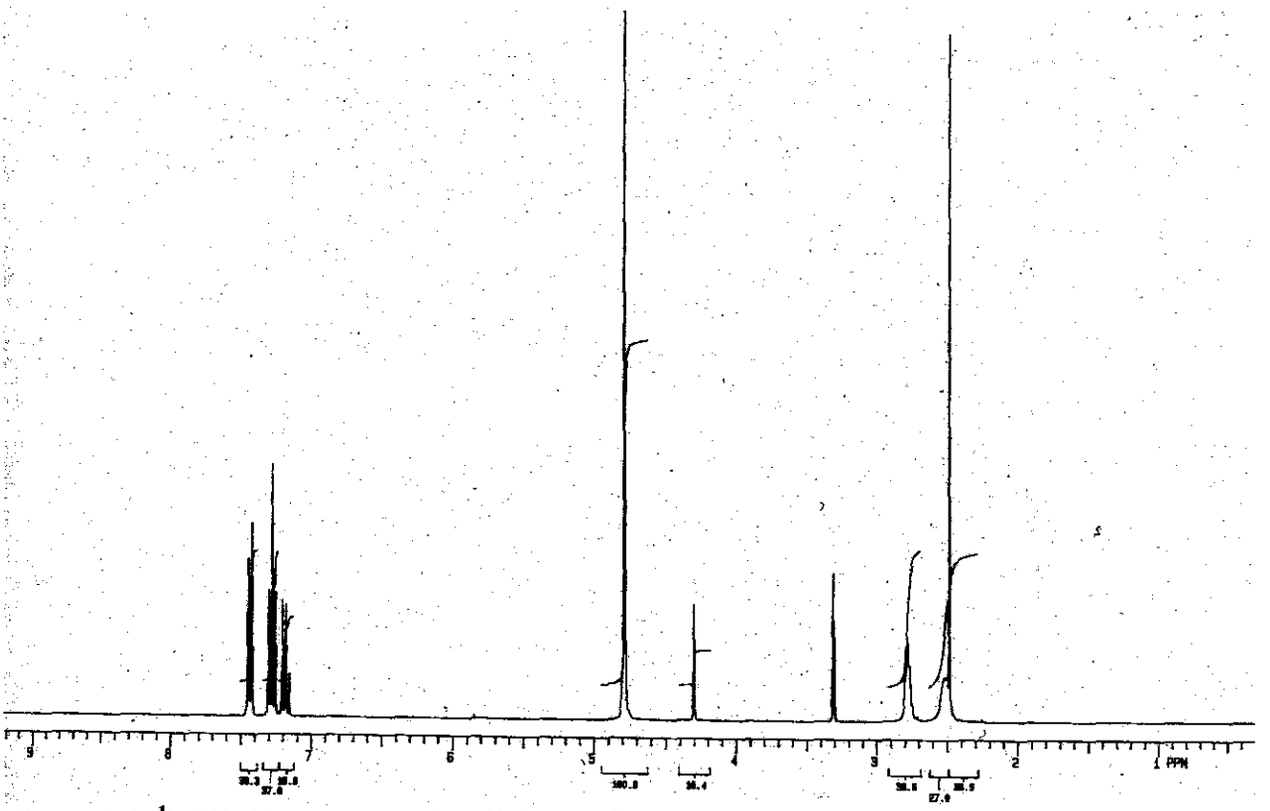


Figure 3.4: ^{1}H NMR spectrum of cyclizine HCl

The ^{1}H NMR spectrum in Figure 3.4 can be described as follows: δ_H 7.47 – 7.43 (dd, 4 H, J 6.97, aromatic H); 7.33-7.28 (t, 4H, J 8.25, aromatic H); 7.24-7.19 (m, 2H, aromatic H);

4.8(br s, 8H, 4x N-CH_2); 4.42 (s, 1H, CH); 2.87 (s, 3H, CH_3). This verifies the functional groups comprising the structure of cyclizine

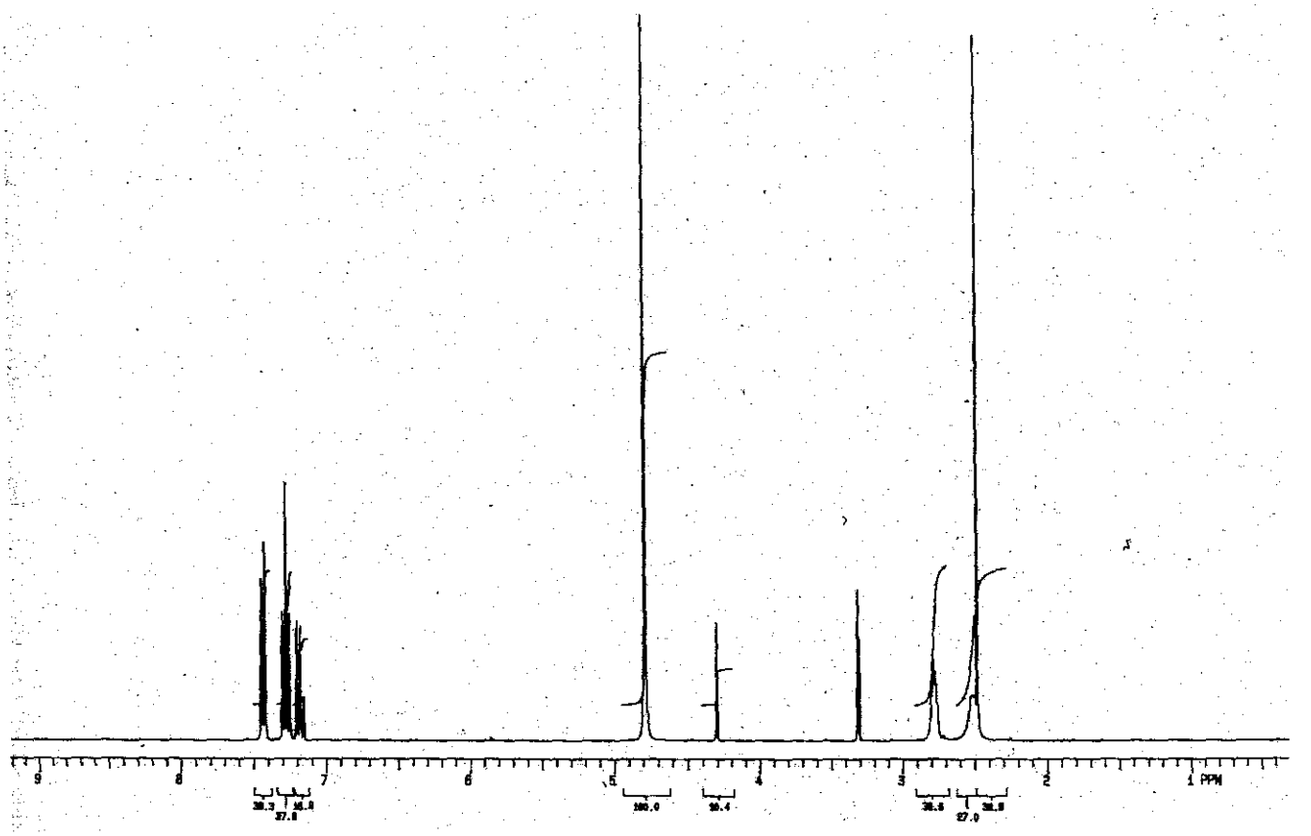


Figure 3.5: ^{1}H NMR spectrum of cyclizine base

The ^{1}H NMR spectrum in Figure 3.5 which is similar to the one in Figure 3.4 can be described as follows: δ_H 7.45 – 7.42 (dd, 4 H, J 10.26, aromatic H); 7.3-7.2 (t, 4H, J 7.14, aromatic H); 7.197-7.15 (m, 2H, aromatic H); 4.79 (br s, 8H, 4x N-CH_2); 4.3 (s,1H, CH); 2.48 (s, 3H, CH_3). This verifies the structural makeup of the predicted compound (cyclizine base).

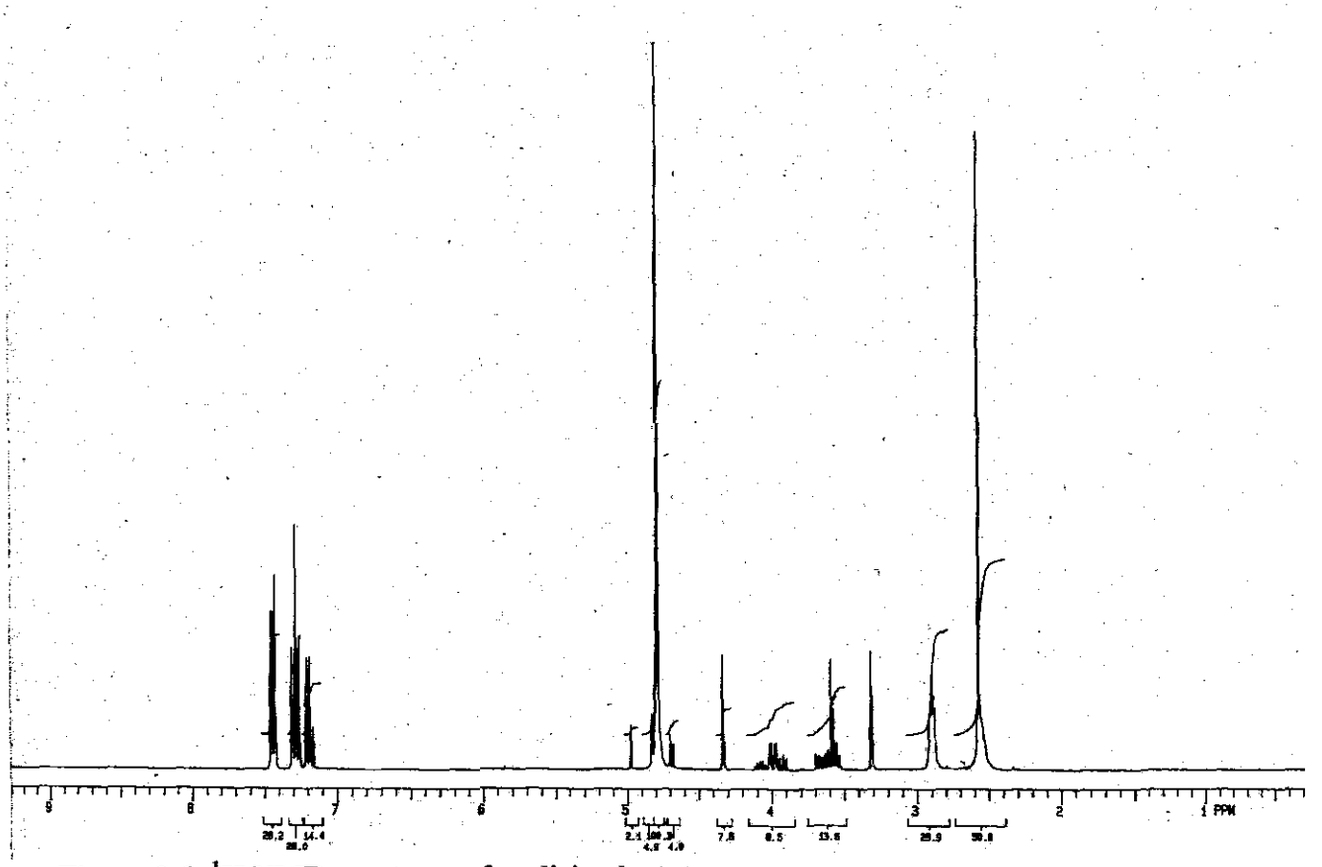


Figure 3.6: ^{1}H NMR spectrum of cyclizine lactate

Similarly the ^{1}H NMR spectrum in Figure 3.6 can be described as follows: δ_H 7.45 – 7.31 (dd, 4 H, J 6.97, aromatic H); 7.31-7.26 (t, 4H, J 8.25, aromatic H); 7.25-7.16 (m, 2H, aromatic H); 4.78(br s, 8H, 4x N-CH_2); 4.33 (s,1H, CH); 2.57 (s, 3H, CH_3).These functional groups are similar to the ones exhibited by the compound in Figure 3.4. In addition, the spectrum exhibits the following signals: 4.01-3.97 (m, 1H, aliphatic CH); 3.59-3.31 (m, 1H, OH); 3.31 (t, 3H, CH_3). This spectrum verifies the structure of the new compound synthesised.

3.3 Solubility studies

3.3.1 Materials and method

Cyclizine HCl powder was donated by the Institute of Industrial Pharmacy (Potchefstroom University for CHE, RSA). Cyclizine lactate was synthesised in the pharmaceutics laboratory (Potchefstroom University for CHE, RSA). 0.05M monobasic

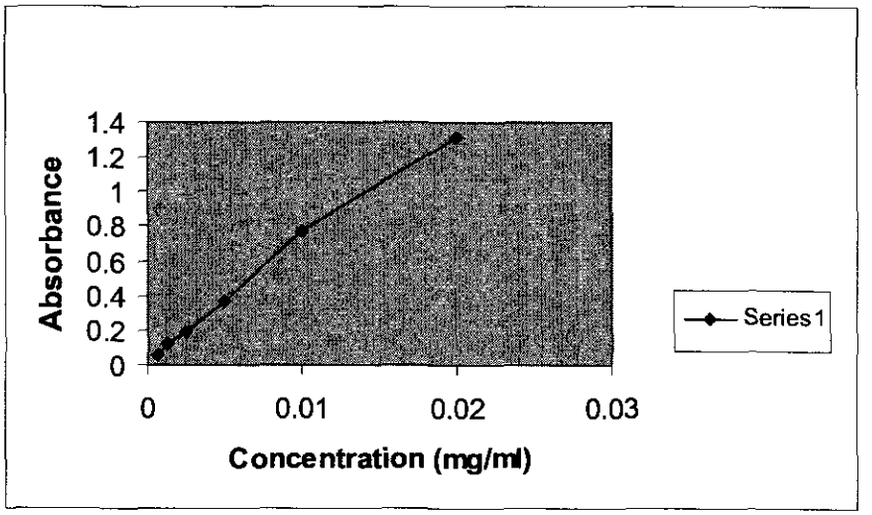
phosphate buffer solution at pH 6.8, HPLC water, polytops, magnetic stirrers, 15-plate water-bath, Shimadzu UV-Vis 160A Spectrophotometer, 1mm cuvets.

The solubility of cyclizine HCl and cyclizine lactate was determined in pure water and in 0.05M monobasic phosphate buffer solution at pH 6.8 (nasal pH) using the 15-well plate based assay. An excess amount of drug was placed in each of the 6 polytops for each compound and about 4 ml of the solvent (pure water or 0.05M monobasic phosphate buffer solution pH 6.8) was added to each container. The suspensions were kept in the water bath at 30° C and continuously stirred for 48 hours. The plate was then covered to avoid light induced degradation of the drugs.

3.3.2 Determination of drug concentration

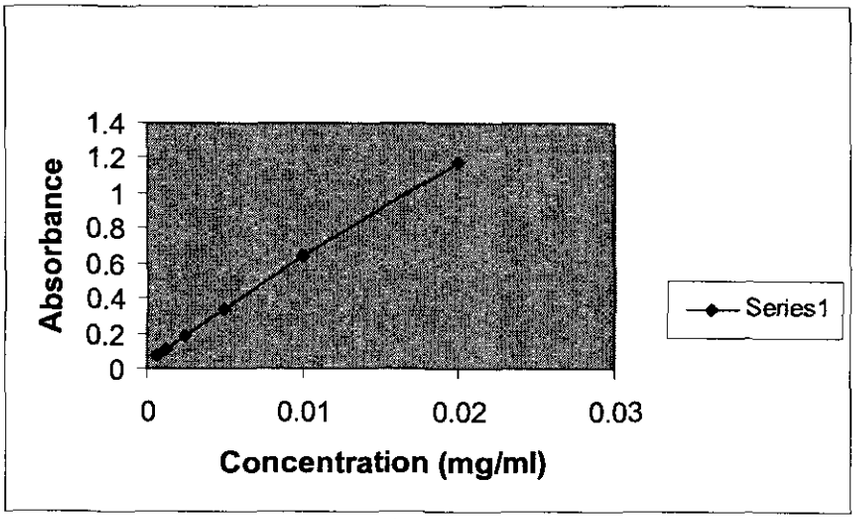
3.3.2.1 Calibration curves

5 standard solutions with known concentrations were prepared in the same solvents for each compound. Calibration curves for both compounds were constructed using absorbencies of the known varying concentrations of these standard solutions for the compounds under study. The drug suspensions were then filtered and the optical absorbency for each filtrate was measured against a blank (water or 0.05M monobasic phosphate buffer solution pH 6.8) at the optimal wavelength of maximum absorption for the particular test compound. The concentration for each compound was calculated using the equation from the corresponding calibration curve or alternatively depending on the absorbance value of the sample by using the closest concentration and the absorbance of the standard, or according to Lambert-Beer's law.



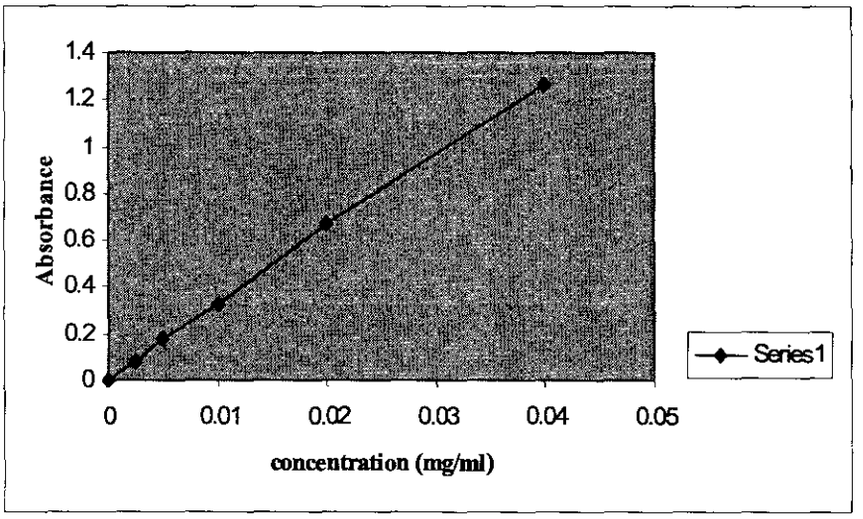
Y = 65.535x + 0.0396 R^2 = 0.9931

Figure 3.7: Calibration curve for cyclizine HCl at pH 6.8



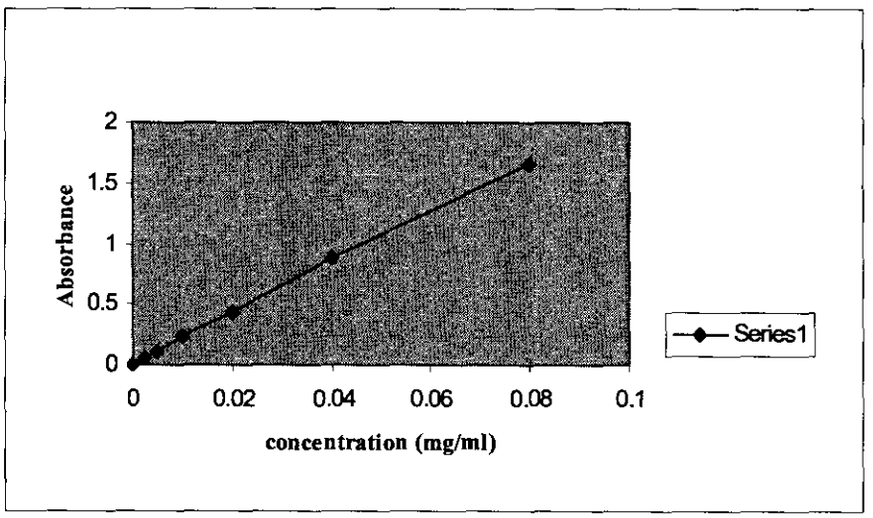
Y = 56.812x + 0.046 R^2 = 0.9989

Figure 3.8: Calibration curve for cyclizine lactate at pH 6.8



Y = 31.634x + 0.0114 R^2 = 0.9999

Figure 3.9: Calibration curve for cyclizine HCl at pH 4.5



Y = 20.689x + 0.0153 R^2 = 0.9992

Figure 3.10: Calibration curve for cyclizine lactate at pH 3.3

3.3.3 Results and Discussion

3.3.3.1 Solubility studies of cyclizine HCl and cyclizine lactate

Solubility studies for both cyclizine HCl and cyclizine lactate at pH ±4 and 6.8 (nasal pH) revealed the following:

Using the Lambert-Beer's law, solubilities for both compounds at the different pH values were found to be as follows:

PH ±4:

Cyclizine HCl: 3.85mg/ml

Cyclizine lactate: 87.35mg/ml

PH 6.8:

Cyclizine HCl: 2.77mg/ml

Cyclizine lactate: 32.15mg/ml

The results obtained indicate an increase in the solubility of cyclizine derivative from 3.8mg/ml of the HCl form to 87.35mg/ml of the lactate. This implies that the formation of an ester has supposedly enhanced the aqueous solubility of the drug (cyclizine) and hence the drug nasal absorption as indicated by Chien at al., (1989). Furthermore, the high aqueous solubility achieved would enable the dissolution of much of the solute in very small volume of solvent of not more than 200µl as required for nasal preparation (Behl et al., 1998).

It was also observed that the solubilities for both derivatives (HCl and lactate) are pH dependent. Thus as the pH increased there was a gradual decrease in solubility of the drug. This is in accordance with the Henderson-Hasselbalch equation as stated by Martin, (1993). The dissolution rate of a weak base decreases with an increase in pH of the surrounding medium, since both cyclizine HCl and cyclizine lactate are weak bases solubility therefore followed a similar trend hence the decrease in solubility at pH 6.8 compared with acidic pH was observed.

References

Behl C R, Pimplaskar A P, Sileno A P, deMeirless J, Romeo V D. Effects of physicochemical properties and other factors on systemic nasal drug delivery. *Advanced Drug Delivery Reviews*; 1998 29 : 89-116

British Pharmacopoeia London Stationaries; 2002 1:531-532

British Pharmacopoeia London Stationaries; 2002 2:A128

Chien Y W, Su K S E, Chang S P. Physicochemical, biopharmaceutical and toxicological considerations. In: *Nasal Systemic Drug Delivery;* 1989 39:49

Clarke E G C. Isolation and identification of drugs in pharmaceuticals, body fluids and post-mortem materials; 1986 2nd Edition: 497-498

Martin A. *Physical Pharmacy* 4th Edition;1993: 169-173

USP 26 NF 21; The Official Compendia Standards; 2003 : 526; 2135-2136; 2178-2181

Vogel A I. *Practical Organic Chemistry* 5th Edition;1989:702

CHAPTER FOUR

IN-VITRO NASAL TOXICITY STUDIES

4.1 INTRODUCTION

Many substances can influence the mucociliary clearance (MCC) system of the airways, either by stimulation or by inhibition. The stimulatory effect of drugs on the nasal MCC is of clinical importance, because these substances can be used to improve the pathological conditions of the mucociliary system. For nasal drug delivery, however, possible inhibitory effects are most relevant, because they can result in undesired side effects on the MCC system. When the MCC system is impaired by components of the nasal formulation, this can be prohibitive of their therapeutic use. Investigating the effects of drugs and excipients on the nasal mucociliary functioning is an important issue, because of the growing number of nasal drug formulations currently investigated for pharmacotherapy. Some of these preparations are meant for long-term treatment. Any adverse effects on the ciliated epithelium may limit the patient's acceptance of the nasal formulation and thus use in chronic nasal drug delivery. In view of the important functions carried out by the cilia, it is obviously desirable that drugs and excipients in general should be as "cilio-friendly " as possible (Merkus, 1992). Screening of pharmacologically active moieties for use in nasal application is therefore of utmost importance since these species can impair the mucociliary system due to their inherent physicochemical properties.

In order to investigate the effects of components of an intranasal dosage form on mucociliary function, various *in-vitro and in-vivo* methods have been developed. Several authors have provided excellent reviews of many of these techniques: Iravani and Melville., (1981); Hermens and Merkus., (1987); Schipper et al., (1991). Studies of radioactive or radio-opaque particle transport in humans or animals and the use of

saccharine test in humans are commonly used methods for measuring the nasal mucociliary transport time (NMCTT). Unfortunately, these methods are often too costly for routine testing, are difficult to use in humans when studying non-approved agents, or cannot be used in whole animal model (Donovan et al., 1995).

The measurement of ciliary beat frequency (CBF) is another method used for studying the mucociliary activity *in-vivo* in humans and in animals and *in-vitro* using respiratory epithelial cells or tissues, excised frog palates, or embryonic chick tracheal tissues (Donovan et al., 1995). CBF has been measured *in-vitro* in a variety of ways by various researchers and these include the use of stroboscopy by Gray, (1930), high-speed cinematographic studies by Proets, (1932), an auditory clicking device by Bleeker et al., (1971) and a photosensitive cell and a microscope with vertical illumination by Dahlman et al., (1962). The Dahlman method gave rise to a photometric technique, which was adapted for direct measurement of CBF on cilia obtained by nasal brushings or biopsy. This technique was found to be quantitatively accurate and reproducible although specialised equipment for data acquisition and analysis was required. Further work using this technique has been published by Yager et al., 1978; Rutland et al., 1980; Smallman et al., 1984; and Hamman, 2001.

In order to make preliminary predictions of the effects of substances on the nasal mucociliary components, not only *in-vitro* CBF studies need to be performed but also *in-vivo* studies on animals, as well such as morphological studies. Various researchers have studied the nasal ciliary ultrastructure using either the scanning electron microscopy (SEM) technique, which normally reveals the surface morphology, or the transmission electron microscopy (TEM) technique, which gives a more detailed cellular structural configuration (Bjork et al 1991; Pereswetoff-Morath et al., 1996; Joki et al., 1998; Ugwoke et al., 2000). Thus both CBF and nasal morphological studies serve as key parameters of the normal mucociliary functioning of the nasal epithelium. *In-vitro* evaluation of ciliary beat frequency of the explants serves as an indicator for the potential damage to the nasal epithelium rather than proof of such damage occurring *in-vivo* (Hermens et al., 1990; Gizurarson et al., 1990). Furthermore, evaluation of the

histopathological changes in the morphology of the nasal epithelium post compound or component exposure would indicate the extent of damage caused (Pereswetoff-Morath et al., 1996).

Although the nasal route has been described as a promising alternative to parenteral administration of therapeutic peptides and proteins as well as drugs used in emergency clinical cases, it has some limitations such as low permeability to large molecular mass and hydrophilic compounds, local enzymatic activity and rapid clearance by the actively beating cilia (Cornaz and Buri, 1994). A number of approaches are being used to counteract the limitations of nasal drug administration. These include: the use of chemical enhancers to improve absorption, incorporation of enzyme inhibitors and increasing the drug local residence time using mucoadhesive polymers (Ugwoke et al., 2000).

Incorporation of mucoadhesive polymers in nasal formulations serves to counteract the rapid nasal clearance of a dosage form within the nasal cavity. Thus it enhances nasal retention time and eventually improves nasal drug absorption (Nagai et al., 1984; Vidgren et al., 1992; Nakamura et al., 1996). However, the incorporation of these compounds into the nasal preparations should not impair the normal functioning of the mucociliary clearance (MCC) system. The components should have minimal or no undesirable effects on the mucociliary system. Nasal absorption enhancement has frequently been associated with tissue incompatibility with the nasal mucosa. These include epithelial necrosis (Chandler et al., 1991; De Fraissinette et al., 1995; Zhou et al., 1996), non-necrotic mucosal inflammation (Bjork et al., 1991; Ugwoke et al., 1999; Pereswetoff-Morath et al., 1996) and cilio inhibition (Romeijn et al., 1996; Aspden et al., 1997; Agu et al., 1999).

The successful use of absorption enhancers depends not only on their absorption enhancement efficacy but also on the safety of the absorption enhancer. The major areas of concern are (Ugwoke et al., 2000):

- Local irritation of the nasal mucosa

- Effect on the mucociliary clearance (MCC)
- Epithelial damage
- Rate of recovery of the damaged mucosa

Due to the potential damage caused by the absorption enhancers it is natural to raise the question of whether the formulation excipients can promote drug absorption through the nasal mucosa without causing any damage (Bjork et al., 1991).

The objective of this study therefore was to perform toxicity investigations on live rats and human nasal epithelium explants, using the study drug (cyclizine HCl) and the possible excipients with mucoadhesive properties for the intranasal preparation. The possible excipients included sodium carboxymethyl cellulose (Na CMC), hydroxypropylmethyl cellulose (HPMC), trimethyl chitosan 36.3% degree of quartenisation (DQ), polysorbate-80 and carbopol 934P.

4.2. Determination of ciliary beat frequency (CBF)

4.2.1 Materials and method

Nasal cytology brushes were purchased from Hobbs Medical Inc, Traunstein, Germany. DMEM cell culture was obtained from Bio-Whittaker, Walkersville, Maryland. Na carboxymethyl cellulose (CMC), hydroxypropylmethyl cellulose (HPMC) 4000, Carbopol 934P and cyclizine HCl were donated by the Institute of Industrial Pharmacy Potchefstroom University for CHE, RSA. Trimethyl chitosan (TMC)36.3% degree of quartenisation (DQ) was synthesised in the Pharmaceutics Laboratory, Pharmaceutics Dept, Potchefstroom University for CHE, RSA. Ciliary beat frequency meter, microscopy slides and a water bath were the properties of the Lung Unit, Pretoria Academic Hospital, Pretoria, RSA. All the excipients were prepared in 0.05M monobasic phosphate buffer solution at pH 6.8.

Before commencement of the CBF studies approval for use of human nasal biopsies was sought from the Ethics Committee of the Potchefstroom University for CHE (Approval number 00M14, appendix 4).

Control batch

Ciliated human nasal epithelia were obtained from the nasal turbine by means of a biopsy. The cell suspension was prepared by shaking the cell bearing cytology brush in DMEM cell culture medium previously maintained at 37°C in a water-bath. About 500µl of the cell suspension was mounted onto the microscopic slide and viewed under the microscope that was coupled to a camera and a ciliary beat frequency (CBF) meter. Frequency measurements of the beating cilia were taken every 5 minutes for 15 minutes and every 15 minutes for 45 minutes and hourly thereafter for 3hours.

Test batch

The effect of varying concentrations of each excipient on the CBF of the ciliated nasal epithelial cells was determined. The concentrations for excipients preparations used were as follows: 0.0625% (w/v), 0.125% (w/v), 0.25% (w/v), 0.5% (w/v) and 1% (w/v) and these were also kept in a water-bath at 37°C.

Procedure

About 500µl of the cell suspension was added to 1ml of the of the test solution. The mixture was shaken to ensure homogeneity and kept in a water-bath at 37°C. About 300µl of the mixture was mounted onto the microscopic slide and viewed under the microscope that was coupled to a camera and a ciliary beat frequency (CBF) meter. Frequency measurements of the beating cilia were taken every 5 minutes for 15 minutes and every 15 minutes for 45 minutes and hourly thereafter for 3 hours.

4.2.2 Morphology studies of the nasal epithelium

Prior to the commencement of the experiments Ethical committee approval for the use of animals for experimental purposes was obtained from the Potchefstroom University for

CHE (Approval number 01D16, appendix 5). The animal experiments adhered to the 1989/90 National code for the handling and use of animals in research, education, diagnosis and testing of drugs and related substances in South Africa.

4.2.2.1 Materials and method

6 Male Sprague-Dawley rats weighing 250 to 300g were bred at the Animal centre, Potchefstroom University for CHE, RSA. Cyclizine HCl powder, hydroxypropylmethyl cellulose, Na carboxymethyl cellulose were all donated by the Institute of Industrial Pharmacy University of Potchefstroom for CHE, RSA. Trimethyl chitosan 36.6% DQ was synthesised in the Pharmaceutics laboratory, Potchefstroom University for CHE, RSA. Normal saline solution, monobasic phosphate buffer solution (pH 6.8) were purchased from BDH RSA. Halothane 2% and 4% were purchased from Safeline Pharmaceuticals, Johannesburg, RSA.

Male Sprague-Dawley rats weighing 250 to 300g were bred and housed at the animal breeding facilities of the laboratory of Potchefstroom University for CHE Animal centre. They were maintained in a light (12 hour light–dark cycle) and temperature controlled environment with free access to water and food. Food was withdrawn approximately 12 hours before the experiments were to be performed.

Control batch

Procedure

6 Sprague-Dawley rats weighing 250 to 300g were anaesthetised with halothane 2% and 4% respectively (see Figure 4.1). About 25µl of the potassium phosphate buffer solution (PBS) at pH 6.8 were instilled into both nasal openings while in the supine position. The rats were then released into a cage with water and food to recover from the anaesthesia. An hour post administration each rat was sacrificed and the nasal septum was removed to expose the nasal epithelium. The treated nasal epithelium was then immersed into a fixative and cut into 1mm to 1.5mm thick sections for the transmission electron microscopy (TEM) procedure.

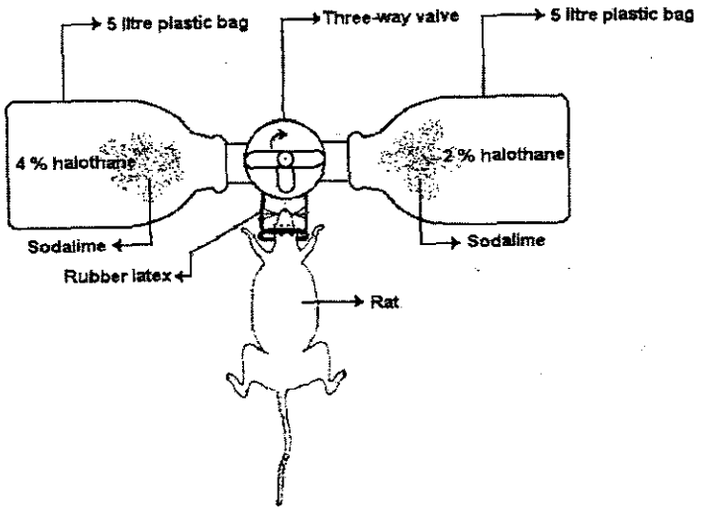


Figure 4.1: Schematic representation of a rat under anaesthesia

Test batch

Procedure

Sprague-Dawley rats weighing 250 to 300g were anaesthetised with halothane 2% and 4% as described before. About 25µl of the test solution at pH 6.8 were instilled into both nasal openings while in supine position. The rats were then released into a cage with water and food to recover from the anaesthesia. An hour post administration each rat was sacrificed and the nasal septum was removed to expose the nasal epithelium. The treated nasal epithelium was then immersed into a fixative and cut into 1mm to 1.5mm thick sections for transmission electron microscopy (TEM) procedure.

4.3 Results and Discussion

4.3.1 Effect of cell culture medium DMEM (pH6.8) on CBF for human nasal epithelia and on morphology of rat nasal epithelium

Control Batch

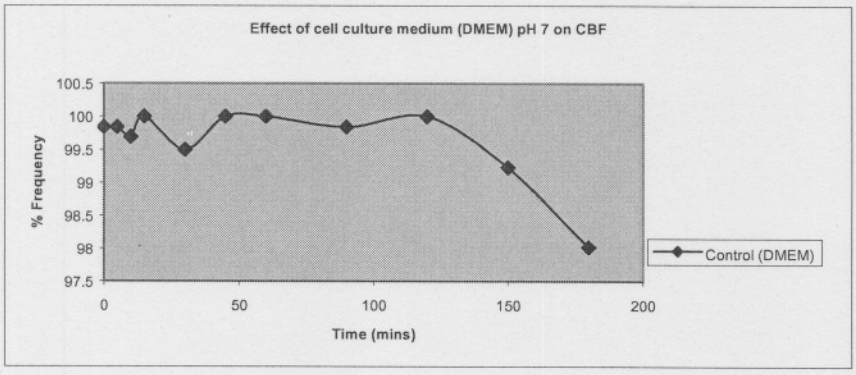


Figure 4.2: Effect of cell culture medium DMEM (pH6.8) on CBF for human nasal epithelia

% frequency is the mean of 5 readings

Control batch:

A mean maximum CBF of 13.14 Hz was observed. This frequency was maintained for approximately 3 hours as indicated in the graph above. According to Wilson et al., (1986); Sykes et al., (1987); Nuutinen et al., (1993) this frequency falls within the ideal range of the normal functioning of cilia.

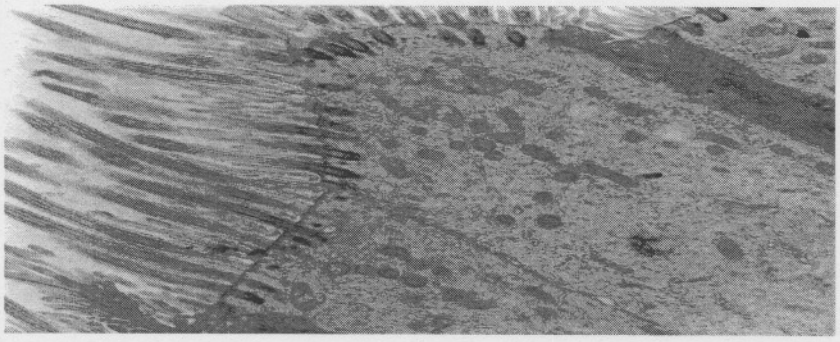


Figure 4.3: TEM micrograph (magnification x8900) of rat nasal epithelium in PBS pH 6.8

Visual assessment of the cell monolayer revealed no structural or morphological changes indicating that no apparent cell damage was caused by the solution. Assessment of the effects of physiologic and hypertonic saline solutions on ciliary activity *in-vitro* by Boek et al., (1999) demonstrated that physiologic saline decreases CBF *in-vitro*. This indicates that saline may impair mucociliary clearance *in-vivo* as well. This effect would be highly undesirable in therapeutic situations. Notwithstanding this possibility, positive results of irrigation with physiologic saline have been well documented (Mejima et al., 1989). It is assumed that the benefits come from evacuating debris and pus rather than from enhancing or protecting the mucociliary transport. These irrigations might have a more beneficial result when a solution without ciliostatic effects is used. This frequency was therefore used as a reference for all CBF measurements performed using this cell suspension.

4.3.2 Effects of cyclizine HCl on CBF of human nasal epithelium explants and on the morphology of the rat nasal epithelia

Immersion of the nasal explants in cyclizine HCl solution at its normal acidic pH of 4.5, caused a decay in CBF. Ciliary motility ceased abruptly. In less than 5 minutes after immersion no movement was observed. Van de Donk et al., (1980) made similar observations using the CBF of chicken embryo trachea at varying pH levels. The values lower than pH 6 and higher or equal to pH 11 resulted in severe decrease in CBF while the highest CBF was recorded between pH 7 and pH 10. At pH 6 there was a decrease of about 20 %. Meinesz et al., (1998) also observed a similar trend of the external pH effect on the CBF of the human bronchial cells. Results indicated ciliary arrest at pH values lower than 6 with irreversible cellular damage i.e. cells showed deleterious effects too fast to allow any CBF) measurements. At pH 11 ciliary motility was observed for the 1st five minutes due to gradual damage. At pH 7 cells remained stable and normal CBF was recorded. Furthermore, studies by Washington et al., (2000) revealed that baseline nasal pH was 6.4 in the anterior of the nose and 6.27 in the posterior of the nose. These findings were in agreement with previous studies showing the pH of the nasal secretions in healthy adults and children to be 6.4 (Chien et al., 1989).

Based on the evidence and observations made, subsequent CBF readings were therefore conducted at nasal physiologic pH 6.8 to pH 7.

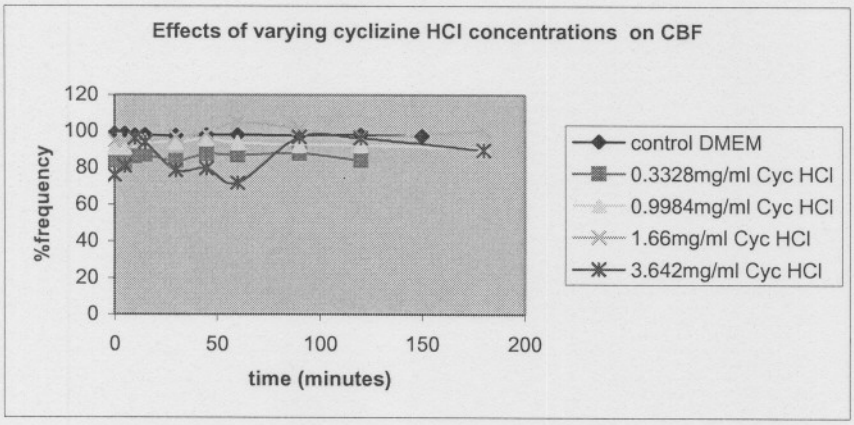


Figure 4.4: Effects of varying concentrations of cyclizine HCl pH 6.8 on CBF of human nasal explants

*** % frequency is the mean of 5 readings**

CBF profiles for the effects of varying concentrations of cyclizine HCl at pH6.8 indicate an overall 20% decline for all drug concentrations with the1.66mg/ml and 0.9984mg/ml drug solutions having almost 5% decline in CBF. Results obtained demonstrate that CBF measured in cyclizine HCl solutions at pH 6.8 is not concentration dependent. Thus cyclizine HCl at pH 6.8 does not inhibit nor stimulate ciliary motility and was shown to be cilio friendly. Thus frequency results were almost most similar to the control and maintained for the entire experimental period. The results indicate that the optimum pH for an intranasal formulation of cyclizine HCl will be 6.8. It is of importance, therefore, to note that the functioning of the mucociliary clearance (MCC) system will not be inhibited by the presence of cyclizine HCl at this pH.

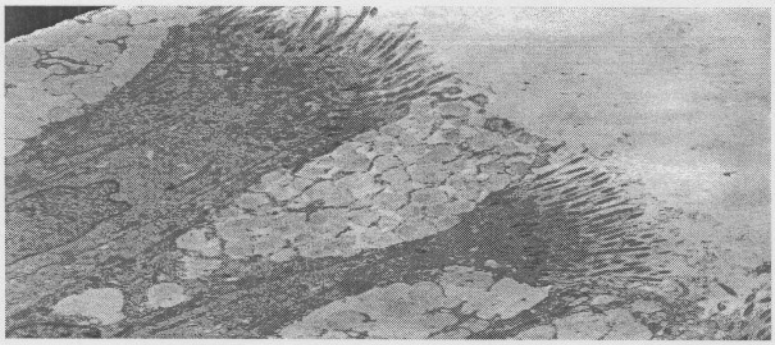


Figure 4.5: TEM micrograph (magnification x2950) of rat nasal epithelium in 1.66mg/ml cyclizine HCl solution

Visual assessment of the transmission electron microscopy (TEM) micrograph did not depict any structural damage nor distortion to the cell. The cellular components maintained their integrity, which was indicative of the cellular tolerance to the drug (cyclizine HCl) at pH 6.8.

4.3.3 Effects of cellulose derivatives on CBF and nasal morphology at varying concentration levels

4.3.3.1 Effects of carboxymethyl cellulose (CMC) on CBF and nasal morphology at varying concentration levels

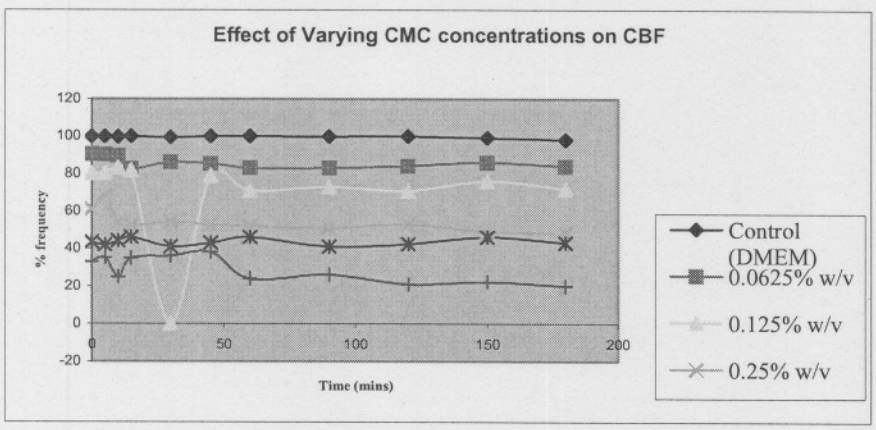


Figure 4.6: Effects of Na carboxymethyl cellulose (CMC) (pH 6.8) at varying concentrations on CBF for human nasal epithelia

*** % frequency is the mean of 5 readings**

The effect of CMC on the CBF was concentration dependent. The higher the concentration, the lower the CBF readings recorded. The results in Figure 4.6 show that at 0.0625% (w/v), there was a mean decrease CBF of 10% although the trend in CMC was similar to the control. At higher viscosities (0.125%(w/v) to 0.25%(w/v)), there was a mild to moderate cilio-inhibition reflected by the decrease in ciliary beat frequency (20% to 40% decrease). At 0.5%(w/v) to 1%(w/v), a marked (60% to 80%) decrease in CBF was observed due to the increase in mechanical hindrance of the solutions with the higher viscosities.

Apart from the viscosity it is possible that other factors like osmotic pressure and polymer adhesive properties may have also contributed to ciliary movement inhibition. An explanation for the ciliostasis by the Na-CMC solutions might be due to the hypo osmotic nature of the system. The osmolarity of the Na-CMC solution is close to that of pure water and cellular exposure caused swelling and disruption of the cell components resulting in a decrease in the ciliary movement. Van de Donk et al., (1980) also made similar observations on the effects of pH and osmolarity on ciliary movement. Studies by Van de Donk et al.,(1980) also showed that the viability of explants depended on salts and glucose in isotonic solutions. Hypo osmotic solutions without glucose decrease ciliary movement. Furthermore, the mucosal dehydration due to water absorption by the polymer for its swelling may be responsible for the observed inflammatory reactions (Bjork et al., 1991).

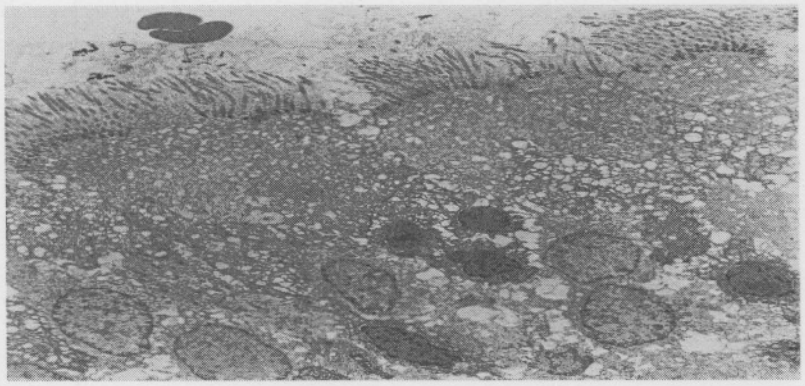


Figure 4.7: TEM micrographs (magnification x2200) of rat nasal epithelium in 1% (w/v) Na-CMC solution pH 6.8

Assessment of the morphological changes of the nasal epithelium by use of TEM after Na-CMC solution exposure, revealed swollen cells which may cause a decline in their beating frequency. The TEM micrograph also revealed no ciliary erosion, no cases of epithelial necrosis that may lead to the loss of both the ciliated and non-ciliated columnar cells, goblet cells and/or basal cells.

Studies by Ugwoke et al., (2000) on the toxicological effects of Na-CMC on CBF of human nasal epithelial cells in primary suspension culture and *in-vivo* in rabbit nasal mucosa also indicated a concentration and time-dependent inhibitory effect on CBF. Thus, the effect on CBF obtained with a 1%(w/v) dispersion was found to be moderate after 15 and 60 minutes, while a 0.25%(w/v) dispersion showed no inhibition after 15 minutes and, mild inhibition after 60 minutes. Furthermore, the cilio-inhibition observed with the 0.1%(w/v) dispersion was partially reversible while washing had no effect on cilio-inhibition caused by the 0.25%(w/v) dispersion. The reversibility of the 1%(w/v) dispersion was not ascertained because of the viscosity. Likewise, at low concentrations of Na-CMC where the dispersion has more free chains available for adhesion, no ciliary motility inhibition was observed. As the concentration of the dispersion increased and hence a gradual onset of adhesion, cilio-inhibition gradually sets in and at high viscosity the dispersion physically prevented ciliary beating due to the mechanical interference with the polymer chains (Ugwoke et al., 2000).

Similar observations were also made by Ugwoke et al., (1996) in a study on the effect of Na-CMC and apomorphine on the CBF of human nasal epithelium in a primary suspension culture and *in-vivo* on rabbit nasal mucosa. Ugwoke et al., (2000) observed some inflammation which may be attributed to local dehydration caused by the polymer during swelling and adhesion as suggested by Bjork et al., (1991). Otherwise no gross necrosis or change in the architecture of the mucosa was observed by Ugwoke et al.,(2000).

4.3.3.2 Effects of various concentrations hydroxypropyl methyl cellulose (HPMC) on CBF and nasal morphology

No documented information on the effects of hydroxypropyl methyl cellulose (HPMC) on CBF and morphology studies of the nasal epithelium explants could be found.

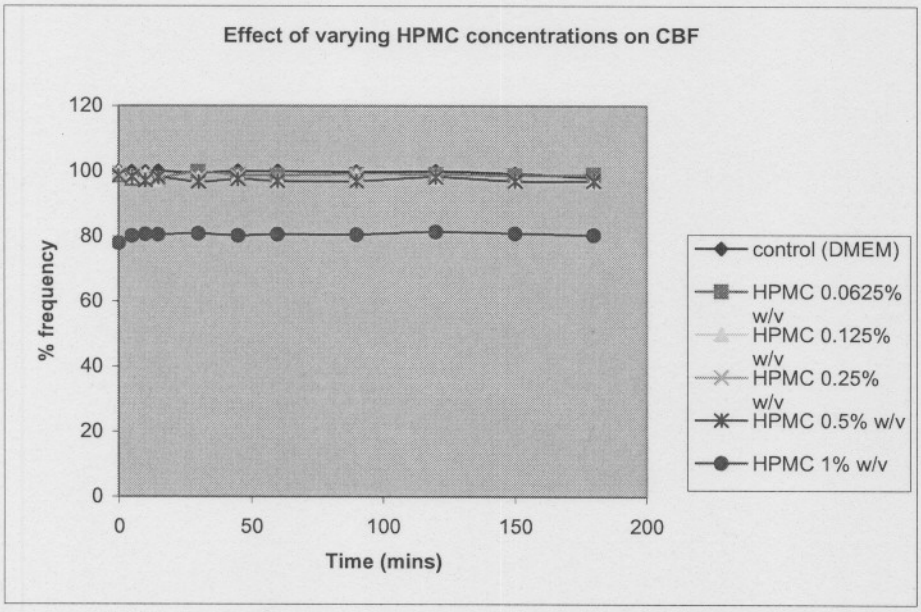


Figure 4.8: Effect of varying concentrations of HPMC pH 6.8 on CBF

*** % frequency is the mean of 5 readings**

As is the case with other cellulose derivatives, the effect of hydroxypropyl methyl cellulose (HPMC) on CBF was found to be both concentration and time-dependent. Thus

there was a slight decrease in the CBF recording as the viscosity of the excipients increased. Dispersions in the 0.0625%(w/v) to 0.5%(w/v) concentration range produced ciliary movement almost equivalent to the control. There was a mild decrease (less than 10%) in ciliary motility. At the highest concentration (1%(w/v)) a 15% decrease was observed but this was maintained throughout the experimental period. This was probably due to the mechanical hindrance that the beating cilia had to move against. Although the trend in ciliary movement was also concentration dependent, the highest concentration caused a slight decrease, which was within the acceptable range.

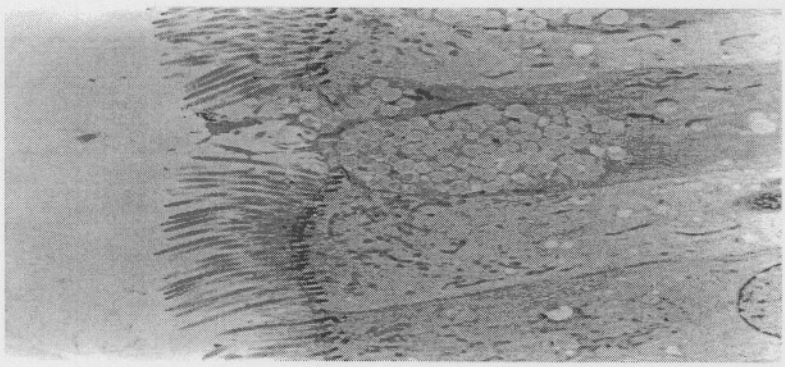


Figure 4.9: TEM micrograph (magnification x2950) of rat nasal epithelium in HPMC pH 6.8

Assessment of the TEM micrographs post-nasal epithelia treatment did not depict any significant morphological changes. Thus the cell monolayer remained intact throughout the entire experimental period.

4.3.4 Effects of polyacrylic acids on CBF and nasal morphology at varying concentration levels

4.3.4.1 Effects of Carbopol 934P on CBF and nasal morphology at varying concentration levels

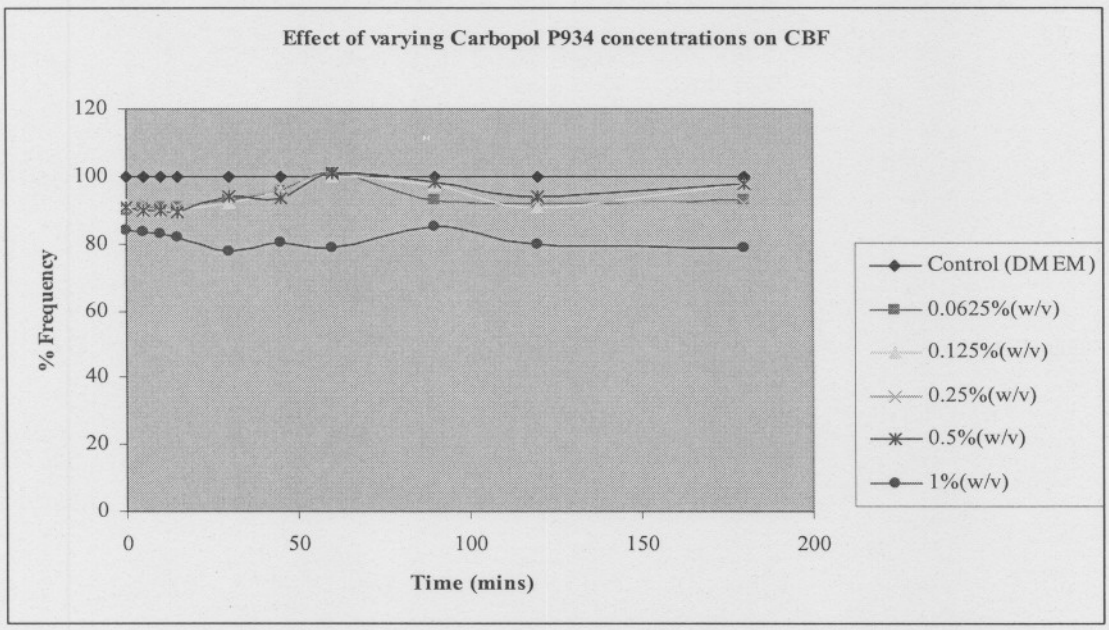


Figure 4.10: Effects of varying concentrations of Carbopol 934P on CBF of nasal human explants

*** % frequency is the mean of 5 readings**

The ciliary movement as depicted by the profiles the graph in Figure 4.10, was found to be concentration dependent. At 0.0625%(w/v) to 0.25%(w/v) concentration range, ciliary movement was almost equivalent to the control. At 0.5%(w/v) concentration a moderate decrease in ciliary movement was observed while at 1%(w/v) only 8% decrease in ciliary movement was recorded. As the concentration of the mucoadhesive increased there was a gradual increase in the viscosity of the solution. Thus the increase in viscosity was inversely proportional to the CBF i.e. as the viscosity increased there was a gradual decrease in the frequency recorded due to the mechanical interference which the beating cilia has to move against. At the highest viscosity, CBF was at its lowest since there existed physical inhibition of ciliary movement. The high viscosity dispersion physically

prevented ciliary movement. Thus although the trend in ciliary motility was found to be concentration dependent, the highest polymer concentration recorded CBF that was within the acceptable range of approximately 93%.

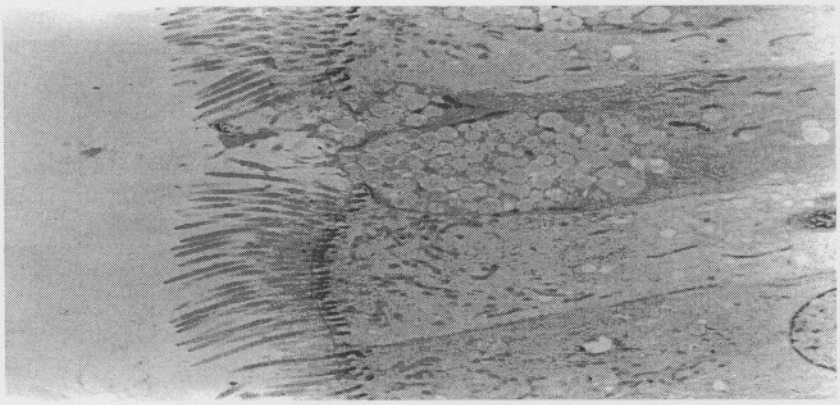


Figure 4.11: TEM micrograph (magnification x1650) of rat nasal epithelium in 1% (w/v) Carbopol 934P pH 6.8

Assessment of the morphology of the nasal epithelia as depicted the by TEM micrograph revealed no necrosis or change in the architecture of the nasal mucosa, reflective of better tolerance of the cell components to the excipients.

Studies conducted by Ugwoke et al., (2000) on the effects of carbopol 974P on CBF and nasal morphology also indicated both a concentration and time dependent inhibitory effect. The inhibitory effect on CBF obtained with 1%(w/v) dispersion was mild after 15 minutes and moderate after 60 minutes while 0.25%(w/v) showed no inhibitory effect after 15 minutes and mild inhibition after 60 minutes. With the 0.1%(w/v) concentration, no inhibitory effect was observed after both time intervals (15 and 60 minutes). The cilio-inhibition observed with 0.25%(w/v) dispersion was partially reversible while reversibility with the 1%(w/v) dispersion was not ascertained due to the inseparable cells from the viscous gel. Cellular ultra-structure remained intact, thus there was no gross necrosis or change in the architecture of the mucosa.

Although Callens et al., (1996) during a study on the toxicological evaluation of a bioadhesive nasal powder containing starch and carbopol 974P on rabbit nasal mucosa and snail mucosa observed that 10% unneutralised pure carbopol 974P induced mucosal irritation to the snails, as reflected by the increased mucus secretion, decrease in body weight and a significant protein release; these results were comparable to the control group. It was therefore concluded that carbopol 974P at 10% did not have any damaging effects on the snail foot.

Furthermore, studies by Ugwoke et al., (2000) on the effects of carbopol 971P on CBF of human nasal primary cell culture and *in-vivo* on rabbit nasal mucosa; indicated that the absence of microbial invasion of the nasal mucosa or mucosal necrosis implied that carbopol 971P while being inflammatory to the nasal mucosa was better tolerated than other chemical absorption enhancers which caused mucosal structural damage.

4.3.5 Effects of chitosan derivative (Trimethyl Chitosan 36.3% DQ) on the CBF and morphology of the nasal epithelia at varying concentration levels

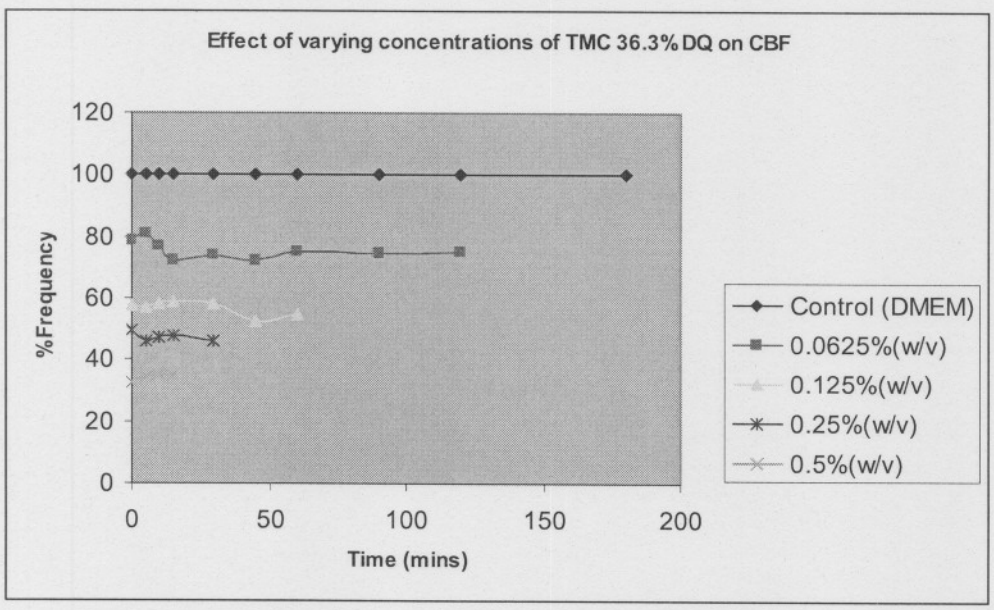


Figure 4.12: Effects of varying concentrations of TMC 36.3% DQ pH 6.8 on CBF of human nasal explants

*** % frequency is the mean of 5 readings**

Visual assessment of the cell monolayer on the monitor depicted definite morphological changes. Cells were gradually swelling and ciliary movement was also slowing down. Marked concentration dependence trend was observed as depicted by the profiles in Figure 4.12. At 0.0625%(w/v) concentration a 20% to 30% decrease in ciliary movement was observed which was maintained for three hours while at 0.25%(w/v), 55% to 60% decrease in ciliary movement was noted and which lasted for less than 40 minutes of the experimental period. Thus as the TMC solution concentration increased, there was a gradual increase in the viscosity of the test solution and a proportional decline in the ciliary movement was observed. The degree of cell inflammation also intensified with an increase in the excipients concentration. The trend observed could be due to the difference in osmotic pressure of the cell contents and the surrounding medium. In this case the potential increase in osmotic pressure within the cell structure impeded the ciliary movement hence the low frequency recorded. Thus in this case, the viscosity and osmolarity of the surrounding medium played a significant role in the decrease in CBF. Moreover, chitosan derivatives at high temperatures possess high flexibility giving enhanced penetration of the mucin network (Soane et al., 1999).

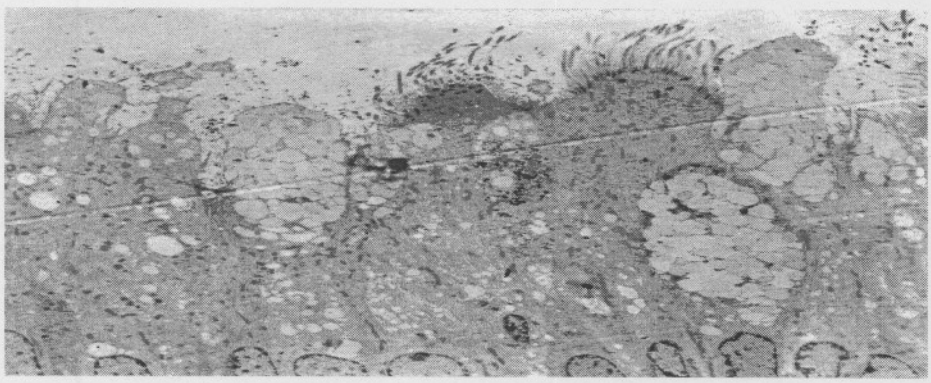


Figure 4.13: TEM micrograph (magnification x3900) of rat nasal epithelium in 0.5% (w/v) TMC 36.3 %DQ pH 6.8

Morphological studies conducted, using TEM, revealed complete ciliary erosion and inflammation of the cell components. The low frequency recordings were therefore

justifiable by the gradual erosion of the cilia with exposure time and a gradual water influx from the solution into the cells. Thus findings were not in accordance with the tonicity phenomenon for it is expected that for hypertonic solution like TMC, water movement should be from a region of low solute concentration to a region of high solute concentration resulting in cell shrinkage not cell inflation as was observed. Literature for the physico chemical properties of the parent compound chitosan, reports this compound to be neutral in nature but the derivative is said to be acidic due to the hydrogen iodide formed as a by product during synthesis. TMC acquired its hypertonicity from the NaCl that was added to the polymer solution to neutralize and displace the iodide ions in the molecular structure.

On the contrary, Tengamnuay et al., (2000) during a study on the enhancing effects of chitosan and its derivatives on intranasal absorption, did not observe any signs such as appearance of epithelial necrosis, sloughing of epithelial cells, nor haemorrhage. All morphological changes observed were reversible despite the high polymer concentration of 1%(w/v).

4.3.6 Effects of surfactants on the morphology of the rat nasal epithelia

4.3.6.1 Effects of polysorbate-80 on the morphology of the rat nasal epithelia

Figure 4.14: TEM micrograph (magnification x1650) of rat nasal epithelium in 2% (w/v) Polysorbate-80 pH 6.8

The TEM micrograph of the rat nasal epithelium exposed to a 2%(w/v) polysorbate-80 solution (Figure 4.14) revealed severe ultrastructural damage. Epithelium disruption, cell loss and subsequent reduction in the monolayer height and total cilia erosion were observed. These reactions are typical of most surfactants. Polysorbate-80 is a cationic surfactant. Like all surfactants or detergents, it interacts with the membrane components (phospholipids and proteins) by solubilising them and thereby increasing the permeability of the cell membrane. This reaction clearly has an irreversible damaging effect on the cell membrane and cell components.

Studies by Chandler et al., (1991) on the histological effects of nasal formulations on the rat nasal mucosa revealed that nasal epithelium exposed to a 1%(w/v) laureth-9 solution, exhibited sites of interaction including epithelium disruption, cell loss and subsequent reduction in epithelium height. In some regions of the nasal cavity complete loss of epithelium occurred and large quantities of free mucus were observed free in the nasal cavity.

Similar observations on the histological effects of some surfactants especially 1% (w/v) laureth-9 solution on the nasal epithelium have been reported. Lee et al., (1988) described cellular erosion, cell-cell separation, dense mucous coating and loss of cilia from rat nasal mucosa 5 minutes post administration of 1%(w/v) laureth-9 solution intranasally. Daugherty et al., (1988) also noted similar destructive effects of this surfactant 30 minutes and 24 hours post nasal dosing. Thus 1%(w/v) laureth-9 was found to be an effective irritant of the rat nasal mucosa with apparent changes in agreement with published reports. Studies by Ennis et al., (1990) revealed that following a 15 minute exposure to selected absorption enhancers (bile salts and laureth-9), which act as surfactants, severe erosion of the nasal mucosa occurred. This was characterised by total loss of cellular and ciliary identity as well as prevalence of extracellular debris. The increasing order of morphological damage resulting from a 5 minute exposure to each enhancer was as follows: 0.5% solulan C-24 $\approx$ 0.5% solulan C-24/0 < 5% Na tauro-24, 25-dihydrofusidate (STDHF) < 0.5% Na tauro-24, 25-dihydrofusidate (STDHF) < 1% Na tauro-24, 25-dihydrofusidate (STDHF) < 1% laureth-9 < 1% Na tauro deoxycholate $\approx$ 1%

Na deoxycholate. It has been postulated that the enhancing effect of the bile salts and these surfactants was due to their ability to erode the epithelial cells thus permanently alter the structure of the mucosal membrane (Ennis et al., 1990). Furthermore, it has been frequently observed that an increase in drug permeability in the presence of different surfactants is invariably accompanied by structural changes in the mucosal membranes (Attwood et al., 1983). Generally, for the surfactants there exists a good correlation between the absorption promoting effects of the surfactants and structural damage (Hirai et al., 1981).

Due to the structural damage and the consequent reduction of the nasal monolayers height, as can be seen by the effect of polysorbate-80, nasal absorption post treatment increased four fold as indicated by Adao et al., (2001) in the study on the influence of change in concentration of methyl cellulose and polysorbate-80 on the bioavailability of metroprolol following intranasal administration. Studies by Mitra et al., (2000) also indicated enhanced nasal absorption of insulin with the aid of polysorbate-80. Thus increased nasal absorption efficacy can be associated with severe and irreversible nasal epithelial damage. Tissue injuries generally cause release of increased quantities of histamine, bradykinnin and serotonin into the inflammed tissue. These in turn cause increased local blood flow and increased capillary permeability (Guyton, 1981).

To highlight the close relationship between tissue damage and absorption enhancement, Chandler et al., (1991) found that a ranked order for increased absorption with the use of surfactants as enhancers for insulin in rats was laureth-9 > lysophosphatydilcholine = STDHF > diethylaminoethyl-dextran > no enhancer. This did not correlate well with the histological damages the surfactants elicited. The ranked order with regards to epithelial damage was laureth-9 > lysophosphatydilcholine > STDHF > diethylaminoethyl-dextran > no enhancer. Other researchers viz. Chandler et al., (1994); Donovan et al., (1990) and Ennis et al., (1990) also observed a similar trend and or relationship for enhanced absorption and severe tissue damage.

4.4 Conclusions

The effects of formulation excipients and drugs on the CBF detected *in-vitro* may be more pronounced than the influence on the mucociliary clearance *in-vivo*. *In-vitro* experiments expose the ciliated nasal epithelia directly to the test solution, whereas in the *in-vivo* situation the cilia are protected by mucus. Furthermore, a solution given intra-nasally will be diluted by the mucus, giving lower concentrations of potentially toxic agents, which, will be cleared from the nasal cavity by the MCC system at intervals of no more than 20 minutes.

Based on the CBF results and nasal epithelium morphology studies of the excipients investigated, the following conclusions were drawn.

Cellulose derivatives

Na carboxymethyl cellulose (CMC)

Na carboxymethyl cellulose (CMC) showed some negative effect on the cell monolayer due to changes in osmotic pressure within the cells. This excipient would therefore not be recommended.

Hydroxypropylmethyl cellulose

CBF results were almost comparable to the control indicating cilio-friendliness of the excipient. An assessment of the morphology of the cell monolayer ultrastructure using the TEM technique revealed no histopathological or morphological changes i.e. there were no significant changes observed after exposure of the tissue to the excipients solution. Thus the cell monolayer maintained its integrity and was almost similar to the control. This therefore indicated that this excipient had no apparent harmful effects on the nasal cell monolayer. High viscosity solutions are not recommendable for use in nasal formulation but overall was found to be better tolerated by the cells and hence its incorporation into nasal formulations is recommended.

Polyacrylic Acids (Carbopol 934P)

Although assessment of the morphology of the cell monolayer post treatment with this compound revealed no cellular ultrastructural damage indicative of cellular components tolerance and no negative effect on ciliary movement, its use as an excipient requires neutralisation with sodium hydroxide which may increase the pH of the solution compound rendering it unsuitable for use intranasally where pH may play a significant role in ciliary movement. Cilia are pH sensitive. Slight pH variation has a marked negative effect on the ciliary movement, thus all compounds for nasal use should be administered at pH 6.8 (physiological pH for the nose).

Chitosan derivatives (trimethyl chitosan 36.3%DQ)

The decline in CBF and the damaging effects of this compound as depicted in the micrographs do not warrant its incorporation into any nasal formulation. It is clear that this compound may cause irreversible damage to the nasal and hence its elimination from the list of possible mucoadhesives for nasal delivery systems.

Surfactants (polysorbate-80)

It is evident that although polysorbate-80 showed excellent absorption enhancement capabilities, it has irreversible damaging effects on the nasal mucosa as depicted in the TEM micrographs. The efficacy of the nasal absorption enhancers should not only be based on its ability to increase permeability and hence absorption but also its histo-pathological effects should be assessed. Thus in the development of a nasal formulation the risk: benefit ratio for the use of such excipients should be weighed.

4.5 Formulation of cyclizine lactate intranasal preparation 125mg/ml (w/v)

4.5.1 Materials

Cyclizine lactate powder was synthesised in the pharmaceutics laboratory, Potchefstroom University for CHE, RSA. Hydroxypropylmethyl cellulose (HPMC) 4000 was donated

by the Institute of Industrial Pharmacy, Potchefstroom University for CHE, RSA. Normal saline was purchased from the local pharmacy.

4.5.1.1 Method of preparation

For 10ml volume:

1253mg of cyclizine lactate were dissolved in 2ml warm normal saline solution and the mixture was kept warm at a temperature of about 30°C. To this, 80.8mg of hydroxypropylmethyl cellulose (HPMC) 4000, which was previously dissolved in 8ml of warm normal saline solution, was gradually added and the mixture stirred continuously with the aid of a magnetic stirrer for approximately 45 minutes.

The viscosity of the resulting gel and the assay were determined.

4.5.2 Determination of the viscosity of the dispersions

4.5.2.1 Introduction

As the viscosity may have an influence on the applicability and or ejection of the preparation through the packaging device orifice, dosage form deposition and distribution within the nasal passages and extent and rate of drug absorption; hydroxypropylmethyl cellulose (HPMC) 4000 dispersions with varying concentrations were prepared and the viscosity determined.

4.5.2.2 Materials and method

Hydroxypropylmethyl cellulose (HPMC) 4000 was donated by the Institute of Industrial Pharmacy, University of Potchefstoom for CHE, RSA. Brookfield DV-II+ viscometer (Brookfield, Stoughton, USA) was used for viscosity determinations.

Hydroxypropylmethyl cellulose (HPMC) 4000 dispersions of concentrations 0.4%(w/v), 0.6%(w/v) and 0.8%(w/v) were prepared by dissolving the corresponding amount of hydroxypropylmethyl cellulose (HPMC) 4000 powder in 600ml of water and stirring overnight till a homogeneous dispersion was obtained. Readings were taken at rotational

speeds of 10, 20, 30, 50, 60 and 100rpm for each dispersion with a 5 minutes time interval between each reading to allow stabilisation of the measurements. The viscosity was determined using spindle sizes L2 for the 0.8%w/v dispersion and L3 for the 0.4%(w/v) and 0.6%(w/v) dispersions at room temperature (20.3°C).

4.5.2.3 Results and Discussion

Viscosity determination of cyclizine lactate gel

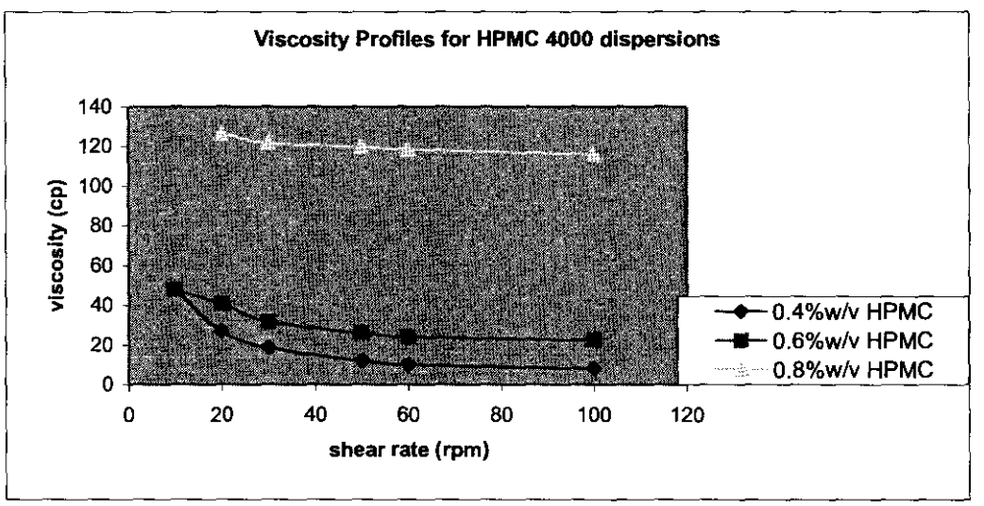


Figure 4.15: Viscosity profiles of the hydroxypropylmethyl cellulose (HPMC) 4000 dispersions at varying concentrations

The viscosity of the HPMC 4000 dispersions was expressed in terms of flow curves of viscosity versus shear rate. The viscosity profiles in Figure 4.15 for all the dispersions showed a similar trend irrespective of the concentration of the dispersion. A decrease in viscosity with an increase in shear rate was observed. The dispersion with the highest concentration (0.8%(w/v)) exhibited the highest viscosity. The 0.6%(w/v) dispersion showed a medium viscosity comparatively and the dispersion with the lowest concentration (0.4%(w/v)) exhibited the lowest viscosity as expected.

Dispersions of HPMC 4000 are non-Newtonian liquids and exhibit shear thinning or pseudoplasticity properties. For non-Newtonian dispersions viscosity is not directly proportional to the shear rate i.e. viscosity changes with the change in shear rate. These dispersions also exhibited the rheological behaviour known as shear thinning, whereby the slope of the curve i.e. the viscosity is high initially and decreases with an increase in the shear rate (Radebaugh et al., 1996). The effect of a sweep in shear rate from high to low gave a hysteresis loop, indicating a thixotropic character. Nonetheless, there is no documented information on the shear rate generated by the spray pump during actuation. However, it may be assumed that the thixotropic nature of HPMC 4000 would mean that the shear rate during actuation of the pump would have little effect on the viscosity of the dispersion in the nasal cavity. Furthermore, it is possible that viscosity may influence the type and size of micelles, which may be responsible for possible drug entrapment (Harris et al., 1988).

Moreover, determination of the viscosity of the preparation is considered of utmost importance for the following reasons: applicability and or ejection of the preparation through the packaging device orifice, dosage form deposition and distribution within the nasal cavity and extent and rate of drug absorption. Although the many advantages of the nasal route have drawn a lot of attention, MCC imposes a time constraint upon contact between the applied formulation and the absorptive surface. Drug absorption can only occur while the drug is retained at the site of absorption, and the time allowed for this is dictated by the rate of mucus transport (Lansley, 1993). This time constraint can be overcome by increasing the drug retention time within the absorptive mucosa or delaying the clearance rate by increasing the viscosity of the dosage form or addition of a mucoadhesive agent. Pennington et al., (1988) in the study on the influence of solution viscosity on nasal spray deposition and clearance, found that the clearance rate of colloidal solutions from the nasal cavity decreased with an increase in the viscosity.

For this study, drug retention time would be increased by increasing the viscosity of the dosage form. Dispersions of varying viscosity were prepared to select the optimal one with regards to applicability and or ease of ejection from the packaging device. Note

should be taken that there exists a correlation between applicability/ease of ejection, deposition and or distribution and extent and rate of absorption.

4.6 Assessment of deposition and distribution patterns of pump spray device

4.6.1 Rationale for Assessment of deposition and distribution patterns of pump spray device

Studies by various researchers have indicated a direct and proportional relationship between dosage form deposition and distribution patterns within the nasal passages and the absorption rate (Thorssons et al., 1999). Metered dose pump sprays normally deposit in a comparatively small area in the anterior part with squamous and transitional non-ciliated epithelium and where there is relatively thick mucosal membrane (Newman et al., 1987). Pump sprays deposit in the turbinates covered by respiratory epithelium, which are the primary sites for systemic absorption of nasally administered drugs (Newman et al., 1987).

4.6.2 Materials

The model of the lateral cross section of the nose was loaned from the Anatomy Department, School of Nursing, Potchefstroom University for CHE, RSA. 10ml metered dose pump spray devices and spray bottles were supplied by Adcock-Ingram Co, Johannesburg, RSA. Hydroxypropylmethyl cellulose (HPMC) 4000, Whatman filter paper size 1 (9cm diameter), blotting paper with an outline of the human face, polytops, orange G colorant were all donated by the Institute of Industrial Pharmacy, Potchefstroom University for CHE, RSA.

4.6.3 Determination of dose per ejection from the pump spray device

4.6.3.1 Method

Reference

12 polytops were weighed and water from the 10ml pump spray device was squeezed into each one of them. The weight of the contents per ejection was determined. The volume per ejection from the pump spray device was determined using the density of water.

Test product

Similarly 12 polytops were weighed and 0.4%(w/v) and 0.6%(w/v) HPMC 4000 dispersions from the 10ml pump spray device was squeezed into each one of them. The weight of the contents per ejection was determined.

4.6.3.2 Results and Discussion

The weight of water and the HPMC 4000 dispersions 0.4%(w/v) and 0.6%(w/v) per ejection from the pump spray device were recorded, the average weight, standard deviation and percentage RSD were calculated. Results obtained are presented in tables 4.1, 4.2 and 4.3 respectively.

Table. 4.1: Weight of water per ejection from the pump spray device

Weight (mg)	49.9	53.3	54.0	53.2	52.3	52.2	49.1	52.1	52.1	51.9	52.3	52.(
Average	52.03											
SD	1.36											
%RSD	2.6											

Table. 4.2: Weight of 0.4%(w/v) HPMC 4000 dispersion per ejection from the pump spray device

Weight (mg)	55.6	55.3	54.2	55.2	56.0	56.0	55.9	55.3	54.9	53.8	55.6	54.8
Average	55.22											
SD	0.695											
%RSD	1.26											

Table. 4.3: Weight of 0.6%(w/v) HPMC) 4000 dispersion per ejection from the pump spray device

Weight (mg)	49.6	54.9	54.9	53.9	54.9	54.7	53.9	53.9	53.9	53.8	53.7	53.9
Average	53.83											
SD	1.42											
%RSD	2.6											

According to the results obtained as reflected in tables 4.1, 4.2 and 4.3, weight per ejection from the pump spray device was found to be reproducible. It is of importance to note that prior priming (4 to 5 actuations) of the device was necessary to achieve a constant weight. Pennington et al., (1988) observed that priming of the insufflator required 6 actuations to achieve a constant ejection volume of 0.13 ± 0.01 ml with water from a nasal spray.

Delivery of this device yielded an accuracy of 49.1mg to 54.0mg and a precision of 2.6% for water, which was used as reference product. For the HPMC 4000, accuracy yielded was 53.8mg to 56.0mg; 49.6mg to 54.9mg and precision of 1.26%; 2.64% respectively.

From the results obtained it was observed that addition of HPMC to water increased the weight per ejection from the pump spray. The size of the pump spray orifice also determines the amount of HPMC dispersion ejected.The thicker the dispersion the bigger the miscelle hence the more difficult the passage through the orifice resulting in less

dispersion being ejected from the pump spray. Thus less maount of the 0.6%w/v of the HPMC dispersion was ejected as compared with the 0.4%w/v dispersion.

The average weight of water as calculated in table 4.1 was used to calculate the volume of water per ejection from the pump spray device using the density of water.

Thus $\rho = \frac{m}{v}$ Equation 4.1

Where:

M = mass

V = volume

Therefore volume of water per ejection from the pump spray was 52.03µl.

4.6.4 Distribution pattern assessment

Method

Reference product

The pump spray device was filled with water coloured with orange G. The coloured water from the spray pump device was then squeezed onto the Whatman filter paper that was placed about 2cm above the device. The distribution pattern was determined by demarcating the orange coloured area and measuring the diameter of each blot. 10 replicates were performed.

Test product

The spray pump device was filled with HPMC 4000 dispersion coloured with orange G. The coloured HPMC 4000 dispersion from the spray pump device was then squeezed onto the Whatman filter paper that was placed about 2cm above the device. The distribution pattern was determined by demarcating the orange coloured area and measuring the diameter of each blot. 10 replicates were performed.

4.6.4.1 Results and Discussion

The diameters of the blot per ejection formed by the pump spray mist of water and the HPMC) 4000 dispersions 0.4%(w/v) and 0.6%(w/v) were measured. The average diameter, standard deviation and percentage RSD were calculated and presented in tables 4.4, 4.5 and 4.6 respectively.

Table. 4.4: Diameter of water blot per ejection from the pump spray device

Diameter (cm)	3.8	4.2	4.3	4.7	4.3	4.7	4.5	4.7	4.5	4.5
Average	4.42									
SD	0.282									
%RSD	6.38									

Table. 4.5: Diameter of the 0.4%(w/v) HPMC 4000 blot per ejection from the pump spray device

Diameter (cm)	2.6	2.7	2.7	2.5	2.4	2.5	2.8	2.6	2.6	2.7
Average	2.61									
SD	0.12									
%RSD	4.59									

Table. 4.6: Diameter of the 0.6%(w/v) HPMC cellulose 4000 blot per ejection from the pump spray device

Diameter (cm)	1.9	2	1.9	2	2	1.9	2	2.3	2	2
Average	2									
SD	0.12									
%RSD	5.83									

According to the results obtained as reflected in tables 4.4, 4.5 and 4.6, diameter per ejection from the pump spray device was found to be reproducible. Delivery of this device yielded an accuracy of 3.8cm to 4.7cm and a precision of 6.38% for water, which

was used as reference product. For the HPMC 4000 dispersions 0.4%(w/v) and 0.6%(w/v), accuracy yielded was 2.4cm to 2.8cm; 1.9cm to 2.3cm and a precision of 4.59%; 5.83% respectively.

From the results obtained it was observed that comparatively as the viscosity of the dispersion increased there was a proportional decrease in the diameter formed by the spray mist. These results indicate that spreading and hence greater coverage can be achieved with a less viscous dispersion. On the contrary clearance of the less viscous dispersion would be faster within the nasal cavity and would lead to a reduced absorption rate and hence a reduced bioavailability due to the limited contact time with the absorbing mucosa. Thus as viscosity of the dispersion increased there was a proportional increase in the particle size of the corresponding mist droplets and hence less spreading and area coverage. Deposition studies by Itoh et al., (1985) in human nasal models suggested that differing particle sizes might produce different deposition patterns. Thus as stated by Hughes at al., (1993) mist droplets with large particle size would be deposited primarily in the anterior region of the nose whereas those with smaller particle size would tend to have deposition in the more distal regions of the nose. Therefore, the distance to the orophanryx would be shorter and hence clearance faster for particles deposited more posteriorly (Hughes et al.,1993).

Studies by Pennington et al., (1988) on the influence of viscosity on the nasal spray deposition and clearance also observed a similar trend as regards the relationship between the area/diameter of deposition of HPMC solutions and the viscosity. Thus, the area/diameter was found to be inversely proportional to the viscosity of the HPMC dispersion ejected from the nasal spray pump device.

Kublik and Vidgren, (1998) stated that drug therapy requires that administration of the dosage form be accurate and very reproducible. This therefore places stringent demands on the device for the nasal drug delivery. The major mechanism of nasal deposition of particles is by inertial impaction that occurs following a change in the direction of air flow. Other contributory mechanisms are gravitational sedimentation and Brownian

diffusion. The choice of a device should therefore take into account the cost and ease of use by the patient, accuracy and reproducibility of dosing and deposition pattern as well as clearance from the nasal cavity, since all these are critical factors that influence nasal absorption (Ugwoke et al., 2001).

The results from this study obtained were in accordance with the above stated facts. Furthermore, the choice of a spray pump device is ideal as the anatomy of the human nose favours inertial impaction of spray preparations in the anterior third of the nasal cavity (Pennington et al., 1988). Deposition of a formulation in the anterior part of the nose provides a greater nasal residence time and allows more contact time between the drug and the absorbing mucosa. Depositing a formulation in the posterior part of the nose will allow a faster ciliary clearance of the formulation (Behl et al., 1998).

4.6.5 Deposition and distribution pattern assessment within the nasal cavity model

Reference product

The pump spray devices were filled with approximately 10ml of water coloured with orange G colorant. To administer the preparation, both the head/nose model and the container were slightly tilted to the back. With the middle finger and forefinger around the bottom of the nozzle and the thumb on the base of the bottle (see diagram below), the device was pressed once to fill the pump mechanism completely. Prior to administration of the preparation, the device was primed five (5) times so as to deliver an accurate and reproducible dose. The nozzle was then inserted into the nostril and actuated repeated times equivalent to the dose required per nostril (e.g. not more than 200µl per nostril) to release the contents into the nasal passages. A blotting paper with an outline of the human face diagram was superimposed onto the model to capture the area and regions of deposition of the device contents within the nasal passages. The deposition area was then demarcated and the diameter of the orange coloured blot was measured. This provided an indication of the available surface area where the pump spray device could deposit and the distribution pattern obtained. 6 replicates were performed.

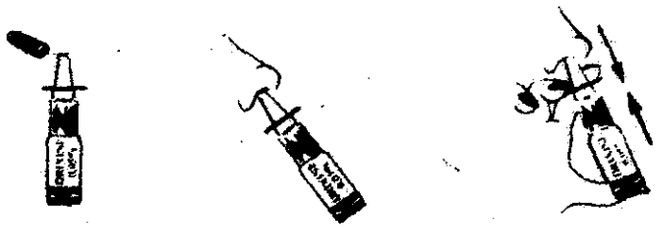


Figure 4.16: Illustration for the handling of a pump spray nasal device

Test product

The pump spray devices were filled with approximately 10ml of HPMC) 4000 dispersions of varying concentrations (0.4%(w/v), 0.6%(w/v) and 0.8%(w/v)) coloured with orange G colorant. Administration procedure for the preparation was similar to the one employed for the reference product. 6 replicas were performed.

4.6.5.2 Results and discussions

The diameter of the blot per ejection formed by the pump spray mist of water and the HPMC 4000 dispersions were measured. The average diameter, standard deviation and percentage RSD were calculated and presented in tables 4.7, 4.8 and 4.9 respectively.

Table. 4.7: Diameter of water blot per ejection from the pump spray device

Diameter (cm)	7	6.8	7	6.9	7	6.9
Average	6.93					
SD	0.019					
%RSD	0.27					

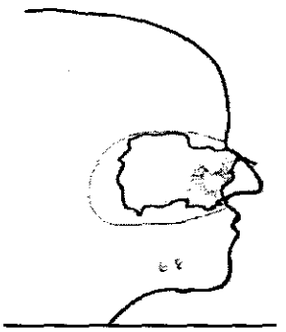


Figure 4.17: Image of sites of deposition and distribution patterns following pump spray administration of water as a reference product.

Table. 4.8: Diameter of the 0.4%(w/v) HPMC 4000 dispersion blot per ejection from the pump spray device

Diameter (cm)	4.7	5.2	4.8	4.7	4.9	5
Average	4.88					
SD	0.19					
%RSD	3.97					

Table. 4.9: Diameter of the 0.6%(w/v) HPMC 4000 dispersion blot per ejection from the pump spray device

Diameter (cm)	3.2	3	3.4	3.	3.3	3.4
Average	3.22					
SD	0.18					
%RSD	5.704					

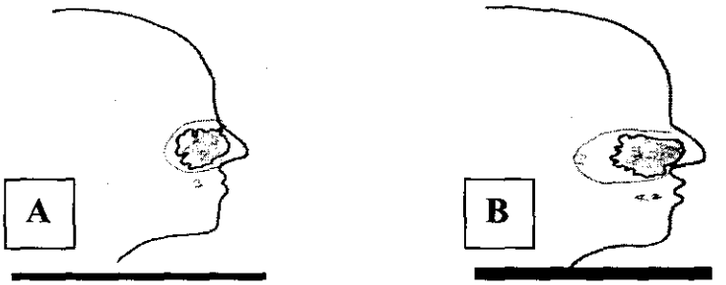


Figure 4.18: Images of sites of deposition and distribution patterns following pump spray administration of 0.6%(w/v) (A) and 0.4%(w/v) (B) HPMC 4000 dispersions respectively

According to the results obtained as reflected in tables 4.7, 4.8 and 4.9, diameter per ejection from the pump spray device was found to be reproducible. Delivery of this device yielded an accuracy of 6.8cm to 7.0cm and a precision of 0.27 % for water, which was used as reference product. For the HPMC 4000 dispersions 0.4%(w/v) and 0.6%(w/v), accuracy yielded was 4.7cm to 5.2cm; 3.0cm to 3.4cm and a precision of 3.97 %; 5.704% respectively.

Results obtained with the nose model as depicted by the images in figures.4.17 and 4.18 correlate with the *in-vitro* findings in section 4.5.6.2, greater area and hence greater coverage of the nasal passage by the contents was achieved with the less viscous dispersion thus using water as a reference. Assessment of the dosage form deposition within the model nasal passages indicated a wider distribution and posterior deposition as opposed to a more localised distribution and anterior deposition with an increase in the viscosity of the HPMC 4000 dispersion. The deposition of a preparation in the posterior region of the nasal cavity leads to faster clearance and hence lesser extent of drug absorption (Harris et al., 1986). This is due to the fact the cilia that are responsible for clearance are primarily located in the posterior two thirds of the nasal cavity (Schipper et al., 1991). It is therefore expected that drugs deposited in the posterior region to be cleared too rapidly for optimal absorption despite the coverage of more surface area (Suman et al., 1999). Contents from pump sprays tested *in-vitro* were chiefly deposited in the anterior zones of the models of the human nose (Kim et al., 1985; Hallworth et al., 1986). The anatomy of the human nose favours inertial impaction of spray preparations in

the anterior third of the nasal cavity (Pennington et al., 1988). Thus the site of deposition and hence the rate of clearance of the drug influences the efficacy of the dose received by the patient. Clearance from the non-ciliated anterior region of the nasal cavity is slow and results from the mucus layer being dragged into the ciliated region (Hardy et al., 1985). The deposition site of the nasally administered drugs can be of importance because of the different cell types in the various regions of the nose (Harkema et al., 1987; Harkema, 1991). Absorption may also be influenced by the residence time of the nasally administered drugs on the nasal mucosa, which in turn is affected by the MCC transport rate, which rapidly clears the material back to the orophanryx (Gonda et al., 1990; Schipper et al., 1991).

Findings by Pennington et al., (1988) indicated that the nasal spray deposited HPMC dispersion anteriorly in the nasal cavity. Furthermore, as the viscosity of the HPMC dispersions increased there was a proportional decrease in the area of the deposition sites. The more viscous preparations exhibited an approximately monoexponential clearance pattern. This may be due to the more viscous dispersions forming a continuous film, which is steadily dragged from the deposition site. It is apparent from these findings that, the duration of residence of the dispersions in the nasal cavity, can be enhanced by increasing the viscosity. Studies by Hardy et al., (1985) on the deposition patterns and clearance rate of the intranasal preparation delivered by spray and drops indicated that the nasal spray deposited anteriorly in the nasal cavity with little of the dose reaching the turbinates. The spray droplets tended to deposit at their impaction sites and produced a more localised distribution. Furthermore, clearance of the tracer from the ciliated region was more rapid and was by movement into the pharynx. Harris et al., (1988) found that the addition of methyl cellulose gave a more localised *in-vivo* deposition in the anterior region of the nasal cavity. By increasing the viscosity of the nasal preparations, a decrease in the clearance rate was observed. Thus, nasal preparation with 0.25%(w/v) methyl cellulose showed a 50% clearance at 75 ± 9 minutes compared to 65 ± 8 minutes for the placebo solution ($p<0.05$). From this study it appears that the effect of viscosity on the retention time in the nasal cavity has a maximum effect, which is related to the droplet size generated by the spray pump. However, the study showed that the effect of

methyl cellulose in the nasal cavity followed a biphasic pattern, with an enhancement in retention time as the viscosity and particle size increased to an optimum level, followed by a decrease in retention time resulting in faster nasal clearance as viscosity and particle size increased still further. Moreover, these studies showed that the blood drug profile resulted in different pharmacokinetics compared with non-viscous solutions, with lower initial blood levels but more sustained action and similar bioavailability.

The action of drugs delivered intranasally is likely dependent in part upon their initial site of deposition. Newman et al., (1987) found that aerosol administered from a pressurised metered dose inhaler was deposited entirely in the anterior one third of the nasal cavity, with a high local concentration in this region. These findings suggested that assuming particles retained in the nose 30 minutes were deposited initially in the non-ciliated region (Proctor, 1982); then on average approximately half the dose from a nasal spray reached the ciliated region in the main passages where it would be required for topical therapy and where rapid absorption of compounds into the systemic circulation may occur.

Based on the above findings 0.6% HPMC dispersion was therefore selected for the formulation of the intranasal preparation.

References

Agu R U, Jorissen M, Willem T, van Den Mooter G, Kinget R, Augustijns P. The effects of pharmaceutical compounds on ciliary beating in human nasal epithelial cells: A comparative study of cell culture models. *Pharmaceutical Research;* 1999 16: 1378-1382

Aspden T J, Mason J D T, Jones N S, Lowe J, Skaugrud O, Illum L. Chitosan as a nasal delivery system: The effect of chitosan solutins on *in-vitro* and *in-vivo* mucociliary transport rates in human turbinates and volunteers. *Journal of Pharmaceutical Sciences;*1997 86: 509-513

Bjork E, Bjurstran S, Edman P. Morphological examination of rabbit nasal mucosa after administration of degradable starch microspheres. *International Journal of Pharmaceutics*; 1991 75 : 73-80

Bleeker J,D J W, Hoeksema P E. A simple method to measure the ciliary beat rate of respiratory epithelium. *Acta Otolaryngologica;*1971 71:426-429

Boek W M, Keles N, Graamans K, Hiuzing E H. Physiologic and hypertonic solutions impair ciliary activity *in-vitro. The Laryngoscope;*1999 109:396-399

Chandler S G, Illum L, Thomas W N. Nasal absorption in rats II: Effects of absorption enhancers absorption and nasal histology. *International Journal of Pharmaceutics;* 1991 76: 61-70

Chandler S G, Thomas N W, Illum L. Nasal absorption in the rat. III. Effect of lysophospholipids on insulin absorption and nasal histology. *Pharmaceutical Research;*1994 11:1623-1630

Chien Y W, Su K S E, Chang S P. Physicochemical, biopharmaceutical and toxicological considerations. In: *Nasal Systemic Drug Delivery;* 1989 39:49

Chien Y W, Su K S E, Chang S. Nasal systemic drug delivery. In: ChienY W (Ed), *Drugs and the pharmaceutical sciences*. Marcel Dekker, New York 1989: 1-29

Dahlman T, Rylander R. Frequency of ciliary beat measured with a photo sensitive cell. *Nature;* 1962 196: 592-593

Daughterty A L, Liggitt H D, McCabe J G, Moore J A, Patton j S. Absorption recombinant methionyl-human growth hormone (Met-hGH) from rat nasal mucosa. *International Journal of Pharmaceutics*; 1988 45: 197-206

De Fraissinette A, Kollop M, Schiller I, Fricker G, Gammert C, Pospischil A., Vondersher J, Ritcher F. *In-vitro* tolerability of human nasal mucosa: Histopathological and scanning electron microscopy evaluation of nasal forms containing Sandostatin®. *Cell Biology Toxicology;*1995 11: 295-301

Donovan M D, Flynn G L, Amidon G L. The molecular weight dependence of nasal absorption. The effect of absorption enhancers. *Pharmaceutical Researchs;*1990 7:808-815

Donovan M D, Zhou M. Drug effects on *in-vivo* nasal clearance in rats. *International Journal of Pharmaceutics*; 1995 116: 77-86

Ennis R D, Borden L, Lee W A. The effects of permeation enhancers on the surface morphology of the rat nasal mucosa: A scanning electron microscopy study. *Pharmaceutical Research;* 1990 7 (5): 468-475

Gizurarson S, Marriot C, Martin G P, Bechgaard E. The influence of insulin and some excipients used in nasal insulin preparations on mucociliary clearance. *International Journal of Pharmaceutics;* 1990 65: 243-247

Gonda I, Gipps E. Model of disposition of drugs administered into the human nasal cavity. *Pharmaceutical Research*; 1990 7: 69-75

Gray J. The mechanism of ciliary movement-VI. Photographic and stroboscopic analysis of ciliary movement. *Proceedings of the Royal Society (London);*1930 107:313-332

Guyton A C. *Textbook of Medical Physiology*, Saunders,Phildelphia; 1981

Hallworth G W, Padfield J M. A comparison of the regional deposition in a model nose of drug discharged from metered aerosol and metered pump nasal spray delivery systems. *Journal of Clinical Immunology;* 1986 37:348-353

Hamman J H. Enhancement of paracellular drug transport in neutral environments with quaternised chitosan: *In-vitro* evaluation in intestinal epithelial cells (Caco-2). *PhD Thesis;* 2001:150

Hardy J G, Lee S W, Wilson C G. Intranasal drug delivery by spray and drops. *Journal of Pharmacy and Pharmacology;* 1985 37: 294-297

Harkema J R *Comparative Biology of the Human Lung*, CRC Press, Boca Raton, Fla;1991 :27-36

Harkema J R, Plopper C G, Hyde D M, St George J A. *Journal of Histochemical Cytochemistry;* 1987 35: 279-286

Harris A S, Nilsson I M, Wagner Z G, Alkner U. Intranasal administration of peptides: nasal deposition, biological response and absorption of desmopressin. *Journal of Pharmaceutical Sciences;* 1986 75: 1085-1088

Harris A S, Svensson E, Wagner Z G, Lethagen S, Nilsson I M. Effect of viscosity on particle size, deposition and clearance of nasal delivery system containing desmopressin. *Journal of Pharmaceutical Sciences;* 1988 77: 405-408

Hermens W A J J, Hooymans P M, Verhoef J C, Mekus F W H M. Effects of absorption enhancers on human nasal tissue ciliary movement *in-vitro*. *Pharmaceutical Research;* 1990 7: 144-146

Hermens W A J J, Merkus F W H M. The influence of drugs on nasal ciliary movement. *Pharmaceutical Research;* 1987 4: 445-449

Hirai S, Takatsuka Y, Mima H. Mechanisms of enhancement of nasal absorption of insulin by surfactants. *International Journal of Pharmaceutics*; 1981 9 (2) : 173-184

Hughes B L, Allen D L, Dorato M A, Wollf R K. Effect of delivery devices on nasal deposition and mucociliary clearance in rhesus monkeys. *Aerosol Science and Technology;*1993 18:241-249

Iravani J, Melville G N. Mucociliary function in the respiratory tract as influenced by physicochemical factors. In: *Widdicombe, Editor, Respiratory Pharmacology, Pergamon, New York;* 1981: 477-500

Itoh H, Smaldone G C, Swift D L, Wagner H. Mechanism of aerosol deposition in a nasal model. *Journal of Aerosol Science;*1985 16:529-534

Kim C S, Eldridge M A, Sackner M A, Swift D L. Deposition of aerosol particles in the human nose. *American Review of Respiratory Diseases;* 1985 131:A370

Kublik H, Vidgren M T. Nasal delivery systems and their effect on deposition and absorption. *Advanced Drug Delivery Reviews;*1998 29: 157-177

Lansley A B. Mucociliary clearance and drug delivery via the respiratory tract. *Advanced Drug Delivery Review;*1993 29: 299-327

Mejima Y, Sakakura Y, Matsubara T, Murai S, Miyoshi Y. Mucociliary clearance in chronic sinusitis: related human nasal clearance and *in-vitro* frog palate clearance. *Biorheology;*1989 20:251-262

Merkus F H W M, Shuster van Hees M T. Influence of levocarbastine suspension on ciliary beat frequency and mucociliary clearance. *Allergy*; 1992 47:230-233

Mitra R, Pezron I, Chu W A, Mitra A K. Lipid emulsions as vehicles for enhanced nasal delivery of insulin. *International Journal of Pharmaceutics*; 2000 205 : 127-205

Nagai T, Artursson P. Mechanism of absorption enhancement and tight junction regulation. *Journal of Controlled Release;*1984 1: 15-22

Nakamura F, Ohta R, Machida Y, Nagai T. *In-vitro and in-vivo* nasal mucoadhesion of some water soluble polymers. *International Journal of Pharmaceutics;* 1996 134: 173-181

Newman S P, Moren F, Clarke S W. Deposition patterns from a nasal pump spray. *Rhinology;*1987 25:77-82

Newman S P, Moren F, Clarke S W. Deposition patterns of nasal sprays in man. *Rhinology;*1987 26:111-120

Nuutinen J, Rauch-Toskal E, Saano V, Joki S. Ciliary beat frequency in chronic sinusitis. *Arch Otolaryngology Head Neck Surgery;* 1993 119:645-647

Pennington A K, Ratcliffe J H, Wilson C G, Hardy J G. The influence of solution viscosity on nasal spray deposition and clearance. *International Journal of Pharmaceutics*; 1988 43 : 221-224

Perewetoff-Morath L, Bjurstrom S, Khan R, Dhalin M Edman P. Toxicological aspects of the use of dextran microspheres and thermogelling ethy(hydroxyethyl) cellulose (EHEC) as nasal drug delivery systems. . *International Journal of Pharmaceutics*; 1996 128 : 9-21

Proctor D F. The upper airway. In: Proctor D F, Anderson I Eds, The Nose. Amsterdam: Biomedical Press;1982: 23-43

Proets A W. Motion picture demonstration of ciliary action and other factors of nasal physiology. *Transactions of the American Laryngology Association;* 1932 54: 264-273

Radebaugh G W. Rheological and mechanical properties of disperse systems. In *Pharmaceutical Dosage forms: Disperse Systems, Marcel Dekker Inc, New York.* Ed Lieberman H A, Rieger M M, Banker G S.1996 1:158-162

Romeijn S G, Verhoef J C, Marttin E, Merkus W F H M. The effect of nasal drug formulations on ciliary beating *in-vitro*. *International Journal of Pharmaceutics*; 1996 135 : 137-145

Rutland J, Cole P J. Non-invasive sampling of nasal cilia for measurement of beat frequency and study of ultrastructure. *Lancet;* 1980 ii: 564-565

Schipper N G M, Verhoef J C, Merkus F W H M. The nasal mucociliary clearance: relevance to nasal drug delivery. *Pharmaceutical Research*; 1991 8:807-814

Smallman L A, Hill S L, Stockley R A. Reduction of ciliary beat frequency and study of ultrastucture. *Thorax;* 1984 39: 2663-667

Soane R J, Freir M, Perkins A C, Jones N S, Davis S S, Illum L. Evaluation of the clearance characteristics of bioadhesive systems in humans. *International Journal of Pharmaceutics*; 1999 178 : 55-65

Suman J D, Laube B L, Dalby R. Comparison of nasal deposition and clearance of aerosol generated by a nebuliser and an aqueous spray pump. *Pharmaceutical Research;*1999 16:1648-1652

Sykes D A, Wilson R, Greenstone M, Currie D C, Steinfort C. Deleriterious effects of purulent sputum sol on human ciliary function *in-vitro*: at least two factors identified. *Thorax;* 1987 42: 256-261

Tengamnuay P, Sahamethapat A, Sailasuta A, Mitra A K. Chitosan as nasal absorption enhancers of peptides: comparison between free amine chitosan and soluble salts. *International Journal of Pharmaceutics;* 2000 197: 53-67

Thorsson L, Borga O, Edsbbacker S. Systemic availability of budesonide after nasal administration of three different formulations: pressurised aerosol, aqueous pump spray and powder. *British Journal of Clinical Pharmacology;* 1999 47: 619-624

toxicological investigation of Carbopol 971P formulation of apomorphine: Effects of ciliary beat frequency of human nasal primary cell culture and *in-vivo* on rabbit nasal mucosa. *European Journal of Pharmaceutical Sciences;* 2000; 9: 387-396

Ugwoke M I, Agu R U, Jorissen M, Augustjins P, Sciot R, Verbeke N, Kingnet R. Toxicological investigations on the effects of carboxymethyl cellulose on ciliary beat frequency of human nasal epithelial cells in primary suspension culture and in-vivo on rabbit nasal mucosa. *International Journal of Pharmaceutics*; 2000 205: 43-51

Ugwoke M I, Verbeke N, Kinget R. The biopharmaceutical aspects of nasal mucoadhesive drug delivery. *Journal of Pharmacy and Pharmacology*; 2001 53: 3-22

Van de Donk H J M, Zuidema J, Merkus F W H M. The influence of pH and osmotic pressure upon tracheal ciliary beat frequency as determined with a new photoelectric registration device. *Rhinology*;1980 18:93-104

Vidgren P, Vidgren M, Arppe J, Laine E, Paronen P. In-vitro evaluation of spray dried mucoadhesive microspheres for nasal administration. *Drug Development and Industrial Pharmacy*; 1992 18: 581-597

Washington N, Steele R C J, Jackson S J, Bush D, Mason J, Gill D A, Pitt K, Rawlins D A. Determination of baseline human nasal pH and the effect of intranasally administered buffers. . *International Journal of Pharmaceutics*; 2000 198: 139-146

Wilson R, Sykes S A, Currie D, Cole P J. Beat frequency of cilia from sites of purulent infection. *Thorax;* 1986 41: 453-458

Yager J, Chen T M, Dulfano M G. Measurement of frequency of ciliary beats of human respiratory epithelium.*Chest;*1978 73:627-633

Zhou M, Donovan M D. Intranasal mucociliary clearance of putatitive bioadhesive polymer gels. *International Journal of Pharmaceutics;* 1996 135: 115-125

CHAPTER FIVE

ANALYSIS OF CYCLIZINE IN BIOLOGICAL FLUIDS

5.1 INTRODUCTION

Cyclizine is a piperazine derivative that has effectively been used for the prevention and treatment of nausea and vomiting associated with motion sickness. Despite its widespread use, little is known about its pharmacokinetics. This paucity of information is more than likely a result of a lack of a suitable analytical method to accurately and precisely quantify the low drug levels found post administration of therapeutic doses to human subjects (Walker, 1996).

The documented methods of analysis for the determination of cyclizine HCl in biological fluids as stated in the official monograph (USP 26 NF 21) which involved the derivatisation of cyclizine HCl in biological fluids with tritiated acetic acid anhydride or measurement with methyl orange, were found by various researchers to be non-specific and relatively inaccurate at low concentrations of the drug following administration (Kuntzman et al., 1965; Kuntzman et al., 1967).

The determination of cyclizine in biological fluids has been investigated using various chromatographic methods and utilising a variety of conditions including the gas chromatography and normal and reverse-phase HPLC (Land et al., 1981; Griffin et al. 1984; Walker, 1987; Walker, 1995). Some of these methods involved complex techniques, and were found to have inadequate sensitivity and selectivity that are essential for the acquisition of appropriate pharmacokinetic data (Walker, 1996).

Several extraction procedures of cyclizine from plasma have been described previously: solid-phase extraction (SPE) with silica cartridges, liquid-liquid extraction (LLE) and protein precipitation with acetonitrile and subsequent evaporation of the organic phase to concentrate the analyte (Poole et al., 2000). The extraction of cyclizine from plasma has been carried out by both liquid-liquid (LLE) and solid phase extraction (SPE) means. The liquid-liquid extraction (LLE) techniques have tended to be complex and labour intensive, involving double or back extractions, frequent centrifugation steps and evaporation phases (Vinas et al., 1997).

Solid phase extraction (SPE) technique was introduced in the 1970's as an alternative to liquid-liquid extraction (LLE) method for the removal of matrix interferences and analyte concentration before analysis (Hearne et al., 1993). The benefits of solid phase extraction (SPE) technique over liquid-liquid extraction (LLE) method are greater recovery for specific analytes, improved selectivity and speed of the extraction process itself. Although the solid phase extraction method is costly, drug recovery rates have been reported high (Walker, 1995).

The isocratic reverse-phase HPLC method using a μBondapack C_{18} packed analytical column for the determination of cyclizine appears to be the most common mode of separation selected. Separation of cyclizine for analysis has been achieved using a wide array of mobile phase conditions (Walker, 1996). In more recent years, phosphate-buffered acetonitrile mobile phase has been frequently chosen (Walker, 1995).

For this present study, the Walker 1987 and Walker 1995 HPLC methods were adapted for the analysis of cyclizine in biological fluids. This method was developed to overcome the complexities and drawbacks of the previous methods and to determine and compare the relative bioavailability of two preparations in human volunteers.

5.1.1 HPLC analysis method development and validation

5.1.1.1 Materials

Cyclizine HCl powder was donated by the Institute for Industrial Pharmacy (Potchefstroom University for CHE, RSA). Cyclizine lactate was synthesised in the pharmaceutics laboratory (Potchefstroom University for CHE, RSA). Protriptylline HCl RS (analytical grade) was purchased from Sigma-Aldrich, Johannesburg, RSA. Methanol, acetonitrile and pentane-sulphonic acid were of HPLC grade, and 32% (w/v) ammonium hydroxide solution, analytical grade, were purchased from Merck, RSA. The HPLC analytical instrument used was a Spectra Physics HPLC system consisting of an auto-sampler AS 3000, UV detector UV 1000, HPLC pump P1000, integrator SN 4000. Integration was done using the chromoquest H3 software. Drug separation was achieved by using the C_{18} Lichrosphere RP 5 microns 250mm x 4mm HPLC column. 1ml C_{18} solid phase extraction (SPE) cartridges with 100mg silica bed were used for sample preparation and all were purchased from Separations, RSA.

5.1.1.2 Preparation of standard solutions

The standard solutions for cyclizine HCl (study drug) and protriptylline HCl (internal standard) were prepared as follows:

Cyclizine HCl:

Cyclizine HCl stock solution with a concentration of 50µg/ml was prepared by dissolving 2.5mg of cyclizine HCl in 50ml of HPLC water. This was sonicated for approximately 5 minutes to aid dissolution of the solute.

Standard solutions with varying concentrations were prepared by dilution from this stock solution.

Protriptylline HCl:

Protriptylline HCl solution with a concentration of 40µg/ml was prepared by dissolving 1mg of Protriptylline HCl in 25ml of HPLC water. This was also sonicated for approximately 5 minutes to aid dissolution of the solute.

All solutions (study drug and internal standard solutions) were freshly prepared daily and stored in the refrigerator to avoid temperature and hydrolysis related degradation.

5.1.1.3 Chromatographic conditions

HPLC equipment: The HPLC system consisted of the following Spectra Physics components: auto-sampler AS 3000, UV detector UV 1000, HPLC pump P1000 and a SN 4000 integrator. Integration was done using the chromoquest H3 software.

HPLC columns: C_{18} Lichrosphere RP 250mm x 4mm; 5 micron particle size HPLC column (purchased from Separations, RSA)

Mobile Phase: 50:50 pentane-sulphonic acid (pH 3.5): acetonitrile

Flow rate: 1ml/minute

Injection volume: 50µl

Wavelength: 205nm

5.1.1.4 HPLC determination for cyclizine HCl

Method

Standard solutions of cyclizine HCl of varying concentrations (0.08µg/ml, 0.2µg/ml, 0.6µg/ml, 1µg/ml, 3µg/ml and 5µg/ml) and 40µg/ml protriptylline HCl solution were prepared as described in 5.1.1.2 and the chromatographic conditions employed were as indicated in 5.1.1.3. 50µl aliquots of either solution were separately injected into the HPLC column in replicates of 3 to determine both the peak area for the varying cyclizine HCl concentrations and the retention times of both compounds.

Results

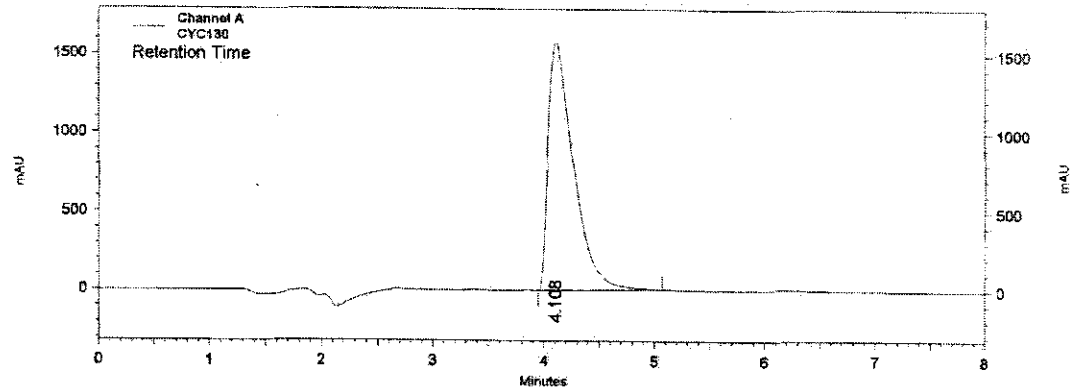


Figure 5.1: HPLC chromatogram for cyclizine HCl raw material

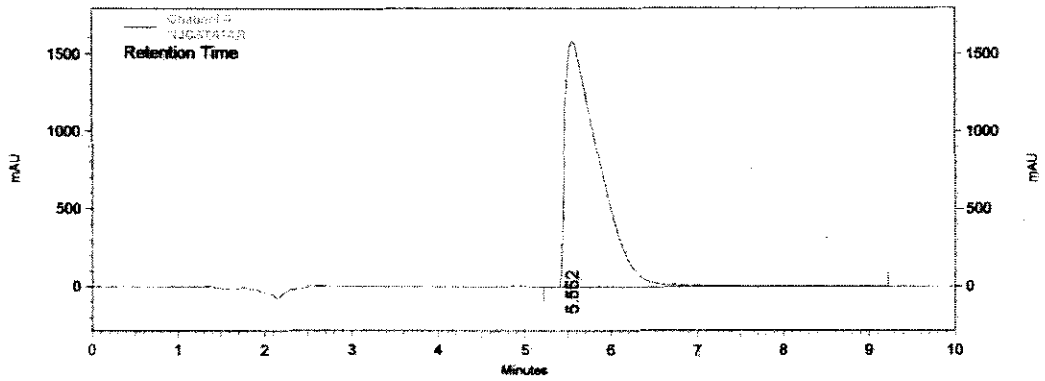


Figure 5.2: HPLC chromatogram for protriptylline HCl RS

Well resolved peaks for either compound were obtained at retention times ±4 and ±5 minutes for cyclizine HCl and protripylline HCl respectively.

To determine peak separation for both compounds, 1ml cyclizine solution 0.2µg/ml was spiked with 100µl of protriptylline HCl solution. The mixture was vortexed and transferred to a Wisp insert. 50µl of the mixture was injected and analysed in triplicate.

Results

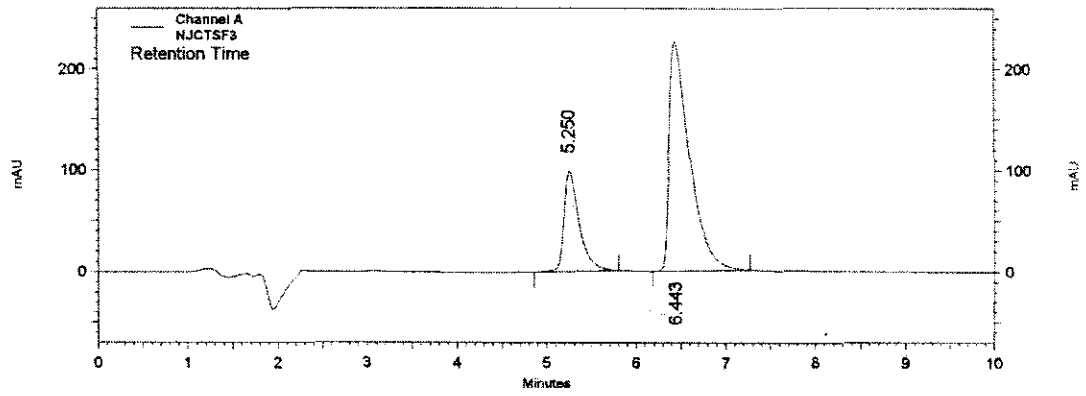


Figure 5.3: HPLC chromatogram for both cyclizine HCl and protriptylline HCl

Well resolved peaks with defined peak separation for both compounds were obtained as indicated in Figure 5.3

Based on the results obtained (well defined peak separation and short retention time for both compounds), the HPLC method was therefore adapted for subsequent HPLC determinations of cyclizine HCl.

5.1.1.5 Calibration Curve

A calibration curve is meant to demonstrate a method's ability within a given concentration range to obtain test results that are directly proportional to the concentration (amount) of analyte in the sample. Generally, the procedures for testing drug products use single point standard calibration; that is, a single concentration of test samples. Because typical calculations assume that the ratio of response to concentration (response factor) is the same for both sample and standard preparations during routine testing (Hokanson, 1994).

In determining the working range, three to six measurements should be made for at least six samples of increasing concentrations within the range from 25% to 125% of the targeted standard concentration specified in the analytical procedure (Hokanson, 1994). From the mean responses obtained, a best fit linear regression analysis is performed and a

linearity response is prepared comparing the actual data points and data calculated from linear regression parameters (slope, and intercept values). Additionally, using the same mean response values, a second plot can be prepared comparing response factors (response divided by concentration, or absorptivity for direct spectrophotometric measurements) and concentration (Dorschel et al., 1989).

In this study, 1ml of cyclizine HCl standard solutions of varying concentrations (0.08μg/ml, 0.2μg/ml, 0.6μg/ml, 1μg/ml, 3μg/ml and 5μg/ml) spiked with 100μl of 40μg/ml protriptylline HCl solution, were selected to cover the concentration range of the targeted plasma concentrations.

Table 5.1:Calibration curve concentrations

Concentration of cyclizine (μg/ml)	**Volume of drug solution (ml)**	**Volume of internal standard (40μg/ml) added (μl)**
0.08	1	100
0.2	1	100
0.6	1	100
1	1	100
3	1	100
5	1	100

Results

The ratio of peak cyclizine HCl area to peak protriptylline HCl area versus the theoretical concentration of cyclizine HCl were used to construct the regression plot as indicated in Figure 5.4.

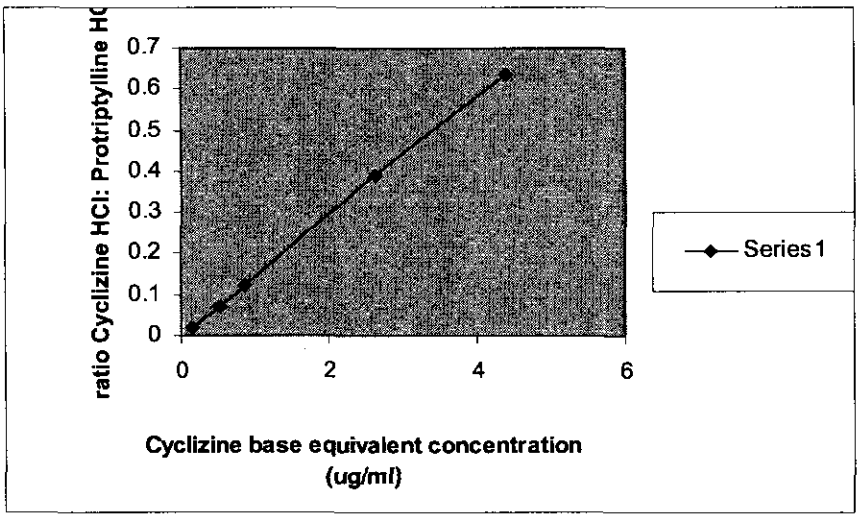


Figure 5.4: Calibration curve for cyclizine HCl

5.1.1.6 Assay of cyclizine

The HPLC method as described previously was employed for the assay of cyclizine. The retention time of cyclizine was found to be ±4 minutes. The assay indicated that the material analysed had a percentage purity of 100.18% which value falls within the USP 26 NF 21 stipulated limits of 98% and 100.5% of drug calculated on the dry basis.

5.1.1.7 HPLC Method for the determination of cyclizine in plasma and water

A modification of the method by Walker 1987 and Walker 1995 was employed for the analysis of cyclizine HCl and cyclizine lactate in water and biological fluids. The procedure was as follows:

Procedure

Pre-conditioning of solid phase extraction (SPE) cartridges

1ml Disposable extraction columns packed with octadecylsilane were utilised for the extraction procedure. Preconditioning of the cartridges was done by washing

them with 2ml of methanol (HPLC grade) followed by 1ml of the 2% (v/v) ammonium hydroxide solution (pH 9.8).

Extraction process

1ml plasma was spiked with drug and internal standard solutions of known concentrations and the mixture was vortexed for a 10 seconds. The sample was then loaded onto the pre-treated column. This sample was washed down with 1ml of 2% (v/v) ammonium hydroxide followed by 1ml of water. The system was kept under vacuum until dry. Both the drug and internal standard were eluted into 3ml tapered collection bottles with 1ml acidified methanol (95% methanol : 5 % HCl solution). The eluted samples were evaporated to dryness under a stream of nitrogen gas. The residue was reconstituted with 250 to 300µl of the mobile phase (50% pentane sulphonic acid (pH 3.5) : 50 % acetonitrile) and the resulting solution was transferred to a Wisp insert using 100µl micropipette. 50µl aliquots of the sample were then injected onto the HPLC column and analysed with the UV detector at 205nm.

Chromatographic conditions

HPLC equipment: The HPLC system consisted of the following Spectra Physics components: auto-sampler AS 3000, UV detector UV 1000, HPLC pump P1000 and a SN 4000 integrator. Integration was done using the chromoquest H3 software.

HPLC columns: C_{18} Lichrospher RP 250mm x 4mm; 5 micron particle size HPLC column (purchased from Separations, RSA)

Separating column: 1ml C_{18} SPE cartridges (purchased from Separations, RSA)

Mobile Phase 50:50 pentane-sulphonic acid (pH 3.5): acetonitrile

Flow rate: 1ml/minute

Injection volume: 50µl

Wavelength: 205nm

Retention times: ± 4 and ± 5 minutes for cyclizine and protriptylline respectively

5.1.1.6 HPLC analysis method validation

Significance of analytical method validation

Analytical methods that are used for the quantitative determination of drugs and their metabolites in biological samples play a significant role in evaluation and interpretation of bioavailability, bioequivalence and pharmacokinetic data. It is essential to use well-characterised and fully validated analytical methods to yield reliable results that can be satisfactorily interpreted (Shah et al., 1992). Pharmaceutical validation protocols rely almost exclusively on liquid chromatography, in particular reversed-phase liquid chromatography. Method validation is therefore a critical phase in process development. The Centre for Evaluation and Research (CDER), a wing of the FDA, in its documented guidelines for the validation of chromatographic methods defines validation of a method as the process by which a method is tested by the developer or user for accuracy, preciseness and reliability of its intended purpose. Thus method validation includes all of the procedures required to demonstrate that a particular method for the quantitative determination of the concentration of an analyte (or series of analytes) in a particular biological matrix is reliable for the intended application (Shah et al., 1992). Method validation should therefore be designed by the developer to ensure ruggedness and /or robustness (Shah et al., 1991).

Ruggedness according to the FDA guidelines is defined as the degree of reproducibility of the test results when the same samples are analysed under a variety of normal test conditions, such as different laboratories, analysts, instruments, with different lots of reagents, elapsed assay times, assay temperatures, and different days.

Robustness is defined as a measure of the method's capability to remain unaffected by the small but deliberate variations in method parameters (Shah et al., 1991).

The parameters essential to ensure the acceptability of the performance of an analytical method are stability of a drug in the matrix under storage conditions of the study, accuracy, precision, sensitivity, specificity (selectivity), response function and

reproducibility (Shah et al., 1992). FDA regulations on validation of HPLC methods also include parameters such as accuracy, detection and quantitation limit, linearity, repeatability, range, recovery, robustness, sample solution stability, specificity/selectivity and system suitability specifications or tests consisting of capacity factor, repeatability of injection/precision, relative retention, resolution, tailing factor and theoretical plate count (Shieh et al., 1998). Although method validation is regarded as a regulatory requirement for analytical processes by the FDA, it must be approached as a process that continually provides maximum confidence in the reliability of test procedures (Hokanson, 1994).

Method validation can be envisaged to consist of two (2) distinct phases (Shah et al., 1992):

- the development phase, in which the assay is defined
- and the application phase, in which the method is applied to the actual analysis of the samples from pharmacokinetic, bioavailability and bioequivalence studies.

The following parameters (linearity, repeatability, recovery, specificity, detection and quantitation limit, accuracy) were tested for the development of an analytical system that would allow determination of very low quantities of cyclizine concentrations in plasma with sufficient accuracy and precision.

5.1.1.6.1 Specificity/Selectivity

The terms specificity and selectivity are often used interchangeably. A method is said to be specific if it produces a response for a single analyte (Karnes et al.,1991). The specificity of the assay of a drug and its metabolites should be investigated during the method development phase to avoid interference that may arise from the presence of the components of the biological fluids or other drugs during analysis (Bruce et al., 1998).

A method can also be selective if it provides responses for several analytes and is able to distinguish between them. Selectivity of a method can be tested by either demonstrating a lack of response in the blank biological matrix or by testing whether the intercept of the calibration curve is significantly different from zero using a one-sided *t* test, which will

provide a quantitative assessment. This test can be very liberal or rigid depending on the reproducibility of the calibration (Karnes et al., 1991).

Results

Figures 5.5, 5.6 and 5.7 show chromatograms of cyclizine HCl and protriptylline HCl (internal standard) in water, plasma and plasma sample spiked with cyclizine HCl and Protriptylline HCl (internal standard) respectively. From these chromatograms it is evident that there were no interfering peaks at the retention times ±4 minutes for cyclizine HCl and ±5 minutes for protriptylline HCl.

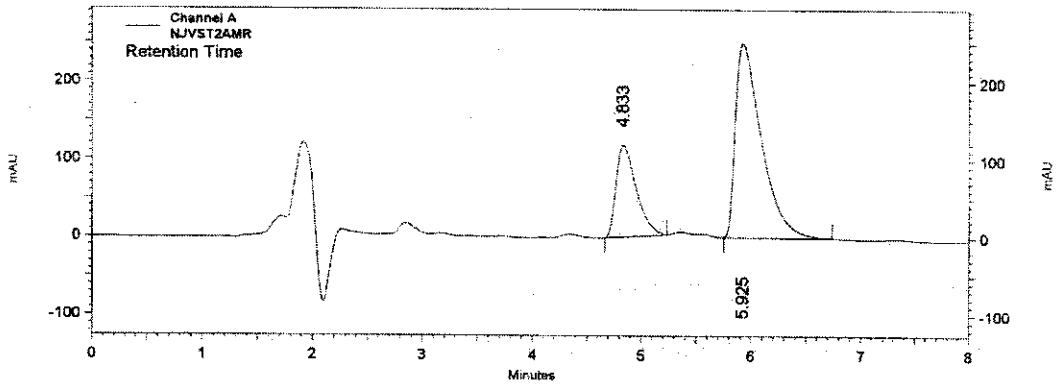


Figure 5.5: Chromatogram of water extract for cyclizine and protriptylline (internal standard)

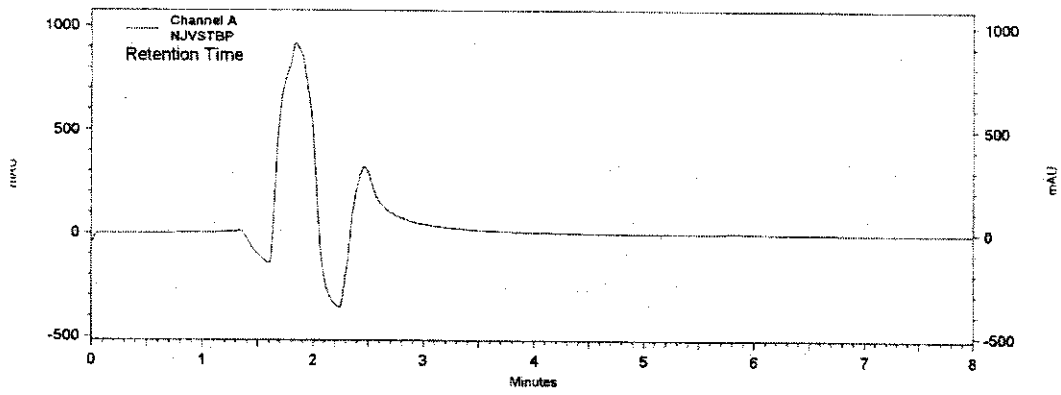


Figure 5.6 : Chromatogram of blank plasma

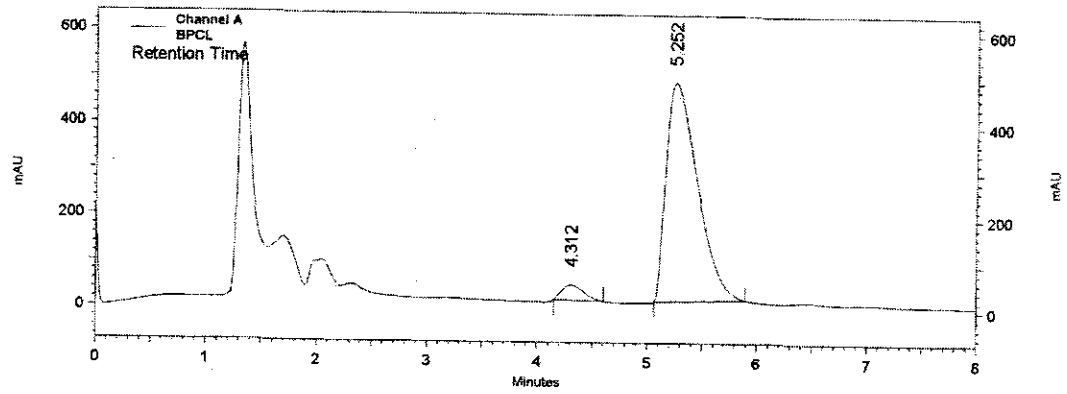


Figure 5.7: Chromatogram of plasma extract for cyclizine and protriptylline (internal standard)

5.1.1.6.2 Linearity

Linearity is defined as the statistical measure of goodness of fit of the regression line for the response versus concentration plot through the data correlation coefficient r. It is conventional to use a linear univariant regression. In this model, the independent variable (x) is concentration and the dependent variable (y) is the response. Univaraint regression response minimises residuals around y and assumes x to be errorless (Karnes et al., 1991).

The plot of log concentration versus log response should ideally provide a slope equal to one for a linear model (Dorschel et al., 1989). The closeness of the slope to one may be used as a criterion of acceptance for linear data (Dorschel et al., 1991; Watson et al., 1999). The y-intercept should be smaller than a few percentages of the response obtained for the analyte at the target level (Bruce et al., 1998). Thus it is the ability of the analytical procedure within a given range to obtain test results, which are directly proportional to the concentration (amount) of the analyte in the sample.

Method

The linearity for cyclizine analysis was determined by performing a linear regression analysis of the peak area ratios over the internal standard (protriptylline) versus cyclizine over a range of 0.08μg/ml to 5μg/ml. The standard curve was obtained by using the

appropriate analytical procedure with at least five (5) standards in the range. The data was best described by a linear equation: $y = mx + c$.

Where:

Y = peak area ratios of cyclizine to protriptylline

M = slope (gradient)

X = concentration of cyclizine

C = y-intercept

1ml of plasma was spiked with cyclizine and protriptylline solution. The various concentrations of the study drug solutions were selected to cover the full range of expected cyclizine plasma concentrations (table 5.2). Each solution concentration was analysed in triplicate and the average of the three concentrations was used to calculate the regression statistics (table 5.4).

Table 5.2:Calibration curve concentrations

Concentration of cyclizine (µg/ml)	**Volume of plasma (ml)**	**Volume of internal standard (40µg/ml) added (µl)**
0.08	1	100
0.2	1	100
0.6	1	100
1	1	100
3	1	100
5	1	100

Results

Table 5.3: Mean peak area ratio of cyclizine:protriptylline as a function of concentration.

Drug concentration (µg/ml)	Peak area ratio (Cyclizine :protriptylline)					
	1	2	3	Mean	SD	%RSD
0.08	0.31663	0.316664	0.31715	0.312823	0.00365	0.116714
0.2	0.32519	0.323298	0.317039	0.321842	0.004266	1.32543
0.6	0.444793	0.436584	0.433026	0.4381345	0.006035	1.377413
1	0.530547	0.53192	0.532813	0.53176	0.11372	0.214671
3	0.848516	0.841306	0.841748	0.8438564	0.004012	0.478896
5	1.390865	1.380908	1.270079	1.347284	0.67647	5.020957

Table 5.4: Regression statistics of the average

R-squared (r^2)	0.9928
Y-intercept	0.2973
Gradient	0.2044

Conclusion

A linear relationship (r^2= 0.9928) was found when the ratio of peak area of cyclizine HCl to peak area of protriptylline HCl were plotted against the various concentrations ranging from 0.08µg/ml to 5µg/ml

5.1.1.6.3 Sensitivity

Sensitivity is defined as the slope of the calibration line provided that the plot is linear and can be measured at any point on the plot. In the regression equation, y =mx + c, the value of c in the equation can be used as a blank since it gives a more accurate estimate of the blank than a single measured value. Thus a method is said to be sensitive if a small change in the concentration causes a large change in instrumental response (Bruce et al.,1998).

5.1.1.6.3.1 Lower limit of quantitation (LOQ)

This is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy.

Results

The limit of quantification for cyclizine was 1.25ng/ml (%RSD <15).

5.1.1.6.3.2 Lower limit of detection (LOD)

This is the lowest amount of analyte in a sample, which can be detected but not necessarily quantitated as an exact value. The analyte peak is usually three times the noise level (baseline disturbances). Although various methods to estimate the lower limit of quantification have been described, an experimental assessment provides the best measure of the operating limits of the equipment (Hokanson, 1994). The lower limit of quantitation (LOQ) varies with different batches of blank controls (plasma, urine etc.), sample type (presence of other drugs and type and condition of instrument). Lower limit of quantitation (LOQ) should therefore not be rigidly fixed at the time the method is validated. It is necessary to reassess it whenever changes in conditions affecting it are suspected (Van Rooyen, 2003).

Results

The limit of detection for cyclizine was 0.625ng/ml (%RSD < 15).

5.1.1.6.4 Accuracy and Precision

Accuracy is defined as the closeness of the measured value. This parameter is determined by comparing the measured value of a sample of known concentration to the true value (Hokanson et al., 1994; Jenke et al., 1996).

Analytical recovery of a method refers to whether the analytical method in question provides a response for the entire analyte that is contained in a sample. Results of experiments that compare the matrix to pure solvent are referred to as relative recovery, and true tests of recovery are referred to as absolute recovery. Absolute recovery is

measured as the response of a processed spiked matrix standard expressed as a percentage of response of pure standard which has not been subjected to sample pre-treatment This is usually expressed as a ratio of slopes measured at several concentration points (Karnes et al., 1991).

Method

Three sets of six blood samples were each spiked with specific drug concentrations. These were analysed and the amount of drug in each was determined using the calibration curve equation. The percentage extraction recovery for cyclizine from plasma was determined by comparing the peak area of known cyclizine concentrations extracted from plasma with the equivalent drug concentrations prepared in water. Accuracy was given as the % recovery and the precision as the %RSD between the values obtained.

Results

From the data presented in table 5.5, the method yielded an accuracy of 96.66% to 97.56%, and a precision of 4.15% to 8.25% for the water extraction and an accuracy of 85.79% to 87.32%, and a precision of 0.519% to 9.45% for the plasma extraction over the concentration range of 0.08µg/ml to 5µg/ml respectively.

Table 5.5: Percentage extraction recovery after solid phase extraction procedure

Original drug concentration(µg/ml)	**Accuracy (%recovered)**		**Precision (%RSD)**	
	Plasma	**H_2O**	**Plasma**	**H_2O**
0.2	87.32	97.18	9.45	8.26
0.6	86.96	96.66	7.04	8.28
1	85.79	97.59	3.415	5.36
3	86.93	97.01	0.52	4.16

5.1.1.6.5 Repeatability

Repeatability means the precision under the same operating conditions over a short interval of time. Repeatability was determined assessing the intra-day and inter-day repeatability assay.

5.1.1.6.5.1 Intra-day repeatability

Method

The intra-day precision and accuracy of the method was evaluated by analysing three (3) replicates of spiked samples at each of all the concentrations (0.08μg/ml, 0.2μg/ml, 0.6μg/ml, 1μg/ml, 3μg/ml and 5μg/ml) on the same day.

Results

The results in table 5.6 indicate insignificant variation in drug content with repeated injections for all concentrations.

Table 5.6: Intra-day repeatability of plasma extracts spiked with cyclizine HCl solutions with varying concentrations

Sample concentration (μg/ml)	% Recovery (Cyclizine :Protriptylline			Mean	SD	%RSD
	1	2	3			
0.08	79.6	82.4	86.4	82.8	3.418	4.128
0.2	84.9	83.9	82.8	83.6	1.473	1.76
0.6	84.0	90.2	88.3	87.6	3.219	3.74
1	87.6	83.9	84.8	85.43	1.93	2.259
3	85.9	86.2	89.1	87.067	1.767	2.03
5	82.3	84.8	81.2	82.767	1.845	2.23

5.1.1.6.5.2 Inter-day repeatability

Method

Inter-day precision and accuracy was assessed by performing the analysis of samples at the same concentration on consecutive days. 6 samples of 0.2µg/ml were analysed on the three consecutive days to determine the inter-day repeatability.

Results

The results obtained in table 5.7 show insignificant variation in drug content determined. These show insignificant variation in drug content, which is within the acceptable range. Thus method is not affected by the period.

Table 5.7: Inter-day repeatability of plasma extracts spiked with 0.2µg/ml drug

Day	% recovered
1	85.24
2	86.57
3	84.3
Mean	85.33
SD	1.14
%RSD	1.34

5.1.1.7 Stability of sample solutions

Stability testing is performed to ensure that the compound integrity is maintained throughout the workup process. Certain stability tests should be performed during the final stages of method development. Stability testing can be performed on the analyte and on the process (Dadgar et al.,1995).

Analyte stability: This test is conducted to determine whether the pure analyte and /or the solutions of the analyte (drug, metabolite and internal standard) are stable under normal laboratory conditions of heat, humidity and light exposure (Dadgar et al., 1995).

In process stability: It is necessary to demonstrate that the drug remains intact if left for several hours at room temperature in the biological matrix. In cases where drug degradation occurs in the biological matrix, decrease of the collecting device temperature may improve stability or extract the samples immediately after collection and store the dried frozen residue for later reconstitution and analysis (Dadgar et al., 1995).

Processed sample stability: Reconstituted samples must remain stable in the reconstituted solvent at the temperature of the auto-injector or at low temperatures (to allow storage of the prepared sample that cannot be analysed immediately) until injection period (Dadgar et al., 1995).

Method

A sample of cyclizine HCl and protriptylline HCl (internal standard) of known concentration was analysed over 7 hours to determine the stability of the drug solution under ordinary laboratory conditions (room temperature and light exposure).

Results

Results as reflected in table 5.8 show no variation of drug content over the test period. Thus this is indicative of the absence of degradation products over an extended time in the autosampler. Taking into consideration the retention times for both the drug and the internal standard, 42 cyclizine sample solutions can therefore be analysed without fear of degradation.

Table 5.8:Stability of a solution of cyclizine and protriptylline over a period of 7 hours

Cumulative hours	% Recovery (Cyclizine:protriptylline)
1	85
2	84.3
3	85.3
5	86.03
7	85.19
Mean	85.34
SD	0.77
%RSD	0.904

5.1.1.8 System suitability

The system suitability tests are an integral part of the HPLC methods. These are used to verify that the resolution and reproducibility of the chromatographic system are adequate for analysis to be performed. These tests are based on the concept that the equipment electronics, analytical operations and samples for analysis constitute an integral system that can be evaluated (USP 26 NF 21, 2003).

5.1.1.9 Peak symmetry

The tailing factor (T), which is a measure of the peak symmetry, is unity for perfectly symmetrical peaks and its value increases as tailing becomes more pronounced. As peak asymmetry increases, integration and hence precision becomes less reliable. Figure 5.8 shows the parameters for equation 5.1 to calculate the tailing factor (T).

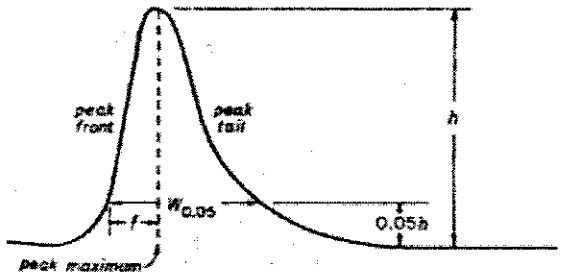


Figure 5.8: An asymmetrical chromatographic peak (USP 26 NF 21, 2003)

$$T = \frac{W_{0.05}}{2f}$$ Equation 5.1

Where:

T = tailing factor

$W_{0.05}$ = width of peak at 5% height

F = the leading edge of the peak, the distance being measured at a 5% point the peak height from the baseline.

Results

The tailing factors for cyclizine and protriptylline were 1.2 and 1.33 respectively which values fall below the stipulated upper limit of 2 (USP 26 NF 21).

5.1.1.10 Resolution

Resolution denotes the degree/magnitude of separation. The resolution is a function of column efficacy and the number of theoretical plates. The resolution gives an indication of how effectively two components (e.g. standard and internal standard) are resolved from each other.

The mathematically resolution can be defined as the difference in the elution volumes of two components divided by the average of the band widths.

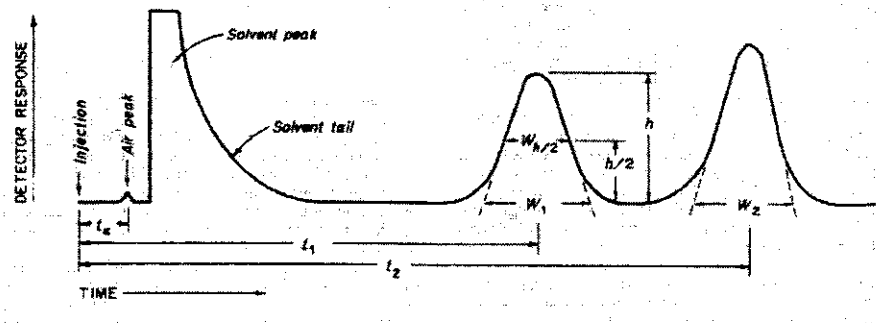


Figure 5.9: Chromatographic separation of two components (USP 26 NF 21, 2003)

$$R = \frac{2(t_1 - t_2)}{(W_1 + W_2)}$$ Equation 5.2

Where:

T_1 = cyclizine retention time

T_2 = protriptylline retention time

W_1 = cyclizine corresponding width at the base of the peak

W_2 = protriptylline corresponding width at the base of the peak

Results

The resolution between the cyclizine and protriptylline peaks was 1.22. According to the resolution determined these peaks were well resolved.

5.1.1.11 Theoretical plate number (N)

The theoretical plate number takes into account the width of the peak (band-spreading) and can therefore be used as a measure of the efficacy of a system. "N" is relative and the number generated is unitless.

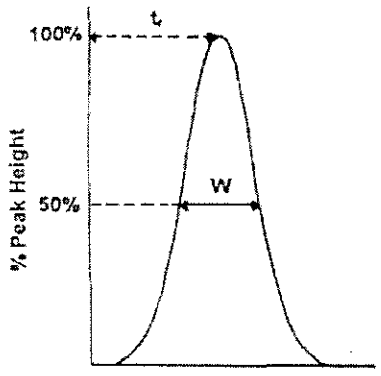


Figure 5.10:Parameters used for calculating the number of theoretical plates (USP 26 NF 21, 2003)

$$N = a\left(\frac{t_r}{W}\right)^2$$ Equation 5.3

Where:

N = theoretical plate number

A	=	constant
t_r	=	retention time
W	=	peak width at 50% height

Results

The theoretical plate number (N) for the column used in the analysis of cyclizine and protriptylline in plasma was 21215 and 21587 for cyclizine and protriptylline respectively which values according to the USP 26 NF 21 limits indicate column efficiency.

References

Bruce P, Minkkinen P, Riekkola M L. Practical method validation: Validation sufficient for an analysis method. *Mikrochimica Acta;*1998 128: 93-106

Dadgar D, Burnett P E, Choc M G, Galliano K, Hooper J W. Application issues in bioanalytical method validation, sample analysis and data reporting. *Journal of Pharmaceutical and Biomedical Analysis;* 1995 13 (2): 89-97

Dorschel C A, Ekmanis J L, Obertholtzer J E, Warren Jnr F V, Bidlingmeyer B A. LC detectors: Evaluation and practical implications of linearity. *Analytical Chemisty;*1989 61 (17): 951A-968A

Griffin D S, Baselt R C. lood urine concentrations of cyclizine by nitrogen –phosphorus gas-liquid chromatography. *Journal of Analytical Toxicology;*1984 8:97-99

Hearne G M, Hall D O. Advances in solid phase extraction technology. *American Laboratory;*1993 :28H-28M

Hokanson G C. A life cycle approach to the validation of analytical methods during pharmaceutical product development, Part 1: The initial method validation process. *Pharmaceutical technology;*1994: 118130

Jenke D R. Chromatographic method validation: A review of current practices and procedures: General concepts and guidelines. *Journal of Liquid Chromatography and Related Technologies;*16 19 (5):719-736

Karnes H T, Shiu G, Shah V P. Validation of bioanalytical methods. *Pharmaceutical Research;*1991 8 (4):421-426

Kuntzman R, Klutch A, Tsai I, Burns J J. Physiological distribution and metabolic inactivation of chlorcyclizine and cyclizine. *Journal of Pharmacological Exp. Therapeutics;*1965 149:29-35

Kuntzman R, Tsai I, Burns J J. Importance of tissue and plasma binding in determining the retention of norchlorcyclizine and norcyclizine in man, dog and rat. *Journal of Pharmacological Exp. Therapeutics;*1967 158:332-339

Land G, Dean K, Bye A. Determination of cyclizine and norcyclizine in plasma and urine using gas chromatography with nitrogen selective detection. *Journal of Chromatography and Biomedical Applications;* 1981 222: 134-140

Poole C F, Gunatilleka A D, Sethuramn R. Contributions of theory to method development in solid phase extraction. *Journal of Chromatography A;* 2000 885: 17-39

Shah V P, Midha K K, McGilveray I J, Skelly J P, Yacobi A, Layloff T, Viswanathan C T, Cook C E , McDowall R D, Pittman K A, Spector S. Analytical method validation: Bioavailability, bioequivalence and pharmacokinetic studies. Shieh P, Cook N, Gant R, Ekssteen. A reliable tool for drug delivery and validation. *Drug Discovery;* 1998 : 66-70

Van Rooyen G F. The development and validation of assay methods for the quantitative determination of drugs and their metabolites in biological specimens clarithromycin, carbamazepine and carbamazepine-10, 11-epoxide. *Masters Dissertation University of the Orange free State RSA;*2003:17-28

Vinas P, Campillo N, Lopez-Erroz C, Hernandez-Cordoba M. Use of post-column fluorescence derivatisation to develop a liquid chromatographic assay for ranitidine and its metabolites in biological fluids. *Journal of Chromatography B;* 1997 673:443-449

Wade A. The Extra Martindale Pharmacopoeia;1992 30th Edition : 1279

Walker R and Kanfer I. High Performance Liquid Chromatographic analysis of cyclizine and its metabolite, norcyclizine in biological fluids using solid phase extraction. *Chromatographia;* 1987 24 : 287-290

Walker R and Kanfer I. Sensitive High Performance Liquid Chromatographic determination of cyclizine and its demethylated metabolite, norcyclizine in biological fluids using coulometric detection. *Journal of Chromatography;* 1995 672: 172-177

Walker R B, Kanfer I. Pharmacokinetics of cyclizine following intravenous administration to human volunteers. *European Journal of Pharmaceutical Sciences*; 1996 4 (5) : 301-306

Watson D G. Control of quality of analytical methods. In: *Pharmaceutical analysis. A text book for pharmacy students and pharmaceutical chemists*. Ed Hancourt Publishers Ltd;1999: 7

CHAPTER SIX

BIOAVAILABILITY STUDIES

6.1 INTRODUCTION

Bioavailability is defined as a measure of the rate and amount of unchanged drug, which reaches the systemic circulation following administration of a drug product. Bioavailability is used as an indication of the bioefficacy of the drug (Abdou, 1989). Bioavailability studies are conducted to ensure the safety and efficacy of the drug product's labelled indications or use as well as to ensure that the applicable standards of utility, strength and quality are met as stipulated in the USP 26 NF 21. The area under the drug-concentration curve (AUC) is used as a measure of the total amount of unaltered drug that reaches the systemic circulation. This parameter is dependent on the total quantity of drug available divided by the elimination rate constant (k_e), and the volume of distribution (V_D) (Shargel et al., 1999).

Relative availability is the availability of a drug in the systemic circulation from a drug product compared with a recognised standard and this is calculated as follows:

$$\text{Relative availability} = \frac{[AUC]_A}{[AUC]_B}$$

Where B is the recognised reference

A is the test product

Absolute availability is the systemic availability of a drug after extravascular administration (oral, rectal, transdermal, subcutaneous). This is generally measured by comparing the AUCs after extravascular and intravenous administration. It is determined as follows:

$$\text{Absolute availability} = \frac{[AUC]_{PO} / dose_{PO}}{[AUC]_{IV} / dose_{IV}}$$

The main aim of exploration of the various routes of drug administration is to attain high plasma drug levels that are comparable to the intravenous route. Studies have been conducted by various researchers to demonstrate the possibility of attaining drug therapeutic levels by the use of other routes without inflicting pain to the patient. The following are some of the successes of non-parenteral preparations, in particular intranasal preparations, that proved comparable bioavailability to the intravenous preparations: sufentanyl (Helmers et al., 1989), fentanyl (Striebel et al., 1993), butorphanol (Abboud et al., 1994), promethazine (Ramanathan et al., 1998), metoclopramide (Ormrod et al., 1999), sumatripan and dihydroergotamine (Logemann et al., 2000), calcitonin (Ahsa et al., 2001), hyoscine (Klocker et al., 2001) and midazolam (Loftsson et al., 2001). It is evident from these studies that the nasal route may be used as an alternative to intravenous route for systemic drug administration.

The present study therefore looks into the bioavailability of cyclizine following oral and intranasal administration.

6.2 Materials and methods

Study Medication:

Reference Product:	Valoid ®
Strength:	50mg Cyclizine HCl
Dose:	A single dose on day 1
Dosage form:	Tablets
Batch No:	1C58C
Expiry Date:	4 2005
Manufacturer:	GlaxoWellcome South Africa (Pty) Ltd
	Old Pretoria Rd

	Midrand, South Africa
	Midrand, South Africa
Test Product:	Cyclizine Lactate intranasal solution
Strength:	125mg/ml
Dose:	A single dose on day 1 (25mg/200µl)
Dosage form:	Intranasal solution
Manufacturer:	Pharmaceutics Dept
	PU for CHE
	Box 36
	Potchefstroom

Rationale for use of human subjects

Testing the safety and efficacy of successful human medicine involves many laboratory animals, which can sometimes be subject to considerable suffering and distress. Also, it is necessary to extrapolate from the test species to humans. The UK and European legislation requires that replacement, reduction and refinement of animal procedures be implemented wherever possible (Combes et al., 2003). In a workshop organised by Volunteers in research and testing in 2001, a group of individuals in the UK which launched an initiative in 1994 to identify where and how human volunteers can participate safely in biomedical studies to replace laboratory animals; it was considered that conducting pre-phase 1 very low dose human studies could enable drug candidates to be assessed earlier for *in-vivo* human pharmacokinetics and metabolism. It was also recommended that some limited animal tests would still be required before the pre-phase 1 studies to take into account the potential risks of early drug/dosage form development. Furthermore, early human volunteer studies should be introduced into the drug/dosage form development process in a way that does not compromise the volunteer's safety, scientific quality or relevance of the resulting data. Thus, improving the selection of drug/excipient candidate and hence reducing the likelihood of drug/dosage form candidate failure by providing *in-vivo* ADME data for pharmacokinetics and metabolism at an earlier stage of candidate development (Combes et al., 2003).

Study subjects

12 healthy adult male volunteers participated in this comparative study at Biocen Clinic Potchefstroom University for CHE. Their mean age was 22 years with a range of 18 to 45 years, mean body weight was 87.0kg with a range of 50 to 95kg and mean body height was 1.81m with a range of 1.75 to 1.88m. The volunteers were free from any significant cardiac, hepatic, renal, pulmonary, neurological, gastrointestinal and haematological diseases, as determined by their medical history, physical examination and routine laboratory tests (haematology, blood biochemistry and urine analysis). All subjects were negative for hepatitis B antigen and were instructed to abstain from taking any drugs including over the counter (OTC) medications for two (2) weeks prior to and during the study period. They were informed about the risks and aims of the study by the clinical investigator. Each subject signed a written informed consent statement before entering the study (Appendix 6). The study was conducted according to the revised Declaration of Helsinki 2000 for Biomedical research involving human subjects and the rules for Good Clinical Practices. Before the start of the study the protocol was approved by the Ethics Committee of the Potchefstroom University for CHE (Appendix 6 and Appendix 7 respectively).

6.2.1 Drug administration and sample collection

Crossover designs are the primary statistical designs for bioavailability and bioequivalence studies (Chow et al., 2000). Such designs allow for comparisons of individual treatments using within–subject variation and thus increase the power of the study (Qu et al., 2003). For this study a single blind crossover design was employed.

The subjects were admitted at the Biocen Clinic at 20h30 prior to the clinic day. After overnight fasting (10 hours) the subjects were given a single dose of either formulation (reference or test in a randomised fashion) of cyclizine. Food and drinks (other than water, which was allowed 2 hours after drug administration) were not allowed until 4 hours post drug administration when a standard lunch was given to all the subjects

according to the time schedule. Beverages and food containing caffeine were not permitted over the entire course of study. The subjects sat or walked around during the first 4 to 5 hours of blood collection, but were prohibited from any strenuous activity. They were under direct medical supervision at the study site. Approximately 8ml of blood samples for cyclizine were drawn into Li-Heparin tubes through the in-dwelling cannula at the following time intervals: 0, 15, 30, 60, 90, 120, 150, 180, 240, 360, 480, 600, 720 minutes, 24 hours, 48 hours and 72 hours. Each blood sample was centrifuged at a minimum of 100-x g for 10 minutes (3000 rpm). Each plasma sample was harvested and transferred in equal volumes into two labelled polypropylene tubes and stored upright at -20°C until analysed. The duplicate sets of plasma samples were placed in separate freezers. Vital signs, BP, pulse rate and temperature for the subjects were recorded at 0, 4 and 10 hours. After a period of 7 days the study was repeated in the same manner to complete the crossover design.

6.2.2 Chromatographic conditions

Plasma samples were analysed for cyclizine concentration using a validated reverse phase (RP) HPLC method with UV detection as described in section 5.1.1.7. All solvents used were of HPLC grade while other chemicals and reagents were of analytical grade. Cyclizine HCl 50mg tablets were obtained from the local pharmacy and the cyclizine lactate intranasal preparation was manufactured in-house.

The HPLC apparatus, accessories and chromatographic conditions used were as described in sections 5.1.1.3. Each analysis required less than 10 minutes. The method was validated by following the international guidelines (Shah et al., 1991).

6.3 Pharmacokinetic/statistical analysis

Pharmacokinetic analysis was performed by means of a model independent method using the SAS System for Windows Release 8.02 TS Level 02M0 computer programme. The area under the curve to the last measurable concentration (AUC_{0-t}) was calculated by the linear trapezoidal rule. The statistical analysis of the pharmacokinetics data obtained for the two phases was done using the two-way analysis of variance (ANOVA) for crossover design to assess the effects of period, product, sequence and subjects on AUC, C_{max} and t_{max}. A difference between two related parameters was considered statistically significant for a P value equal or less than 0.05. Parametric 90% confidence interval based on the ANOVA of the mean test/reference (T/R) ratios of the AUC, C_{max} and t_{max} were computed.

6.4 Results and Discussion

Both formulations were relatively well tolerated by the study subjects. Sneezing was observed in 50% of the subjects during both phases 25 minutes post intranasal drug administration; however, unexpected incidents, which could have influenced the outcome of the study did not occur.

During this study Valoid® tablets containing 50mg of cyclizine HCl were administered orally and an intranasal preparation containing 125mg/ml of cyclizine lactate was administered nasally. Both formulations were readily absorbed from the gastro intestinal tract (GIT) (oral preparation) and from the nasal mucosa (intranasal preparation). Cyclizine was measurable at the 2^{nd} sampling time (0.25h) in some subjects while at 0.5h in the others.

The extent of absorption is the key characteristic of the drug formulation and therefore the area under the curve is an important parameter for comparative bioavailability studies. However, the other pharmacokinetic parameters like C_{max} and t_{max} are also important features of the plasma level profile and could affect the therapeutic use of a drug and hence should be considered in all pharmacokinetic studies (Najib et al., 2003).

In order to calculate the relative bioavailability of cyclizine for each route of administration it was assumed that the drug follows linear kinetics.

Table 6.1: Mean (± SD) of the cyclizine concentrations (ng/ml) in plasma following oral and intranasal administration as a function of time (hours) (n=12)

Concentration (ng/ml)		
Time (hours)	**Oral**	**Intranasal**
0	0±0	0±0
0.25	251.17±68.64	3278.14±4973.75
0.5	282.30±97.26	3292.76±5204.77
1	321.25±105.72	4501.72±5393.45
1.5	314.12±92.93	3183.98±4063.23
2	338.53±116.53	2662.52±3886.68
3	344.22±113.63	3001.12±381.06
4	317.73±102.41	2723.25±3910.62
6	344.56±116.52	2685.63±3929.5
8	350.81±179.22	2603.68±3880.95
10	331.05±97.63	2449.29±3269.61
12	323.99±110.62	2510.93±3586.61
24	289.29±57.0	2462.02±3710.98
48	288.66±43.89	2587.3±3870.7
72	281.23±64.23	2784.86±4441.75

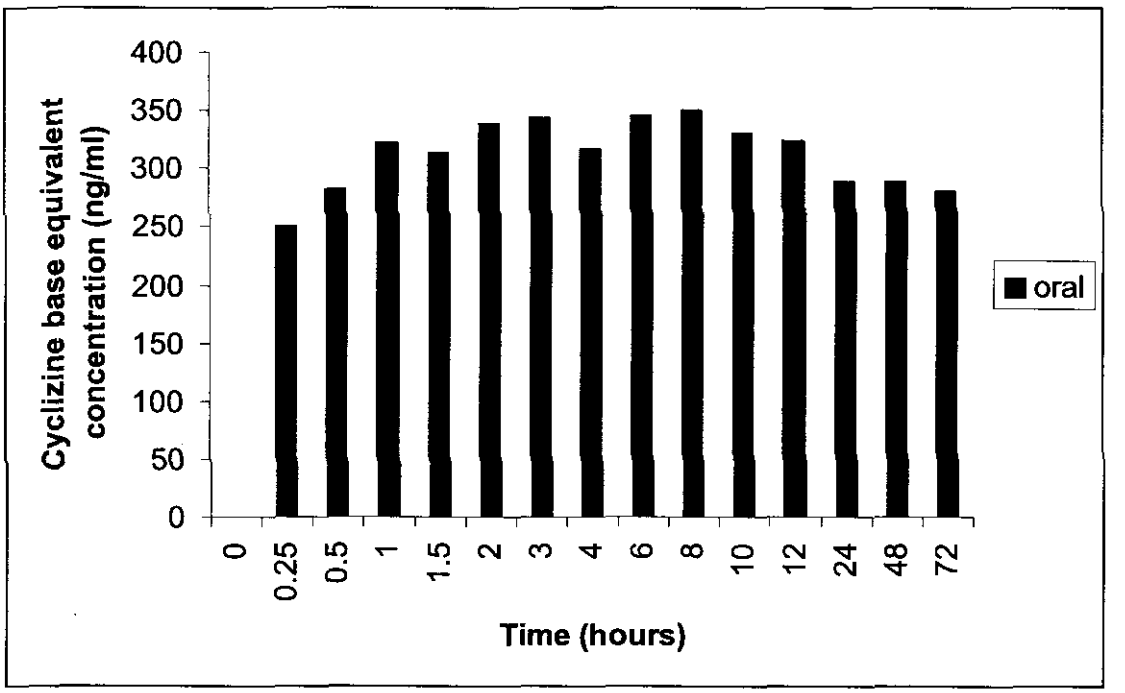


Figure 6.1:The concentration of cyclizine (ng/ml) as a function of time (hours) post oral administration (n=12)

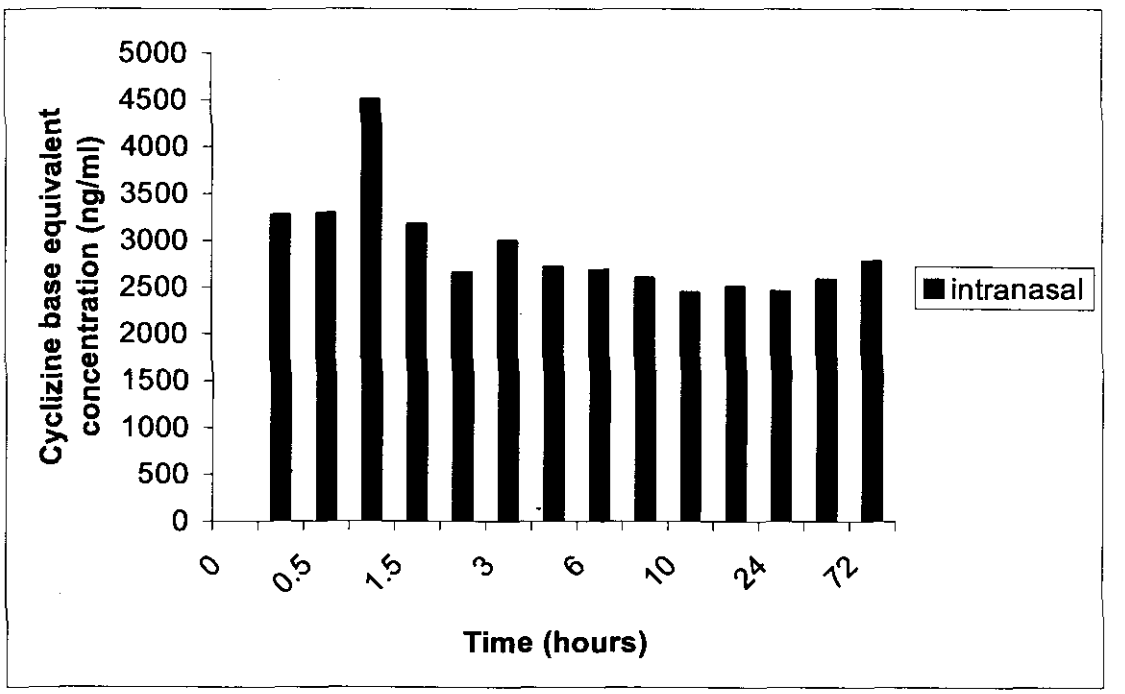


Figure 6.2: The concentration of cyclizine (ng/ml) as a function of time (hours) post intranasal administration (n=12)

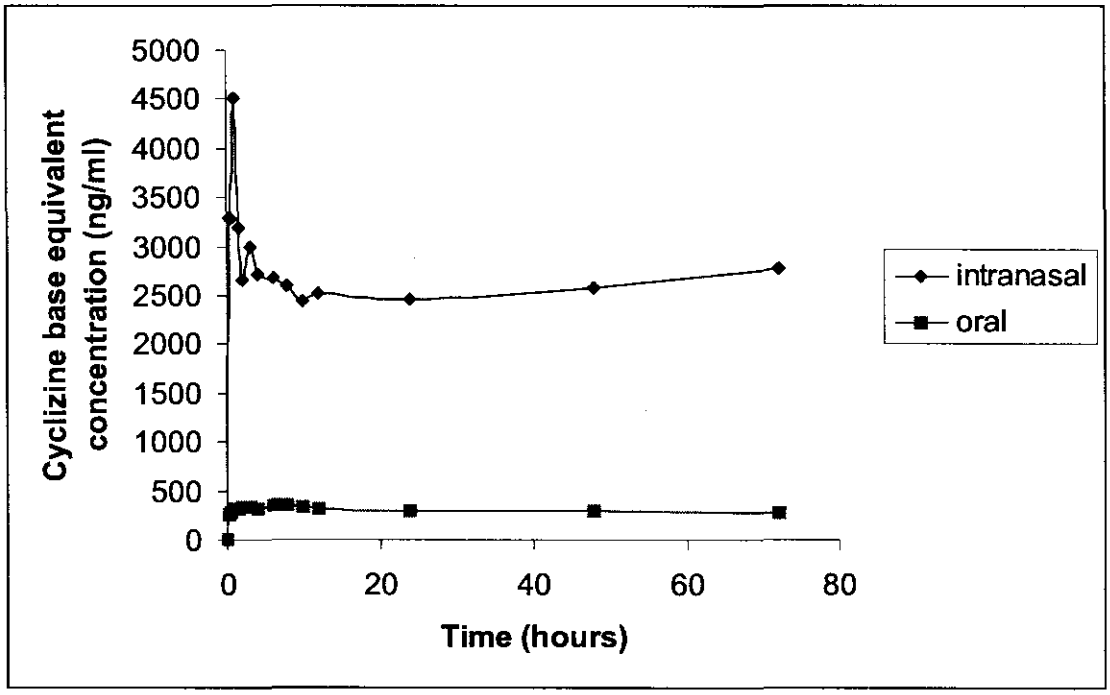


Figure 6.3: concentration of cyclizine (ng/ml) as a function of time (hours) post oral and intranasal administration (n=12)

The mean cyclizine concentrations and the standard deviations (SD) after oral and intranasal administration are presented in table 6.1. These data as a function of time are shown in Figures 6.1 and 6.2 respectively. Figure 6.3 shows the mean cyclizine concentrations following oral and intranasal administration as a function of time.

For both routes of administration, intranasal and the oral route, the terminal phase (48^{th} and 72^{nd} hour samples) showed a slight incline due to the fact that the analysis method was not able to distinguish between the parent compound and the metabolite.

The pharmacokinetic parameters (n=12) of the two routes are summarised in tables 6.2 and 6.3

Table 6.2: Pharmacokinetic parameters for both the intranasal and oral routes of administration.

Pharmacokinetic Parameter	N	Oral Route	Intranasal Route
AUC	12	5943.48ng/ml/h	122860.7ng/ml/h
C_{max}	12	200.79ng/ml	5354.22ng/ml
t_{max}	12	9.46h	3.17h

Table 6.3: Mean bioavailability ratio parameters (AUC, C_{max}, t_{max}) post oral and intranasal administration (mean ± SD) (n=12)

Bioavailability parameter	N	*Mean ratio ± SD (Test /Reference)	Coefficient of Variance (C.V)
AUC	12	19.2± 23.8	124
C_{max}	12	25.6 ± 26.4	103
t_{max}	12	0.41 ± 0.33	79.6

***Mean ratio is calculated as the mean of the individual ratios (test /reference) for all 12 subjects (table 6.3) while table 6.2 reflects the mean of all references versus tests**

The mean AUC ratio indicates that an average of 19.2 fold more cyclizine was registered in blood plasma with the intranasal route compared with the oral route; that the highest plasma concentration which was on average 25.6 fold higher with the intranasal route and was reached in a shorter period compared with the oral route. The 90% cross over t-interval for bioequivalence testing of the two formulations showed statistically significant difference for all parameters AUC [8.69; 18.9]; C_{max} [10.5; 27.8] and t_{max} [0.20; 0.41]. The non-parametric 90% Mann-Whitney intervals also gave similar results.

Table 6.4: The ANOVA log transformed data for the pharmacokinetic parameters (AUC, C_{max}, t_{max})

Source of Variation	p-Values		
	AUC	**C_{max}**	**t_{max}**
Sequence	0.519	0.911	0.787
Period	0.097	0.714	0.00311
Product	0.0000	0.0000	0.00006

Table 6.4 presents p-values for the statistical analysis of the different effects on the pharmacokinetic parameters (AUC, C_{max}, t_{max}). ANOVA for these parameters after log-transformation of the data, showed no statistical difference between the two phases in sequence as indicated by the high p-values for AUC (p=0.519), C_{max} (p=0.825), and t_{max} (p=0.787). Similarly, for period (sampling time) effect no statistical difference was apparent for AUC (p=0.97) and C_{max} (p=0.714) while t_{max} (p=0.00311) shows significantly statistical difference. The product effect for all parameters was found to be statistically significantly different with p-values<0.05.

For t_{max} the parametric point estimate of the difference (test to reference) was 6.28h, and was found to be within the acceptable limits (±20% of the reference mean).

The pharmacokinetic parameters such as AUC, C_{max} and t_{max} were found to be statistically significantly different (p<0.01) following intranasal administration compared with oral administration. This therefore is indicative of the amount of drug absorbed as well as the rate of absorption was found to be statistically significantly higher after intranasal administration compared with post oral administration.

The AUC-values for the two different routes of administration were calculated from the mean cyclizine concentrations determined in plasma (Table 6.1). The AUC-value for cyclizine following oral administration was found to be 5943.48ng/ml/h, which is only 4.8% of the AUC-value of 122860.70ng/ml/h found after intranasal administration. Thus cyclizine was found to be 20.67 times more bioavailable via the intranasal route

compared with the oral route. In other words the intranasal dose can possibly be reduced to 5mg cyclizine compared with the 100mg oral dose to reach the therapeutic blood concentration.

The time to peak plasma concentration of cyclizine lactate after intranasal administration was found to be lower (t_{max} =3.17h), t_{max} geometric mean value of 1.59h than that of the oral route (t_{max} =9.46h), t_{max} geometric mean value of 5.57h and the peak plasma concentration was much higher after intranasal administration (C_{max} =5354.22ng/ml), C_{max} geometric mean value of 3213.92ng/ml, than after oral administration (C_{max} = 200.79ng/ml), C_{max} geometric mean value of 188.596ng/ml/h.

Plasma levels may be used as surrogate parameters for clinical activity. Therefore, the data in this study by providing appropriate statistical results suggest a major difference in the clinical efficacy of the two preparations.

The enhanced bioavailability of cyclizine as reflected by the high AUC-value 122860.7ng/ml/h, (geometric mean value of 74213.75ng/ml/h), post intranasal administration compared with the low AUC-value 5943.48ng/ml/h, (geometric mean value of 5798.11ng/ml/h), following oral administration, can be attributed not only to the anatomical configuration and physiological characteristics of the nasal mucosa but also to the physicochemical properties inherent to both the drug and the excipients employed in the formulation of the intranasal preparation.

The extent of nasal absorption is dependent on the physiological factors such as the rate of MCC. Thus the faster the ciliary movement and the greater the nasal secretion the smaller the bioavailability will be (Harris et al., 1986). The effect of the above mentioned physiological factors can be minimised by formulation approaches such as the utilisation of non-irritating water-miscible gels.

Hydroxypropylmethyl cellulose (HPMC) is said to be both a mucoadhesive and an absorption enhancer. It conferred its mucoadhesive properties to the intranasal

preparation by forming temporary bonds (tethered structure) with the nasal mucosal components. This temporary cohesion localised the preparation at the site of absorption thus promoting intimate contact between the absorbing mucosa and the dosage form for a prolonged period. The theoretically increased retention time of the preparation within the nasal passages exploited maximal release and diffusion of the active moiety from the dosage form into the highly vacularised nasal mucosa bed and hence into the systemic circulation. Another possibility is that the strong bond formation between the cellulose excipient (HPMC) and the nasal mucosal components possibly modified the tissue permeability to enable paracellular transport of the active moiety solution.

The use of absorption enhancers to increase drug permeability through the nasal mucosa has been demonstrated by various researchers to be successful. Suzuki et al., (1999) attained comparable bioavailability for salmon (CT) via the transnasal route as the intravenous route by employing hydroxypropyl cellulose as an enhancer. Hydroxypropyl cellulose acts by transiently widening the tight junctions as well as localising the preparation at the site of absorption which allows for increased residence time and drug diffusion and hence permeation into the systemic circulation (Nagai et al., 1984). Studies by Loftsson et al., (2001) demonstrated increased bioavailability of benzodiazepines with the use of hydroxypropyl methyl cellulose. Absolute bioavailability of 73% was attained intranasally with C_{max} of 543ng/ml/h at 15 minutes. Sasaki et al., (2003) achieved approximately 80-fold increase in the bioavailability of glycyrrhizin via the intranasal route compared with the oral route in rats with surfactants, while Katagani et al., (1998) obtained 21-fold increase in bioavailability of azetirelin intranasally with the use of acylcarnitine compared with the oral route.

The chemical modification of the sparingly water soluble cyclizine HCl to the freely water soluble cyclizine lactate form facilitated the dissolution of a larger amount of solute in a limited volume of solvent (25mg/200µl); a prerequisite for nasal preparations (Behl et al., 1998). Preparations of high concentrations of the lactate form were therefore achievable. Relative aqueous solubility of an active moiety is a prerequisite for permeation and/or absorption across any biological site of absorption (Behl et al., 1998).

Thus high concentrations of this freely water soluble cyclizine salt solute permeated the systemic circulation by paracellular transport through the widened tight junctions of the nasal mucosa (Nagai et al. 1984). Studies by Huang et al., (1985) indicated that structural modification improved nasal absorption of some amino acids. The carboxylic acid esters with higher water-octanol partition coefficient than the parent compound were absorbed 4 to 10 times faster than the parent amino acid. This improved absorption rate is not solely attributable to the partition coefficient but to the absence of the negative charges on the carboxylate moiety. Thus apart from the high water solubility of cyclizine lactate one other factor, which may have contributed to the increased absorption could have been the absence of the negative charge on the carboxylate moiety.

Furthermore, the mucoadhesive cellulose excipients (HPMC) not only allowed for an increased retention time of the dosage form within the nasal cavity but its viscosity also allowed for a more localised *in-vivo* deposition and even distribution of the preparation to the non-ciliated region of the nasal passages where the mucociliary clearance rate is slow and hence flushing of the preparation out of the nasal cavity by the actively beating cilia, is delayed. Thus the formulation tool used for enhancing nasal absorption and hence bioavailability included the use of a viscosity-enhancing agent (Harris et al., 1988). This gave the preparation sufficient contact time with the absorbing mucosa and hence penetration of the active moiety into the systemic circulation. Studies by Pennington et al., (1988) on the effect of solution viscosity on nasal spray deposition and clearance indicated that an increase in the solution viscosity is inversely proportional to the clearance rate of the same solution from the nasal passages. This is reported as a means of providing prolongation of the residence time of the preparation within the nasal cavity, increased drug plasma concentrations and hence improved therapeutic effect of nasal preparations. Hussain et al., (1998) recommended the use of non-irritating water miscible gels to counteract the premature flushing out of an intranasal dosage form by the actively beating cilia and nasal secretions and hence increase in the nasal bioavailability.

The employment of a pump spray device for the administration of the nasal preparation also contributed indirectly to the increased bioavailability of the drug. The pump spray

deposited mainly in the anterior third part of the nose where clearance is very slow. This slow clearance of the preparation exploited maximal contact with the absorbing mucosa resulting in enhanced drug permeation across the nasal mucosa and eventual increase in drug bioavailability. These results indicated that the drug delivery device type probably determines the deposition site of the preparation within the nasal cavity and the extent and rate of absorption of the drug. Studies by Hughes et al., (1993) on the effect of delivery device on nasal deposition and mucociliary clearance in monkeys, found that the different delivery devices (dropper bottle and spray pump) exhibited differing deposition patterns and also deposited at differing sites within the nasal cavity. Dropper bottles deposited more posteriorily and therefore the distance to the oropharynx was shorter and hence clearance faster as opposed to the spray pump. Hardy et al., (1985) also found rapid clearance rates following nasal administration with dropper bottles averaged half times ranging from 6 to 9 minutes while with spray pumps clearance was much slower and deposition was mainly in the third anterior region of the nasal passages where maximal drug absorption occurs. Harris et al., (1986) on comparing the nasal deposition, biological response and the bioavailability of desmopressin by use of different delivery devices found high plasma levels with the use of a pump spray compared with the dropper bottle. The biological response was clearly enhanced after spray administration and produced similar increases in F VIII activity as with the intravenous route. Thus the spray deposited in the non-ciliated anterior third part of the nasal cavity where mucociliary clearance is very slow allowing for maximum drug retention time and hence increase in the drug absorption. It is evident from these studies that the anatomy of the human nose favours inertial impaction of spray preparations in the anterior third part of the nasal cavity.

The cyclizine nasal preparation also exhibited a reduced t_{max} of 1.59h compared with that of the oral route (t_{max} =5.57h). Thus high plasma drug concentrations were reached at a much shorter time interval with the intranasal route than with the oral route. This implies that a more rapid onset of action is achievable with the intranasal route as opposed to the oral route. For antiemetics, anxiolytics, analgesics and possibly anticonvulsants rapid onset of action is required for fast relief of the symptoms. These results demonstrate that

cyclizine intranasal preparation is a practical alternative to the conventional route in cases of emesis, motion sickness, nausea and vomiting where rapid onset of action is required and also where therapeutic drug levels are unachievable due to non-absorbance caused by vomiting.

The reduced t_{max} obtained with the nasal route is in accordance with previous studies by various researchers. Ramanathan et al., (1998) achieved high nasal bioavailability (94%) of promethazine with a t_{max} of 7.3min compared with the low oral bioavailability averaging 22 to 25% with a t_{max} of 50min. The intranasal preparation would therefore give a faster relief in gastric complaints and gastric motility experienced during space flights. Hussain et al., (1997) also achieved a reduced t_{max} of 0.1h with intranasal absorption of oxymorphone, narcotic analgesic used for the treatment of chronic pain (for post caesarean and cancer pain). Nasal bioavailability was also found to be 43% compared with 15% of the oral preparation. Gizurarson et al., (1999) also achieved high bioavailability of 50.4% and a reduced t_{max} of 18minutes with diazepam intranasal administration. The results obtained indicated that the intranasally administered diazepam may be an effective alternative route in the relief of seizure in an acute situation when medical personnel is not available on location.

Documented research on drug bioavailability improvement using the nasal route includes the following. Studies by Li et al., (2002) on the development of an intranasal delivery of diazepam revealed an increased bioavailability of 77% with a t_{max} of 2 minutes. The achievement of a rapid onset of action for diazepam would meet the emergency therapeutic purpose of the drug in the treatment of the seizure termination and control. Studies by Katagani et al., (1998) showed an increased nasal bioavailability for azetirelino of 17% compared with that of the oral route, which was 0.8%. Incorporation of an absorption enhancer lauroycarnitine (LCC) further increased the nasal bioavailability to 96.9% although morphological studies of the nasal mucosa post treatment revealed ultrastructural damage possibly due to the enhancer. Dahlin et al., (2001) demonstrated comparable nasal bioavailability (100±30%) as intravenous for the physiostigmine analogue (NXX-066). Van de Kuy et al., (1999) obtained nasal

bioavailability to be 25% compared with the oral bioavailability of 8% for dihydroergtamine mesilate.

6.5 Conclusion

Based on the results obtained it is evident that high drug plasma levels (C_{max}) and hence increased bioavailability (AUC) were achievable within a short period of time (t_{max}) with the intranasal route compared with the oral route due to the following:

The favourable anatomical configuration and physiological characteristics of the nasal mucosa allowed for enhanced drug permeation to the systemic circulation and avoidance of the first pass effect and proteolytic degradation of the drug.

Chemical modification of the parent compound lead to high aqueous solubility thus facilitating a high dose in a limited volume of solvent as required for nasal administration. Furthermore, the absence of a negative charge on the carboxylate moiety, lactate group, could have contributed to the increased absorption.

Incorporation of a ciliofriendly mucoadhesive, which, localised the dosage form for a prolonged period of time at the site of absorption, allowing for the exploitation of an intimate contact between the dosage form and the absorbing mucosa.

The use of a spray pump device deposited the preparation in the non-ciliated anterior third region of the nasal passages allowing for maximal residence time of the preparation due to slow mucociliary drug clearance in this region.

Selection of the ideal viscosity of the preparation (0.6%(w/v)) which allowed for a more localised distribution and anterior deposition hence slower clearance and prolonged residence time at the site of maximum absorption and eventual increase in the drug bioavailability.

References

Abboud T K, Zhu J, Longhitano M. Transnasal butorphanol: Efficacy and safety of butaphanol nasal spray for the relief of postepisiotomy pain. *Current Therapeutic Research;* 1994 55:500-509

Abdou H M. Dissolution, bioavailability and bioequivalence, Pennsylvania: Mack;1989:546

Ahsan F, Arnold J, Meezan E. Enhanced bioavailability of calcitonin formulated with alkylglycosides following nasal and ocular administration in rats. *Pharmaceutical Research;* 2001 18 (12):1742-1746

Behl C R, Pimplaskar H K, Sileno A P, deMeireles J, Romeo V D. Effects of physicochemical properties and other factors on systemic nasal drug delivery. *Advanced Drug Delivery Reviews;*1998 29:89-116

Chow S C, Liu J P. Design and analysis of bioavailability and bioequivalence studies, Marcel Dekker, Inc, New York; 2000

Combe R D, Berridge T, Connelly J, Eve M D, Garner R C, Toon S, Wilcox P. Early microdose drug studies in human volunteers can minimise animal testing: Proceedings of a workshop organised by Volunteers in research and testing. *European Journal of Pharmaceutical Sciences*; 2003 19 (1): 1-15

Dahlin M, Bjork E. Nasal administration of physiostigmine analogue for Alzheimer's disease to rats. *International Journal of Pharmaceutics;*2001 212:267-274

Gizurarson S, Gudbrandsson F K, Jonsson H, Bechgaard E. Intranasal administration of diazepam aiming at the treatment of acute seizures: Clinical trials in healthy volunteers. *Biological Pharmaceutical Bulletin;* 1999 22 (4): 425-427

Hardy J G, Lee S W, Wilson C G. Intranasal drug delivery by spray and drops. *Journal of Pharmacy and Pharmacology;* 1985 37: 294-297

Harris A S, Nilsson I M, Wagner Z G, Alkner U. Intranasal administration of peptides; nasal deposition, biological response and absorption of desmopressin. *Journal of Pharmaceutical Sciences;* 1986 75 (11) 1085-1088

Harris A S, Nilsson I M, Wagner Z G, Alkner U. Intranasal administration of peptides: Nasal deposition, biological response and absorption of desmopressin. *Journal of Pharmaceutical Sciences;* 1986 75 (11): 1085-1088

Harris A S, Svensson E, Wagner Z G, Lethagen S, Nilson I M. Effect of viscosity on particle size, deposition ad clearance of nasal delivery systems containing desmopressin. *Journal of Pharmaceutical Sciences;* 1988 77 (5):405-408

Helmers J H, Keifer A T, Rosenberger J L. Comparison of intravenous and intranasal sufentanyl absorption and sedation. *Canadian Journal of Anaesthesia;* 1989 36: 494-497

Huang C H, Kimura R, Bawarshi- Nassar R, Hussain A. Mechanism of nasal absorption of drugs II: Absorption of L-tyrosine and the effect of structural modification on its absorption. *Journal of Pharmaceutical Sciences;* 1985 74 (12): 1298-1301

Hughes B L, Allen D L, Dorato M A, Wolff R K. Effect of delivery devices on nasal deposition and mucociliary clearance in Rhesus monkeys. *Aerosol Science and Technology;* 1993 18:241-249

Hussain A A. Intranasal drug delivery. *Advanced Drug Delivery Reviews;*1998 29 (1-2):39-49

Hussain M A, Aungst B J. Intranasal absorption of oxymorphone. *Journal of Pharmaceutical Sciences;* 1997 86 (8): 975-976

Katagani S, Inaba N, Fuki M, Sonobe T. Nasal absorption kinetic behaviour of azetirelin and its enhancement by acylcarnitines in rats. *Pharmaceutical Research;* 1998 15 (1): 77-81

Klocker N, Hanschke W, Toussaint S, Verse T. Scopolamine nasal spray in motion sickness: a randomised, controlled, and crossover study for the comparison of two scopolamine nasal sprays with oral dimenhydrinate and placebo. *European Journal of Pharmaceutical Sciences;* 2001 13 (2): 227-232.

Li L, Nandi I, Kim K H. Development of an ethyl laurate-based microemulsion for rapid onset intranasal delivery of diazepam. *International Journal of Pharmaceutics;* 2002 237 (1-2): 77-85

Loftsson T, Guomundsdottir H, Sigurjonsdottir J F, Sigurosson H H, Sigfusson S D, Masson M, Stefansson E. Cyclodextrin solubilisation of benzodiazepines: formulation of midazolam nasal spray. *International Journal of Pharmaceutics;* 2001 212:29-40

Logemann C D, Rankin L M. Newer intranasal migraine medications. *American Family Physician;*2000 61 (1) 180-186

Nagai T, Nishimoto Y, Nambu N, Suzuki Y, Sekine K. Powder dosage form of insulin for nasal administration. *Journal of Controlled Release;* 1984 1: 15-22

Najib N M, Idkaidek N, Adel A, Mohammed B, Al-Masri S, Admour I, Alam S M, Dham R, Qumaruzaman. Comparison of two cyclosporin formulations in healthy Middle Eastern volunteers: Bioequivalence of the new Sigmasporin Microoral and Sandimmun Neoral. *European Journal of Pharmaceutics and Biopharmaceutics;* 2003 55:67-70

Ormrod D, Goa K L. Intranasal metoclopramide. *Drugs;* 1999 58 (2): 315-324

Pennington A K, Ratcliffe J H, Wilson C G, Hardy J G. The influence of solution viscosity on nasal spray deposition ad clearance. *International Journal of Pharmaceutics;* 1988 43:221-224

Qu R P, Zheng H. Sample size calculation for bioequivalence studies with high order crossover designs. *Controlled Clinical Trials;* 2003 in press

Ramanathan R, Geary R S, Bourne D AW A, Putcha L. Bioavailability of intranasal promethazine dosage forms in dogs. *Pharmacological Research;* 1998 38 (1):35-39

Ramanathan R, geary R S, Bourne D W A, Putcha L. Bioavailability of intranasal promethazine dosage forms in dogs. *Pharmacological Research;* 1998 38 (1): 35-39

SAS Institute Inc., *The SAS System for Windows Release 8.02* TS Level 02M0 Copyright© 1999-2001 by SAS Institute Inc., Cary, NC, USA:1999.

Sasaki K, Yonebayashi, Yoshida M, Shimizu K, Aotsuka T, Takayama K. Improvement in the bioavailability of poorly absorbed glycyrrhizin via various non-vascular administration routes in rats. *International Journal of Pharmaceutics;* 2003 265 (1-2): 95-102

Shah V P, Midha K K, Sighe S, McGilveray I J, Skelly J P, Yacobi A, Layloft T, Viswanathan C T, Cook C E, McDowall R D, Pitman K A, Spector S. Analytical methods validation: bioavailability, bioequivalence and pharmacokinetic studies. *European Journal of Drug Metabolism and Pharmacokinetics;* 1991 16 (4): 249-255

Shargel L, Yu A. Bioavailability and bioequivalence. In *Applied Pharmaceutics and Pharmacokinetics* 4th Edition, McGraw-Hill Medical Publishing Division, New York;1999:250

Striebel H, Pommerning J, Rieger A. Intranasal fentanyl titration for post operative pain management in an unselected population. *Anaesthesia;*1993 48:753-757

Suzuki Y, Makino Y. Mucosal drug delivery using cellulose derivatives as a functional polymer. *Journal of Controlled Release;* 1999 62:101-107

Van de Kuy, Lohman J J H M, Hooymans P M, Treberg J W M, Merkus F W H M. Bioavailability of intranasal formulations of dihydroergotamine. *European Journal of Pharmacology;*1999 55:677-680

CHAPTER SEVEN

LIMITATIONS AND RECOMMENDATIONS

7.1 LIMITATIONS

7.1.1 Ciliary beat frequency and nasal morphology assessment

The importance of this type of study lies in the fact that interference with mucociliary clearance (MCC) can lead to lower airways infections. If the drug or formulation components inhibit ciliary beating, this effect must be completely reversible upon removal of the noxious compound. This reversibility is best ascertained *in-vivo* under normal physiologic conditions of an animal, but of course *in-vitro* systems offer quick convenient means for such toxicity screenings. While being very easy to use, *in-vitro* systems have the limitation of the absence of the protective mucus layer, which dilutes the noxious compounds prior to contact with the cilia.

7.1.2 Deposition and assessment of spray pump device

Although spray pump device was preferred in terms of reproducibility and accuracy of delivery of dosing, Ugwoke et al., (2001) stated that the duration and condition of storage as well as the physico-chemical properties of the formulation such as viscosity, homogeneity (e.g. of suspensions) affect the dosing accuracy. Furthermore, spray pump device requires thorough and regular cleaning for it is prone to microbial contamination and frequently requires the incorporation of a preservative, which could be cilio-toxic (van de Donk et al., 1980; Batts et al., 1990).

7.1.3 Pharmacokinetic Studies

7.3.1.1 Clinical trials

Subject recruitment

Subject recruitment for this trial was mainly based on monetary payment .An outline of the study was read and the subjects gave in an informed consent. No prior thorough planning for this activity was conducted. Halpern, (2002) describes participant recruitment for randomised controlled trials as the most difficult and challenging aspect of clinical trials. Various researchers (Cassileth et al., 1980; Applebaum et al., 1987; Lavelle-Jones et al., 1993; Davis et al., 1998; Taylor et al., 1998; Veatch, 1999) stipulate that the most widely recognised deficiency of informed consent is that subjects typically have a poor understanding of the information provided by the investigators. Moreover, subjects considering enrolling in trials may be intimidated by investigators and thus participate in studies even when doing so is inconsistent with their true interests (Drazen et al., 2000). Typically informed consent is granted at the time of enrolment decision when receipt of the perceived benefits of participation is contingent upon consenting. Furthermore, the traditional informed consent process is often inadequate to respect the research participants' autonomy. Past efforts to overcome these problems are not universally applicable. A new method, the prospective preference assessment (PPA) is suggested as a way to simultaneously enhance participant accrual, identify groups of subjects to whom the trial's results may apply and promote subjects' interests. PPA is said to be a method by which investigators would evaluate the potential trial subjects' motivations for and concerns about enrolling in a planned trial prior to the formal recruitment (Halpern, 2000). Moreover, the informed consent for this trial consisted of a clause, which stipulates that the subject participates at his own risk and that no investigating authority/body should be held responsible. Protecting the rights, interests and safety of the subjects in research is an ethical mandate. Although risk is inevitable in clinical research, it is essential that the risk is minimised and that any unanticipated harm be rapidly detected and contained. It is a widely held belief that the current system of oversight of clinical research, particularly the means of assessing risks and minimising harms to the subjects in clinical trials, could be improved. Research ethics review groups

are not able to perform safety monitoring of individual adverse events and are often overburdened by duplicate reviews of large multicenter studies. There are no standards for data monitoring committees (DMC) to ensure they can reliably identify safety issues (Califf et al., 2003). Theoretically, the activities of all the research groups (Research Ethics Review Boards) should be integrated to avoid duplication and inefficiency. An ideal system would be the one in which careful study of adverse reactions, with input from each group commensurate with its role, would lead to a reduction in harms and risks without creating unnecessary consternation and possibly inappropriate response to individual adverse effects (Califf et al., 2003).

Clinical trial monitoring

No proper dissemination of trial-related information was conducted during the preparation period for this study. Yet section 5.18.4 subsection (g) and section 4.2.2 of the Good Clinical Practice guidelines state that it is the duty of the investigator to ensure that persons assisting with the trial are adequately informed about the protocol, the investigational product(s) and their trial-related duties and functions (ICH Guidelines for GCP, 1996).

Sample analysis

All analytical procedures require rigorous adherence to Good Laboratory Practice GLP) and Good Clinical Practice (GCP).Thus there should be uniformity in terms of laboratory conditions under which the samples are subjected, to minimise analytical variation. In this study analytical samples were stressed by movement among four (4) sites/ laboratories under abnormal conditions for various analytical procedures (biosample thawing, drug extraction, sample drying and HPLC analysis). It is of utmost importance that analytical conditions are kept optimal and uniform for all procedures to eliminate inter-laboratory variation and/or stress to the analytical samples. Good Laboratory Practice guidelines stipulate that for research purposes particular importance should be attached to installations and equipment in order to ensure that research is carried out under adequate conditions (Wallin, 1996).

References

Applebaum P S, Roth L H, Lidz C W, Benson P, Winslade W. False hopes and best data: consent to research and the therapeutic misconception. *Hastings Centre for Rep;*1987 17:20-24

Batts A H, Marriott C, Martin G P, Wood C F, Bond S W. The effect of some preservatives used in nasal preparations on the mucus and ciliary components of mucociliary clearance. *Journal of Pharmacy and Pharmacology;*1990 42: 145-151

Califf R M, Morse M A, Wittes J, Goodman S N, Nelson D K, DeMets M S, Iafrate R P, Sugarman J. Towards protecting the safety of participants in clinical trials. *Controlled Clinical Trials;* 2003 24 (3): 256-271

Cassileth B R, Zupkis R V, Sutton-Smith K, March V. Informed consent- why are its goals imperfectly realised? *North England Journal of Medicine*; 1980 302: 896-900

Davis T C, Holcombe R F, Berkel H J, Pramanik S, Divers S G. Informed consent for clinical trials: a comparative study of standards versus simplified forms. *Journal of National Cancer Institute;* 1998 90:668-674

Drazen J M, Koski G. To protect those who serve. *North England Journal of Medicine*; 2000 343: 1643-1645

Halpern S D. Prospective preference assessment a method to enhance the ethics and efficiency of randomised controlled trials. *Controlled Clinical Trials;* 2002 23 (3): 274-288

ICH. Monitoring In: ICH Harmonised Tripartite Guideline for Good Clinical Practice; 1996: 21, 38-39

Lavelle-Jones C, Byrne D J, Rice P, Cuschieri A. Factors affecting quality of informed consent. *British Medical Journal;* 1993 306:885-890

Taylor K M, Bezjak A, Fraser R H S. Informed consent for clinical trials: is simpler better? *Journal of National Cancer Institute;* 1998 90:644-645

Ugwoke M I, Verbeke N, Kinget R. The biopharmaceutical aspects of nasal mucoadhesive drug delivery. *Journal of Pharmacy and Pharmacology;* 2001 53: 3-22

Van de Donk H J M, Zuidema J, Merkus F W H M. The influence of pH and osmotic pressure upon tracheal ciliary beat frequency as determined with a new photoelectric registration device. *Rhinology;*1980 18:93-104

Veatch R M. Abandoning informed consent. In *Bioethics: an anthology* Eds Kuhse H and Singer. *Blackwell Publishers, Malden Massachusetts*; 1999: 523-531

Wallin R F. Fundamentals of Good Laboratory Practice. In: *Good Clinical Practices;* 1996:2

7.2 RECOMMENDATIONS

7.2.1 Ciliary beat frequency (CBF) and nasal epithelium morphology assessment

In-vitro experiments should be designed so as to take into account the 10 times mucus dilution which occurs *in-vivo* as stated by Streichenberger et al., (1992).

7.2.2 Clinical trials

Although the suggested prospective preference assessment (PPA) method may seem costly and time-consuming (Halpern, 2000), randomised controlled trials should adapt PPA into their procedures. This would make clinical trials more democratic by allowing subjects to provide input, alongside investigators, as to the relative merits of the interventions being compared and the costs and the benefits of alternate study design to make such comparisons. Potential research subjects could assist the investigators in designing studies to best serve their interests (Karlawish et al., 1997). Thus just as political and scientific developments have fostered a growing emphasis on the contributions subjects may make to their clinical care, similar forces coupled with basic ethical principles suggest that potential research subjects' perspectives should also be elicited (Heymann, 1995; Blumenthal, 1996; Marks 1997; Bayer et al., 2000).

The proposal to heed the views of potential subjects and their clinicians does not imply a radical overhaul of the research process. Clinical trials must continue to be designed and conducted by those uniquely qualified to do so. When evaluations of potential subjects' preferences reveal strong reservations about enrolling in a particular trial, such information may guide investigators' choice of alternate designs that may be more appealing and thus more efficient. In addition to increasing enrolment and broadening the diversity of subjects, enrolling subjects who genuinely identify with the goals of the research may minimise post randomisation losses. (Vollmer et al., 1994; Heymann, 1995; Silverman et al., 1996).

References

Bayer R, Oppenheimer G M. Towards a more democratic medicine: sharing the burden of ignorance In: *Voices from the epidemic*, New York, Oxford University Press AIDS Doctors; 2000:156-169

Blumenthal D. Quality of care-What is it? *North England Journal of Medicine*;1996 335:891-894

Heymann S J. Patients in research: not just subjects, but partners. *Science*;1995 269:797-798

Karlawish J H T, Lantos J. Community equipoise and the architecture of clinical research. *Camb Q Health Ethics*;1997 6:385-396

Marks H. The dreams of reason: retrospect and prospect. In: *The progress of experiment: science and therapeutic reform in the United State*, Cambridge University Press , Cambridge, United KIngdom;1997: 229-248

Silverman W A, Altman D G. Patients' preference and randomised trials. *Lancet*; 1996 347:171-174

Streichenberger G, Domage G, Domage L. Microsphere for nasal administration. In: Duchene D (Ed), *Buccal and nasal administration as an alternative to parenteral administration*. Edition de Sante Paris. 1992; :85-98

Vollmer V M, Osborn M L, Hertert S et al. Recruiting hard to reach subjects: is it worth the effort? *Controlled Clinical Trials*;1994 15:154-159

APPENDICES

APPENDIX 1

almu, s.a.
PRODUCTOS QUÍMICOS FARMACÉUTICOS
Ctra. de Zeneta nº 149, 30.130 BENIEL (Murcia)
Phone (968) 85 42 66 – 77 Fax: (968) 85 40 61 –
http:// www.almu.es

CERTIFICATE OF ANALISYS

PRODUCT: CYCLIZINE HYDROCHLORIDE

BATCH NUMBER: A-111-99100

MANUFACTURING DATE: JUN-1999 **EXPIRY DATE:** JUN-2001

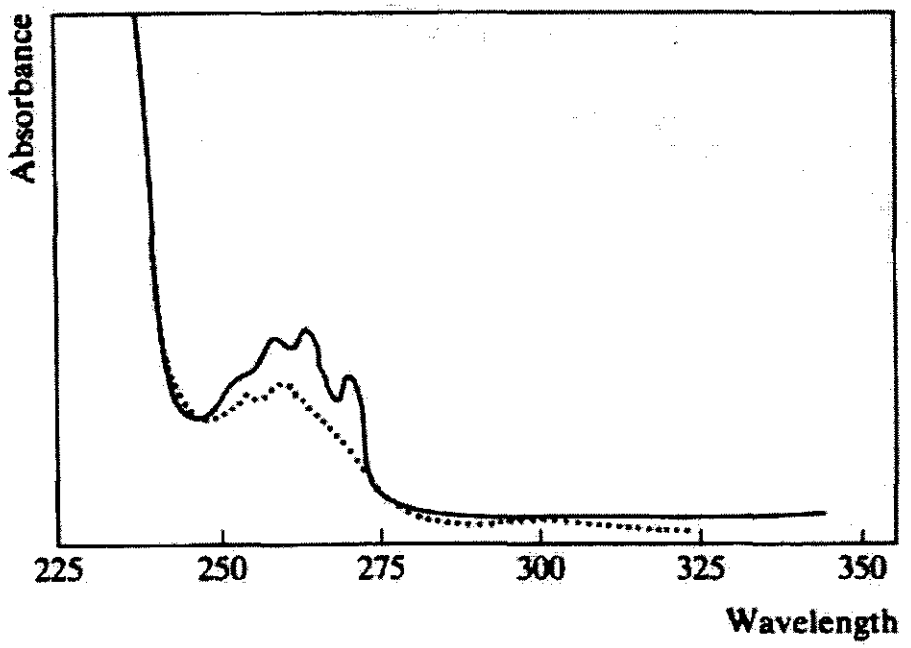
PARAMETERS	SPECIFICATIONS	RESULTS
Description	White, crystalline powder.	Complies
Solubility	Slightly soluble in water and in alcohol; sparingly soluble in chloroform; insoluble in ether	Complies
Identification		
A)IR Spectrum	Concordant with the Reference.	Complies
B)UV Spectrum 0,002% in sulphuric acid	Abs max. At 225 nm= 0,78 abt	Complies
C)M.P. of the precipitate	Abt. 107° C	Complies
D)Characteristic chlorides reaction	Positive	Complies
Assay (on dried basis)	98,5% - 100,5%	99,7%
Loss on drying (at 130°C)	≤ 1,0 %	0,29%
Sulfated ash	≤ 0,1 %	0,03%
pH	4,5 – 5,5	4,7
Clarity and colour of solution	2% solution in ETOH/H_2O (2:3) is clear and colourless	Complies

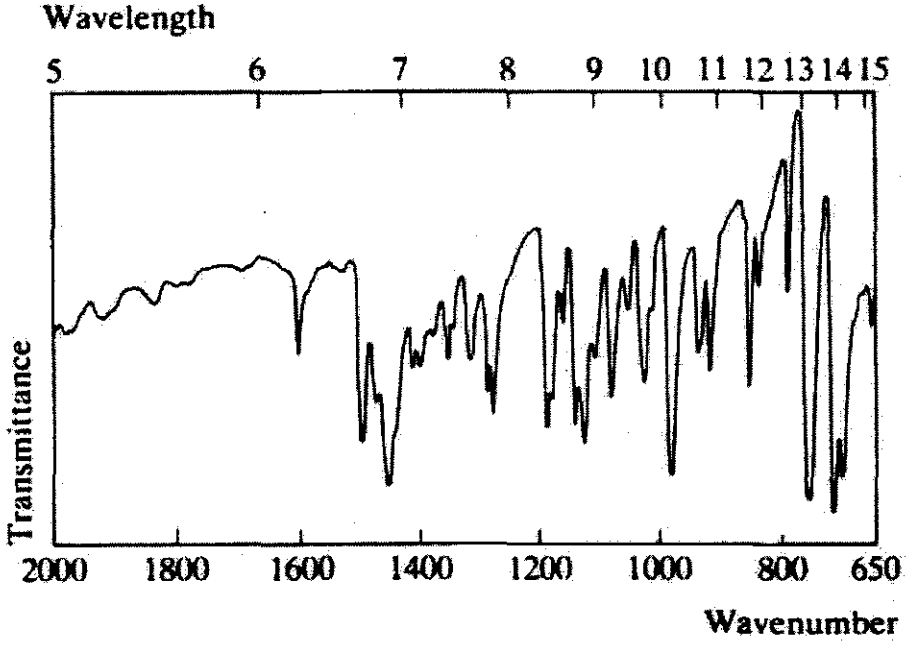
21/10/99

Represented By :
Malachite Chemicals
Phone : (011) 455-1201
Fax : (011) 455-1306

21/10/99

APPENDIX 2: UV AND IR SPECTRA FOR CYCLIZINE HCl (ADAPTED FROM CLARKE, 1986)



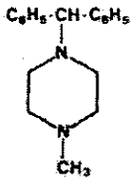


APPENDIX 3

Cyclizine

Antihistamine/Anti-emetic

1-Benzhydryl-4-methylpiperazine
$C_{18}H_{22}N_2 = 266.4$
CAS—82-92-8



A white or creamy-white crystalline powder. M.p. 106° to 109°. Practically **insoluble** in water; soluble 1 in 6 of ethanol, 1 in 1 of chloroform, and 1 in 6 of ether.

Cyclizine Hydrochloride

Synonym. Cyclizinium Chloride

Proprietary Names. Marezine (tablets); Marzine; Valoid (tablets). It is an ingredient of Diconal, Migril, and Wellconal.

$C_{18}H_{22}N_2,HCl = 302.8$
CAS—303-25-3

A white crystalline powder, or small colourless crystals. M.p. about 285°, with decomposition.

Soluble 1 in about 125 of water, 1 in about 120 of ethanol, and 1 in 75 of chloroform; practically insoluble in ether.

Cyclizine Lactate

Proprietary Names. Marezine (injection); Valoid (injection).

$C_{18}H_{22}N_2,C_3H_6O_3 = 356.5$
CAS—5897-19-8

Freely **soluble** in water.

Dissociation Constant. pK_a 2.4, 7.8.

Colour Tests. Liebermann's Test—orange; Marquis Test—yellow.

Thin-layer Chromatography. *System TA*—Rf 57; *system TB*—Rf 49; *system TC*—Rf 41. (*Dragendorff spray*, positive; *acidified iodoplatinate solution*, positive; *Marquis reagent*, yellow.)

Gas Chromatography. *System GA*—cyclizine RI 2020, norcyclizine RI 2050; *system GB*—RI 2081; *system GC*—RI 2348; *system GF*—RI 2320.

High Pressure Liquid Chromatography. *System HA*—cyclizine k' 2.9, norcyclizine k' 2.2.

Ultraviolet Spectrum. Aqueous acid—257 nm, 262 nm ($A^{1\%}_{1cm}$ = 28 a), 268 nm; aqueous alkali—260 nm ($A^{1\%}_{1cm}$ = 16 b). (See below)

Infra-red Spectrum. Principal peaks at wavenumbers 716, 756, 701, 984, 1125, 1496 (cyclizine hydrochloride, KBr disk). (See below)

Mass Spectrum. Principal peaks at *m/z* 99, 56, 167, 207, 194, 266, 195, 165.

Quantification. GAS CHROMATOGRAPHY. In plasma or urine: cyclizine and norcyclizine, sensitivity 10 ng/ml for cyclizine, AFID—G. Land *et al.*, *J. Chromat.*, 1981, *222*; *Biomed. Appl.*, *11*, 135–140. In blood or urine: sensitivity 10 ng/ml and 5 ng/ml,

APPENDIX 4: LETTER OF APPROVAL FOR USE OF HUMAN NASAL BIOPSIES

Potchefstroomse Universiteit
vir Christelike Hoër Onderwys

Privaatsak X6001 Potchefstroom 2520
Etiekkomitee
Tel (018) 299 2263
Faks (018) 299 2225
E-Pos fchjcb@puknet.puk.ac.za
8 Junie 2000

Dr AF Kotzé
Bussie 36
PU vir CHO
2520

GeagteDr AF Kotzé,

GOEDKEURING VIR EKSPERIMENTERING MET MENSE

Hiermee wens ek u in kennis te stel dat u projek getiteld *"In Vitro evaluation of the effect of pharmaceutical compounds on the ciliary beat frequency of human nasal epithelia"* deur die Etiekkomitee van hierdie universiteit goedgekeur is onder die nommer **00M14.**

Die volgende kommentaar is van die beoordelaars ontvang en word vir u kennisname en, waar nodig aksie, aan u deurgegee:

> *Hier word nie gesê 1) hoeveel proefpersone gebruik gaan word, 2) of dit belangrik is dat van verskillende persone gebruik gemaak gaan word, 3) wie die proefpersone is en hoe hulle gewerf word, 4) uitsluitingskriteria (bv. Rokers ens.).*
>
> *Die projek is nie 'n etiese probleem nie, maar die tersaaklike inligting moet gegee word.*

Gebruik asseblief laasgenoemde nommer in alle korrespondensie rakende bogenoemde projek en let daarop dat daar van projekleiers verwag word om jaarliks in Junie op die voorgeskrewe vorm (wat voorsien sal word) aan die Etiekkomitee verslag te doen insake etiese aspekte van hulle projekte asook van publikasies wat daaruit voortgespruit het.

Goedkeuring van die Etiekkomitee is vir 'n termyn van hoogstens 5 jaar geldig (volgens Senaatsbesluit van 4 November 1992, art. 9.13.2). Vir die voortsetting van projekte na verstryking van hierdie tydperk moet opnuut goedkeuring verkry word.
Die Etiekkomitee wens u alle voorspoed met u werk toe.

Vriendelike groete

p/p
JC Breytenbach
Sekretaris: Etiekkomitee
FCHc:\fiona2\farmch~1\profbr~1\goedmns.doc

APPENDIX 5: LETTER OF APPROVAL FOR ANIMAL STUDIES

FROM : PHONE NO. : Nov. 27 2003 11:30AM P1

01D16

Potchefstroomse Universiteit
vir Christelike Hoër Onderwys

Privaatsak X6001 Potchefstroom 2520
Tel (018) 299 1111 Faks (018) 299 2799
http://www.puk.ac.za

Etiekkomitee
Tel (018) 299-2256
Faks (018) 299-2284
E-Pos fchjcb@puknet.puk.ac.za

Prof DG Muller
Bussie 36
PU vir CHO

3 Augustus 2001

Geagte prof Muller

GOEDKEURING VIR EKSPERIMENTERING MET DIERE

Hiermee wens ek u in kennis te stel dat u projek getiteld *"Loodsstudie 2: die bepaling van intra- en inter rot variasie ten opsigte van die transport siklosporien in rotte met die doel om 'n in-vitro weefselmodel te vestig"* deur die Etiekkomitee van hierdie universiteit goedgekeur is onder die nommer **01D16**.

Die kommentaar gegee by aansoek 01D15 is ook hier van toepassing.

Gebruik asseblief laasgenoemde nommer in alle korrespondensie rakende bogenoemde projek en let daarop dat daar van projekleiers verwag word om jaarliks in Junie op die voorgeskrewe vorm (wat voorsien sal word) aan die Etiekkomitee verslag te doen insake etiese aspekte van hulle projekte asook van publikasies wat daaruit voortgespruit het.

Goedkeuring van die Etiekkomitee is vir 'n termyn van hoogstens 5 jaar geldig (volgens Senaatsbesluit van 4 November 1992, art. 9.13.2). Vir die voortsetting van projekte na verstryking van hierdie tydperk moet opnuut goedkeuring verkry word.

Die Etiekkomitee wens u alle voorspoed met u werk toe.

Vriendelike groete

J C BREYTENBACH
Sekretaris: Etiekkomitee

BIOCEN

1.1. Study Co-ordinating Site:BIOCEN, Potchefstroom Universiteit vir CHO, Potchefstroom.

Clinical Site: BIOCEN,
University of Potchefstroom, South Africa

Sponsor: CSIR, Pretoria

STRICTLY CONFIDENTIAL

CLINICAL RESEARCH PROTOCOL

STATUS = Final

TITLE

DETERMINATION OF THE PHARMACOKINETICS OF CYCLIZINE HCL AFTER SINGLE DOSE INTRANASAL AND ORAL ADMINISTRATION TO HEALTHY VOLUNTEERS

PROTOCOL NO. BIO-010203

Date of first draft: 27/03/03

Date of Final:

Clinical Development Phase: 1

Test Products: Cyclizine HCl 50 mg tablets
: Cyclizine Lactate 50 mg IN gel

CONFIDENTIAL/PROPRIETARY STATEMENT

Title	Determination of the pharmacokinetics of cyclizine HCl after single dose intranasal and oral administration to healthy volunteers.	
1.2. Protocol No:		BIO-010203
Study Design:	Mode	A single blind three phase cross-over stud
	Dosing	Single dose
	Participants	12 healthy volunteers
	Specimens	Plasma samples collected pre-dosing on Day 1 and post-dosing for 72 hr
	Analyte	Cyclizine
Study Medication:		
A)	Valoid ®	
	Strength:	50 mg
	Dose:	A single dose on day 1
	Form:	Tablets
	Batch No:	TO BE SUPPLIED
	Expiry Date:	TO BE SUPPLIED
	Manufacturer:	GlaxoWellcome South Africa (Pty) Ltd Old Pretoria Rd Midrand, South Africa
B)	Valoid ®	
	Strength:	25mg/ml
	Dose:	A single dose on day 1
	Form:	Intranasal gel
	Batch No:	TO BE SUPPLIED

Expiry date:	TO BE SUPPLIED
Manufacturer:	Pharmaceutics Dept PU for CHE Box 36 Potchefstroom

Assessment Criteria:
Concentrations of cyclizine in plasma.

Primary Variables
- Area under the plasma concentration time curves (AUC_{0-t})
- Mean plasma level after 72 hours (C_{av} [4])
- Maximum measured concentrations (C_{max})

Secondary Variables
- Time to maximum concentration after dosing (t_{max})

Sponsor:

APPROVAL SIGNATURES

Site Investigator: Prof D G Müller, B Sc, B Sc Hons (Chem), B Sc (Pharm), M Med Sc, D Sc

Signed:_____ Date:_____

Project Manager: Dr M M Malan, B Sc (Pharm), M Pharm (Pharmaceutics) Ph D

Signed:_____ Date:_____

Sponsor: Me J van Oudshoorn, M Sc

Signed:_____ Date:_____

Clinician: Dr G E Y Schulze, B Sc, Hon B Sc, MB Ch B

Signed:_____ Date:_____

Biostatistics: Prof H S Steyn, B Sc, M Sc, Ph D

Signed:_____ Date:_____

The *curricula vitarum* of the Investigators are kept on file in the BIOCEN Clinic. (Copies Appended).

PROTOCOL SUMMARY

Title: Determination of the pharmacokinetics of cyclizine HCl after single dose intravenous, intranasal and oral administration to healthy volunteers

1.3. Background

Little is known about the pharmacokionetic and pharmacodynamic propertiesof cyclizine, available literature reports that the drug undergoes an extensive first pass effect post oral administration thus reducing the bioavailability intensively (Walker, 1995).

This low bioavailability of cyclizine can be overcome by the use of a different route of administration i.e. the intranasal route. Studies based on the application of the intranasal route of administration for most problem drugs have indicated rapid kinetics of absorption and enhanced bioavailability comparable to the parenteral route due to:

- The direct accessibility of the highly vascularised nasal mucosa surface to the drug delivery system and to the systemic circulation.
- Avoidance of the gut wall metabolism and hepatic extraction effect by direct blood drainage from the nose to the systemic circulation.
- Furthermore, the intranasal route offers ease of administration for the drug preparation, which increases the likelihood of patience compliance especially in geriatric and paediatric patients.
- Normally for patients with nausea and vomiting whereby oral administration is unsuitable since vomiting prevents the absorption of the drug, application of the intranasal route would be a feasible, non-invasive alternative form of drug administration.

It is therefore the objective of this study to compare the bioavailability of the drug using the three different routes of administration viz. Intravenous, intranasal and oral.

Objectives

To assess/compare the bioavailability of cyclizine from each of the three routes of administrations after 72 hours and after administration to 12 healthy volunteers.

Study Design

Single dose, randomised, three-way crossover with a washout period of at least 7 days between each dosing period.

Volunteers

Twelve (12) healthy volunteers, aged between 18 and 45 years.

1.4. Treatment

Valoid 100mg tablet,GlaxoWelcome(Pty) Ltd, Midrand South Africa
Cyclizine 125mg/ml intranasal gel, Pharmaceutics Dept, PU for CHE, South Africa.

1.5. Dosage Regimen

Cyclizine 100 mg tablet per dosing period per volunteer.
Cyclizine 125mg/ml i.eintranasal gel per dosing period per volunteer

Blood Sampling

Blood samples will be collected prior to dosing and at 15, 30,45, 60, 120, 180, 240, 360, 480, 600, 720 mins, 24 hrs, 48 hrs and 72hrs after drug administration.

Each blood sample will be collected into Li-Heparin tubes and centrifuged at a minimum of 100 x g for 10 minutes (3000 rpm). The plasma will be harvested and transferred in equal volumes into two labeled polypropylene tubes. The samples will be stored upright at -20^0 C until analysed.. The duplicate sets of plasma samples will be placed in separate freezers.

Analytical Procedure
The concentration of cyclizine in each blood sample will be measured by means of a fully validated HPLC chromatographic method.

Assessment Criteria
Concentrations of cyclizine in plasma will be determined and the data found after the three phases for each route of administration will be statistically compared.

Primary Variables

- Area under the plasma concentration time curves (AUC_{0-t})
- Mean plasma level after 72 hours (C_{av} [4])
- Maximum measured concentrations (C_{max})

Secondary Variables

Time to maximum concentration after dosing (t_{max})

Expected Start of Study

Upon receipt of an approved protocol, a photocopy of Sponsor Insurance Policy and all relevant pharmacy data, applications will be made to the Ethics Committee and Medicines Control Council. Clinical dosing will commence approximately one week after written approval has been received.

Expected Duration of Study

Approximately 8 weeks from clinical study start to report completion.

Location

BIOCEN Clinic, site at the Department of Pharmaceutics, University of Potchefstroom, South Africa.

CONTENTS **PAGE NO.**

(1) INTRODUCTION

Cyclizine is an H_1 receptor antagonist with central nervous system depressant, anti -cholinergic, anti-emetic, anti-spasmodic and local anaesthesia effects. It is mainly used as an anti-emetic and anti-motion sickness due to its prominent anti-cholinergic activity and action on the vomiting centre (Martindale, 1992). Although little is known about its pharmacokionetic and pharmacodynamic properties, available literature reports that the drug undergoes an extensive first pass effect post oral administration thus reducing the bioavailability intensively (Walker, 1995).

This low bioavailability of cyclizine can be overcome by the use of a different route of administration i.e. the intranasal route. Studies based on the application of the intranasal route of administration for most problem drugs have indicated rapid kinetics of absorption and enhanced bioavailability comparable to the parenteral route due to:

- The direct accessibility of the highly vascularised nasal mucosa surface to the drug delivery system and to the systemic circulation.
- Avoidance of the gut wall metabolism and hepatic extraction effect by direct blood drainage from the nose to the systemic circulation.
- Furthermore, the intranasal route offers ease of administration for the drug preparation, which increases the likelihood of patience compliance especially in geriatric and paediatric patients.
- Normally for patients with nausea and vomiting whereby oral administration is unsuitable since vomiting prevents the absorption of the drug, application of the intranasal route would be a feasible, non-invasive alternative form of drug administration. .....

References

Wade A. *The Extra Martindale Pharmacopoeia;*1992 30th Edition : 1279

Walker R and Kanfer I. Sensitive High Performance Liquid Chromatographic determination of cyclizine and its demethylated metabolite, norcyclizine in biological fluids using coulometric detection. *Journal of Chromatography;* 1995 : 172-177

Mechanism of action

The mechanism by which cyclizine exerts its anti-emetic and anti-motion sickness is not precisely known but may be related to its central anticholinergic actions thus it tends to diminish vestibular stimulation and depresses the labyrinth function. An action on the medullary chemoreceptor trigger zone may be involved in the anti-emetic effects (USP DI, 1998).

Like all antihistamines, cyclizine competitively inhibits the H_1-receptors of the smooth muscles to histamine stimulation. Within the vascular tree, it also inhibits both vasoconstrictor effects of histamine and to a degree the more rapid vasodilator effects that are mediated by H_1-receptors on the endothelial cells (Hardma, 1996).

Effects on capillary permeability: It strongly blocks the action of histamine that results in the increase in capillary permeability and the formation of oedema and wheal.

Effects on exocrine glands: Cyclizine does not inhibit the secretion of gastric fluids but suppresses histamine evoked salivary, lachrymal and other exocrimal secretions with variable responses (Hardma, 1996).

Clinical applications and dosage

Cyclizine is an H_1-receptor antagonist with central nervous system depressant, anticholinergic, anti-emetic, antispasmodic and local anaesthesia effects. It is mainly used as an anti-emetic and anti-motion sickness due to its prominent anticholinergic activity and actions on the vomiting centre. It is therefore indicated for the following:

- Prevention and treatment of motion sickness
- Treatment of irradiation sickness
- Control of post-operative and drug induced vomiting
- Treatment of nausea and vomiting
- Symptomatic treatment of vertigo caused by Mernier's disease and other labyrinth disturbances (Martindale, 1992).

The different clinical uses and doses for each disease state are presented in the table 4 below. The dose largely depends on the indication presented. Usually the intravenous and

oral dose range between 25mg to 300mg and the rectal dose is between 100mg and 300mg.

Table 4: Clinical presentations and recommended dose

Indication	IV/ Oral/rectal dose Adult	IV/ Oral/rectal dose Paediatric
Motion sickness	100mg tid supp, 50mg tid po/im	50mg tid supp 12.5 mg tid po/im
Post–operative nausea	50mg im before end of operation	12.5mg im before end of operation
Nausea and Vomiting/Labyrinth disorders	100mg tid supp, 50mg tid po/im	50mg tid supp 12.5mg tid po/im

Pharmacokinetics

There is limited information on the pharmacokinetics of antihistamines and cyclizine in particular.

Pharmacodynamics

Absorption

Cyclizine is well absorbed following oral or parenteral administration. Peak plasma concentrations after a single oral dosing of 50mg occurs after 2 to 3 hours and last for 4 to 6 hours following oral administration. Usually symptomatic relief begins after 15 to 30 minutes post oral administration (Drugs, 1988_g).

Metabolism

The drug is extensively metabolised by N-demethylation in the liver to form an inactive metabolite, norcyclizine that is widely distributed throughout the tissues especially the kidneys, liver, lungs, and spleen. In plasma, norcyclizine is 60 % protein bound. Due mainly to the drug's extensive hepatic extraction, its bioavailability after oral administration is reported to be low (Clarke, 1986).

Distribution

The distribution of cyclizine has not been well characterised but available literature indicate that high concentrations of both the drug and the inactive metabolite (norcyclizine) are found in the kidneys, liver, lungs and spleen (Clarke, 1986).

Elimination

The metabolic fate of cyclizine is not clearly stated however, reports indicate that the drug undergoes an extensive hepatic metabolism and is excreted as an inactive metabolite (norcyclizine) in urine.

Half-life

- There is no documented information on the pharmacokinetics of cyclizine however, studi
- Dryness of mouth, nose and throat
- Tachycardia
- Loss of appetite
- Nervousness
- Restlessness
- Trouble in sleep/skin rash
- Input stomach (USP DI, 1998)

Contraindications

The use of cyclizine is contraindicated in patients with the following diseases or disorders:

- Acute asthma
- Glaucoma
- Urinary retention
- Prostrate hypertrophy
- Chronic pulmonary disease
- Shortness/difficulty of breathing
- For geriatrics, no documented information is available on the relationship of age to effects of cyclizine but caution should taken as regards usage by geriatrics. This group of patients tends to exhibit sensitivity to anticholinergics, which are pharmacologically related to cyclizine and therefore constipation, dryness of mouth, and urinary retention may occur (USP DI, 1998).

Drug Interaction

1. Concurrent use of cyclizine with:

- Anticholinergics and other drugs with anticholinergic activity potentiates the anticholinergic side effects.
- Central nervous system depressants potentiates the sedative effect of these medications.

2. Prior administration of cyclizine may reduce the anti-emetic response to apomorphine in the treatment of poisoning (USP DI, 1998).

2. STUDY OBJECTIVES

2.1 *Pharmacokinetics*

1.1.1.1 The objectives of this study are:

- to evaluate and compare the bioavailability profiles of cyclizine found after the administration of Valoid 100 mg tablets (GlaxoWellcome South Africa), and cyclizine 125mg/ml (test product) over 72 hours after administration. A washout period of at least 7 days between periods and using a randomised, two way cross-over design involving 12 healthy male volunteers, will be applied.

Assessment Criteria:

Concentrations of cyclizine in plasma:

Primary Variables

- Area under the plasma concentration time curves (AUC_{0-t})
- Mean plasma level after 72 hours (C_{av} [4])
- Maximum measured concentrations (C_{max})

Secondary Variables

Time to maximum concentration after dosing (t_{max})

Plasma samples collected after dosing on day 1 will be assessed for C_{max}, AUC_{0-4} as measures of bioavailability and bioequivalence of the test and reference formulations.

2.2 *Adverse Events*

To monitor the safety and tolerability of cyclizine in the volunteers.

3. STUDY MEDICATION

The detailed procedures for handling of the study medication are described under Section 7.4, Drug Administration and Section 11 Pharmacy Supplies.

3.1 *Test Medication (A)*

Cyclizine	
Strength:	125mg/ml
Dose:	A single dose on day 1
Form:	Intranasal gel
Batch No:	TO BE SUPPLIED
Expiry date:	TO BE SUPPLIED
Manufacturer:	Pharmaceutics Dept PU for CHE Box 36 Potchefstroom

3.2 *Reference Medication (B)*

Valoid ®	
Strength:	50mg
Dose:	A single dose on day 1
Form:	Tablets
Batch No:	TO BE SUPPLIED
Expiry Date:	TO BE SUPPLIED
Manufacturer:	GlaxoWellcome South Africa (Pty) Ltd Old Pretoria Rd Midrand, South Africa

3.3 *Drug Identification*

The test and reference drugs will be supplied by the office of the Study Sponsor. Test and reference drugs will be identified by the following labelling:
Name and Address of Manufacturer
Drug Name
Batch No. and Expiry Date
Dosage Form
Number of Supplies
Storage Instructions
"For Clinical Trial Purposes Only"

In addition the study supplies will be relabelled by BIOCEN Clinic staff as follows:
Volunteer No.
Study No.

Study Day
Period No.
Dosage Form
Drug (generic) Name
Drug Administration Instructions e.g. 'Take orally with 240 ml water'
"For Clinical Trial Purposes Only"
Investigator's Name

A medication dosing label will be attached to each volunteer CRF at dosing on each study day.

Prior to initiating this study, the Sponsor will conduct drug identification tests and assay drug content for the test and reference products. Dissolution test results will be sent together with the drugs.

4. INVESTIGATOR AND FACILITIES

4.1 *Clinical Centre*

Investigator
Prof. D.G. Muller, B Sc (Pharm), Hon B Sc (Chem), M Med Sc, DSc
Professor of Pharmaceutics,
University of Potchefstroom,
South Africa.

Co-Investigators
Dr G E Y Schulze, B Sc, B Sc Hon, MB Ch B

Project Manager
Dr M M Malan BSc (Pharm), M Sc, Ph D

Quality Assurance
Me. J Handford, BSc (Pharm), RIIP, Potchefstroom

4.3 *Biometric Centre*
Prof H S Steyn, B Sc, M Sc, Ph D
Statistical Consultant Services,
Potchefstroom University for CHE
Potchefstroom.

5. STUDY DESIGN

5.1 *Design*

The study will be an open label, single dose, randomised, balanced, two-way cross-over study involving 12 healthy male volunteers with a washout period of at least 6 days between study periods. There will be 5 blood samples taken during each study period (5 x 3 samples in total). These will be as follows: pre-dose on Days 1 and 7 over 72 hours, and the cyclizine concentrations in plasma will be measured.

5.2 *Randomisation*

Volunteers will be assigned to a balanced treatment sequence as they enter the study. Drug allocation will be carried out by means of a randomisation code (Appendix A).

5.3 *Expected Study Time Schedule*

Upon receipt of an approved protocol and all relevant pharmacy data, applications will be made to the Ethics Committee and the Medicines Control Council. The study will commence approximately one to two weeks after written approval has been received.

Expected Duration of Study:

Approximately 10 weeks from clinical study start to report completion.

6. PARTICIPANTS

6.1 *Number of volunteers*

Twelve volunteers will be tested to adequately cover the optimal sample size needed.

Assuming that there is :

- no interaction between formulations or period
- the observations arise from a log normal distribution
- the variances of the test and reference parameters are the same

The bioavailability of each formulation after first dosing (Day 1 of each period) will be measured with calculations of C_{max} and AUC values. The data collected will be analysed and commented upon but the assessment of bioequivalence will be based upon data collected on Day 1 of each dosing period.

Volunteers will be screened on an out-patient basis within 14 days prior to entry and participate in the study according to the inclusion and exclusion criteria.

The results of screening tests will be available to the Investigator before first dosing and will be recorded in the volunteer case record form.

At the end of the study another clinical assessment consisting of a physical examination and laboratory tests will be carried out within 14 days of completing Treatment period 2. Any abnormality will be followed up, reported and treated appropriately.

6.2 *Source of Volunteers*

Non-institutionalised volunteers consisting of university students, teachers, members of civic groups and members of the community at large will be used in this study. Volunteers will be recruited using methods approved by the local Research Ethics Committee. Afrikaans translation of the Volunteer Information will be provided when requested.

6.3 *Inclusion Criteria*

- Caucasians
- Healthy volunteers.
- Aged between 18 and 45 years.
- Weight range 50-95kg and within 15% of the 1983 Metropolitan Life Insurance Actuary Tables.
- Clinically normal vital signs.
- Clinically normal medical history.
- Clinically normal findings on physical examination.
- Clinically normal findings for haematology and clinical chemistry of blood and urine or showing clinically insignificant deviations only. These assessments will involve (quantitative) measurement of the following:

Blood:

Haematology		*Biochemistry*
WBC	BASO	Sodium
RBC	LYMPH	Potassium
HGB	MONO	Bilirubin
MCV	MCHC	Alk. Phos.
Plt	EOS	AST, ALT and LDA
	LUC	Creatinine
	NEUT	

Urine:

Ketones	Glucose	pH	Leucocytes
Protein	Specific Gravity	Blood Pigments	
Urobilinogen	Bilirubin	Nitrites	

- Screening results for drug abuse (taken within 14 days of study start) must be negative for opiates, cannabinoids, amphetamines (including methamphetamines), benzodiazepines and cocaine.
- HIV, Hepatitis B and C tests, taken within 3 months of study start, must be negative.
- Capable of comprehending and communicating effectively with the Investigator and staff.
- Able to give written informed consent.
- Electrocardiogram recording (12-lead) within the normal range.

6.4 *Exclusion Criteria*

- Resting heart rate outside the range 45 - 90 beats per minute or exhibiting any clinically significant degree of heart block.
- Resting, seated blood pressure less than 90/60 mmHg or more than 160/100mmHg.
- Severe electrolyte imbalance.
- Aged less than 18 or more than 45 years.
- Evidence of clinically significant cardiovascular, haematological, hepatic, gastrointestinal, renal, pulmonary, neurological, or psychiatric disease.
- History of significant drug or drug related hypersensitivity/intolerance or food allergies.
- History of medication with any psycho-pharmacologically active agents in the last five years.
- Significant illness within 5 days of start of study.
- Hospitalisation within the previous 3 months for major surgery or medical illness.
- Mental handicap as defined by clinical evaluation.
- Volunteer not willing to refrain from smoking during each treatment period.
- Smoking of more than 10 cigarettes per day.
- Participation in a clinical drug study within 12 weeks prior to the start of the study.
- Donation of blood within previous 90 days or a volume exceeding 1500 ml in the previous 12 months.
- Any indication of current or previous abuse of alcohol, solvents or drugs.
- Treatment with a full or regular course of medication during the 28 days prior to the start of the study.
- Use of alcohol on study days or within 24 hours prior to commencement of the study.

- Taking medication (prescription or OTC) within 14 days prior to the study start. Paracetamol is allowed up to 72 hours prior to either study period.
- Diet, which in the opinion of the Investigator, deviates from a normal diet.
- Intake of quantities of methylxanthine-containing beverages* which, in the opinion of the Investigator is abnormal (*tea, coffee, cola, chocolate etc).
- Not able/not willing to give informed consent.
- Not able to be contacted in case of emergency.

6.5 *Responsibility for Replacement of Volunteers*

In the event of a volunteer who, in the opinion of the Investigator withdraws/is withdrawn from the study for a reason related to the study drug, the individual is to be considered a drop out and replaced at the cost of the Sponsor.

In the event of the Investigator withdrawing a volunteer from the study for any reason unrelated to the study drug, then that individual is considered a non-completer and will be replaced at the cost of the Investigator.
Replacements will be allocated the same number and randomisation as the proband replaced. The letter R will be added to the relevant study number.

6.6 *Volunteer Screening*

All volunteers will be assessed within 14 days prior to enrolment in order to determine their status with regard to the criteria for inclusion and exclusion (sections 6.3 and 6.4). Volunteers will undergo a physical examination and a medical history will be obtained. Vital signs will be recorded while the volunteer is sitting (heart rate and blood pressure). Physical measurements (height without shoes, weight) taken and recorded. This data will be recorded directly in the CRF's (Case record forms) with no prior written or electronic record. Volunteers will also have a 12 lead ECG recording and undergo haematology, biochemistry, and urinalysis testing. A written Informed Consent Form to participate in the trial will be obtained from the volunteer in accordance with ICH GCP Guidelines and BIOCEN Clinic SOP, when he attends the physical examination. An Afrikaans translation of the study Informed Consent Form will be provided if requested. The volunteer only becomes an official participant on the trial when all his haematology, biochemistry, virology and urinalysis results have been received and meet with the inclusion criteria.

The results of all screening tests will be available to an Investigator before the first dose is administered and will be recorded in the appropriate case record form. Volunteers with screening values outside the laboratory range will be accepted into the study only if the Investigator or his clinical designate sign off the values as "of no clinical significance".

6.7 *Management*

Recruitment of volunteers, compliance with protocol, administration of drugs, sampling and recording times of sampling will be the responsibility of the Investigator and his deputies.

6.8 *Withdrawal Criteria*

Volunteers may withdraw from the study at any time and for any reason. Should any volunteer so withdraw the Investigator will be informed immediately.
The Investigator may decide to terminate the participation of any volunteer according to the following criteria:

1) The occurrence of serious or unexpected unwanted effects.
2) In the event of abnormal laboratory results judged to be of clinical significance.
3) Any protocol violation
4) Serious difficulty in obtaining blood samples.
5) Lack of co-operation by the volunteer.
6) Intercurrent illness requiring medication.

Any volunteers withdrawn in accordance with criteria 2, 3, 4, 5 or 6 will be replaced at the cost of the Investigator.

The Study Monitor will be informed promptly of all withdrawals. The reason(s) for such withdrawal will be entered into the volunteer's case record form (withdrawal form). If withdrawal of the volunteer is on medical grounds then the volunteer concerned will remain under supervision of the Medical Investigator until satisfactory health has returned.
Volunteers who fail to complete all periods of the study will be replaced by suitable substitutes. Data referring to volunteers who withdraw from the study will be retained for analysis. Any unwanted effects reported by the volunteers who withdraw will be incorporated into the final study report.

7 STUDY PROCEDURES

7.1 *General*

The volunteers will be institutionalised and supervised from approximately 20h30 on the day preceding the clinical days (Days 1and 7). At 21h00 all volunteers will

be given a light snack and a mug of decaffeinated tea and/or coffee. Fasting will begin at 21h30 (at least 10 hours prior to dosing).

7.2 *Screening Phase*

On the initial and all subsequent visits to the clinical center each participant will be evaluated to ensure compliance with the inclusion and exclusion criteria as specified in Section 6.

7.2.1 *Concomitant Medication*

Concomitant medication is generally not permitted for the duration of the trial. If this is considered to be necessary for the volunteer's welfare it may be given at the discretion of the Investigator. The volunteers have to inform the Investigator about any intake of other drugs in the course of the trial. Any intake of concomitant medication has to be documented in the case record form and is to be regarded as an exclusion criterion.

7.3 *Dosage Schedule*

The dosage schedule is described in Appendix A.

7.4 *Drug Administration*

In accordance with the randomisation code, the test or reference drug(s) will be given during Periods 1 and 2 of the study, with a washout period of at least 6 days between dosing periods.

A total of 2 x tablets (cyclizine 100 mg) will be administered to each volunteer over the entire study period. The volunteers will be supervised in-house until after the 4 hr blood sample has been taken on Study days 1 and 7. During institutionalisation participants will follow a standard meal and refreshment schedule (see Section 7.7). Blood samples will be collected at defined times following the dose on Days 1 and 7.

At each study period all volunteers will fast overnight for at least 10 hours prior to dosing on the study Days 1 and 7.During the overnight fast, water may be taken *ad libitum,* up to one hour pre-dosing. Each volunteer will be given a single oral dose of either the test or reference medication at 08h00, with volunteer 1 and dosing of subsequent volunteers at two minute intervals. Dosing times will be recorded on the CRF*. The medication will be swallowed, without chewing or crushing, with 240 ml water. A mouth check will be carried out to ensure the medication is swallowed. The actual clock time of each dose administered will be recorded to the nearest whole minute in the case record form (time sheet). Water will be restricted one hour prior to dosing and through the two hour post dosing

period. After the two hour post-dosing period, water will again be available *ad libitum*.

In order to minimise the influence of posture on gastric emptying, volunteers will not be permitted to lie down for a period of 4 hours post-dosing. Vigorous exercise will not be permitted during the first 4 hours after dosing. On Study Days 1 and 7 fasting will be continued until the 72 hour plasma sample has been taken.

7.4.1 *Compliance*

Administration of the study medication will always be supervised by QA/QC personnel in order to verify the compliance of the volunteers. The administration of the study medication is to be documented in the CRF and certified by an Investigator.

7.5 *Blood Sampling Times*

A 5 ml venous blood sample will be aseptically aspirated from each volunteer according to the following schedule (= 5 blood samples during each Period):

	Scheme Time	*Clock Time*
Study Day 1, 7	0	08:00 (pre-dose)
	15	
	30	
	60	
	90	
	120	
	180	
	240	
	360	
	480	
	600	
	720	
	24	
	48	
	72	
		09:00
		00

The actual clock time of each sample will be recorded to the nearest minute in the case record form.

7.6 *Sample Handling.*

The 7 ml blood samples will be collected in appropriately labelled Li-Heparin tubes. The whole-blood samples will be centrifuged at 3000 rpm (100 g) for 10 minutes at +4° C in a refrigerated centrifuge. Samples will be kept refrigerated and centrifuged within approximately 15 minutes of sampling. Supernatant plasma will be divided into two aliquots and placed in polypropylene tubes. The duplicate sample tubes will be labelled with the following information

- study code
- number of volunteer and code
- date of birth
- time of sampling and study day
- drug name
- study period

All plasma samples will be frozen (upright) and stored at - 20^0 C until analysis. The total volume of blood taken from individual volunteers over the entire study will not exceed 250 ml.

7.7 *Food and Fluid intake*

Volunteers will be in a fasting state from 10 hours before until 4 hours after dosing on the study Days 1, 7.

Water will be available *ad libitum* during the pre-dose period up to one hour pre-dosing. Water will be restricted one hour prior to dosing and during the two hour post dosing period. After the two hour post-dosing period, water will again be available *ad libitum*.

7.8 *Volunteer Restrictions*

Volunteers will be instructed to abstain from the consumption of alcohol and beverages/food that contain methylxanthines (coffee, tea, cola, chocolate, etc.) for a period lasting from 24 hours prior to each period of the study commencing (08h00 on day 1 of dosing) and until 72 hrs post dosing on study Day 1.

Volunteers will avoid taking any medication other than study drugs (see Section 6 for details). If any medication is taken (including non-prescription medicines) the Investigator is to be informed immediately. The Investigator will note the nature, dose, time, occurrence and possible reaction with the study drug and will record this in the appropriate case record form. If regular administration of concomitant drugs is necessary the volunteers will be withdrawn from the study.

7.9 *Volunteer Monitoring*

7.9.1 *Vital Signs*

Between 07h00 and 08h00 and prior to the administration of the study medication, on dosing days, the following parameters will be measured (having allowed each volunteer a rest period of at least 5 minutes in the sitting position):

Blood pressure (mm Hg) (sitting)
Pulse rate (bpm) (sitting)

If the pulse rate is between 45 - 90 beats per minute, the systolic blood pressure between 90-100 mm Hg and the diastolic blood pressure between 60-100 mm Hg the volunteer will be eligible to receive the study drug. Blood pressure and heart rate will be recorded at 4 hr on each of the study days. These measurements will be recorded whilst the volunteer is sitting. All vital sign observations will be recorded in the appropriate case record form (timesheet), with no prior written or electronic record.

7.9.2 *Adverse Events*

7.9.2.1 Definitions

Adverse Event: Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporarily associated with the use of a medicinal (investigational) product.

7.9.2.2 *Adverse Events:*

- Worsening (change in nature, severity or frequency) of conditions present at the onset of the study
- Patient deterioration due to the primary illness
- Intercurrent illness
- Drug interactions
- Events related or possibly related to concomitant medications
- Important abnormal laboratory values, as well as significant shifts from baseline
- Within the range of normal, which the Investigator considers to be important

An adverse event is an adverse drug reaction (ADR) if there is a founded suspicion that the event is caused by the study medication.

7.9.2.3 *Serious Adverse Events*:

A serious adverse event is an adverse event that is:

- Fatal
- Life threatening
- Permanently disabling/persistent or significant incapacitation
- Results in in-patient hospitalisation or prolongation of hospitalisation
- A congenital abnormality/birth defect
- A cancer
- Overdose

A serious adverse event has to be reported to the Sponsor within 24 hours (by telephone or fax) after becoming aware of the occurrence of such an event. All serious and unexpected adverse events must be reported to the Ethics Committee and Regulatory Authorities.
If the Investigator considers an adverse event to be so serious as to require specific knowledge of identity and dose of the study substance, the Investigator may (only if necessary) break the code for that volunteer only.

7.9.2.4 *Classification of Adverse Events*

Adverse events have to be recorded on an adverse event information sheet in the volunteer's Case Report Form and graded as mild, moderate or severe according to the following definitions:

- Mild: Causing no limitation of usual activities, the patient may experience slight discomfort
- Moderate: Causing some limitation of usual activities, the patient may experience annoying discomfort
- Severe: Causing inability to carry out usual activities, the patient may experience intolerable discomfort or pain

7.9.2.5 *Definition of Adverse Event Causality (modified to the classification of Karch/Lasagna, Journal of the American Association, 234: 1235 - 1241; 1975)*

The Investigator will determine the relationship of any adverse event to study medication according to the following criteria:

- Definite: A reaction that follows a reasonable temporal sequence from administration of the drug, and that is confirmed by improvement on stopping or reducing the dosage of the drug, and the reappearance of the reaction on repeated exposure.

- Probable: A reaction that follows a reasonable temporal sequence from administration of the drug, that follows a known or expected response pattern to the suspected drug, that is confirmed by improvement on stopping or reducing the dosage of the drug and that could not be reasonably explained by the known characteristics of the patients clinical state.

- Possible: A reaction that follows a reasonable temporal sequence from administration of the drug that follows a known or expected response pattern to the suspected drug but that could readily have been produced by a number of other factors.

- Unlikely: A reaction that follows a reasonable temporal sequence from administration of the drug, that follows a known or expected response pattern to the suspected drug but that could reasonably be explained by the known characteristics of the volunteers clinical state.

- Not related: Any event that does not meet the above criteria, there is sufficient information that the aetiology of the event is not related to the study drug.

- Not possible to judge: A judgement of the relation to study drug is not possible. The statements that are available won't suffice for a definite judgment.

7.9.2.6 *Adverse Event Documentation*

The recording of every single AE/SAE has to meet special requirements :

- Detailed patient data
- Exact documentation of the event
- Exact description of temporal sequence to the therapy course
- Documentation of severity
- Documentation of the results of diagnostic and therapeutic measurements
- Results of a repeated exposure (reexposition) if possible
- Details of the development and outcome including medical judgment
- As much data as possible has to be obtained which is important for judgement concerning the relationship of the AE/SAE to study drug
- Critical examination of the relationship to study drug

All AE's have to be dealt with in accordance with this scheme whether spontaneously reported by a volunteer, observed by the Investigator or elicited by general questioning.

7.9.2.7 *Registration procedures of AE's/SAE's*

The Investigator is responsible for recording all adverse events on the CRF which occur during the study (including all deviations of laboratory values from normal ranges), regardless of their relationship to the study medication.

Occurrence of any serious adverse event (including death, irrespective of reason), has to be notified immediately by the principal Investigator (within 24 hours of the event, at latest the next working day), to the Sponsor at the corresponding address (see below)

CSIR
C/o J & B Pharmaceutical Consultants (Y52269)
P O Box 25395
Monument Park
0105
South Africa

Telephone (+27 12) 347 1392
Telefax (+27 12) 347 7687
Mobile 083 460 5534

The first report should contain a detailed description of the observed symptoms and the concomitant therapy. The Investigator must judge the possible causal relationship between the event and the study drug.

The Investigator should arrange additional examinations at his own discretion to clarify if the event is connected with the study medication and should consult a specialist if necessary.
All adverse events and serious adverse events have to be followed up until an outcome is known. This outcome has to be reported to the Sponsor.

7.9.3 *Procedures for eliciting reports of AEs*

In addition to recording spontaneous complaints, clinical staff must follow a procedure of daily questioning of each volunteer at dosing and an hourly questionnaire on Study days 1 and 6 in accordance with Shandon Clinic SOP 5.

7.10 *End of Study Clinical Assessments*

Physical examination, laboratory tests and 12 lead ECG as at entry (with the exception of virology) will be repeated at the end of the study, within 14 days of completing Treatment Period 2. Any abnormality will be followed up, reported and treated appropriately.

7.11 *Reserves and Volunteer Replacement*

Volunteers selected to take part in the study will be asked to report to the clinic on the evening before the first dosing day and will remain institutionalised, under supervision overnight. Any volunteer who fails to reach this stage, having complied satisfactorily with the protocol, will be replaced by a reserve. Any volunteer who is withdrawn or drops out during the course of the study will be replaced by a reserve. Reserves who replace one of the original volunteers will be allocated the same number to which the initial R will be added. This schedule will be followed for both treatment periods.

7.12 *Premature Termination of the Study*

If it is deemed justifiable to discontinue a study for medical or safety reasons, the Investigator must consult the Sponsor or the Sponsor may terminate the trial for safety, administrative, or other reasons. Documentation explaining reasons for terminating the study must be forwarded to the Sponsor and Ethics Committee. A complete final examination must still be carried out on the volunteers who participated in the terminated study concerned.

8. LABORATORY TESTS

8.1 *Personnel* The people responsible for quality assurance of these tests is:
Dr. Caryl Ann Richmond
BARC,
Napier House,

Napier Road,
Richmond, Johannesburg
South Africa.

8.2 *Haematology*

A 5 ml blood sample will be drawn from each volunteer and the following investigations carried out:

WBC	PLT	LUC
RBC	LYMP	
HGB	MONO	
HCT	NEUT	
MCV	BASO	
MCHC	EOS	

All haematological parameters will be assessed using a Technicon H*1 Automated Haematology Analyser or equivalent system.

Normal ranges for haematology screening will be supplied by the laboratory prior to study start.

8.3 *Biochemistry*

A 7 – 10 ml blood sample will be taken for assay for the following:

BILIRUBIN
ALK. PHOS
AST
CREATININE
POTASSIUM
SODIUM

These will be assayed using a Hitachi 91/917 Analyzer or equivalent systems.
Normal ranges for biochemistry screening will be supplied by the laboratory prior to study start.

8.4 *Urinalysis*

A mid-stream urine specimen will be collected into a sterile container and assayed for the following:

Ketones	Glucose	pH	Leucocytes
Protein	Specific Gravity	Blood Pigments	
Urobilinogen	Bilirubin	Nitrites	

The above assays will be performed using a Multistix® 10 SG (Bayer Diagnostics) system.

8.5 *Virology*

Tests for the detection of Hepatitis B, C and HIV infection will be performed within 3 months of study start.

9. SAMPLE MANAGEMENT

9.1 *Labeling of samples*

All samples will be labelled with the following information:

1) Study Number/Code
2) Study Number of the Volunteer
3) Date of birth
4) Study Period
5) Sample Time and Study Day
6) Drug Name (generic)

9.2 *Storage of samples*

The 5 ml blood samples for drug assay will be collected into appropriately labeled Li - Heparin tubes. These heparinised whole blood samples will be centrifuged at 3000 rpm (100 g) for 10 minutes at +4° C in a refrigerated centrifuge. Samples will be kept refrigerated and centrifuged within approximately 15 minutes of sampling. The supernatant plasma will be separated into two aliquots and placed into labeled polypropylene tubes and frozen at - 20^0 C until analysis.

9.3 *Assay of drug*

The concentration of cyclizine in each blood sample will be measured by means of a fully validated chromatographic method(s), in accordance with GLP.

The randomisation code will not be available to the analyst, who will analyse the samples taken during Phases 1, 2 from each volunteer at the same time. A fully validated description of the methods will be supplied in the report.
An analytical protocol/study design shall be supplied by the analyst prior to the start of the analysis.

10. ASSESSMENT PARAMETERS

10.1 *Parameters requiring evaluation will be:*

a) Pharmacokinetic, covering those parameters detailed in Section 2.1 above.
b) Statistical analysis of pharmacokinetic data (Section 10.5).
c) Adverse events (Section 7.9.2).
d) Clinically significant, abnormal laboratory findings.

10.2 *Quality Control*

10.2.1 *Revisions to and deviations from Protocol*

With the exception of an emergency, no deviations or changes in this protocol will be permitted without the documented approval of the Sponsors. Any deviations, irrespective of their causation and nature will be fully and explicitly recorded. The Ethics Committee which granted the original approval must be notified of any significant changes to the protocol and must provide documented approval of any change which may increase the risk to the volunteer or adversely affect his rights or jeopardise the validity of the investigation.

10.2.2 *Study Monitoring*

The monitor for the Sponsor may visit the Investigators and the study facility at any time in order to maintain current and personal knowledge of the study through review of the records, comparison with source documents, observation and discussion of the conduct and the progress of the study. Prior to the start of the study, the Investigator will be contacted and informed of any impending visits and the frequency of such visits. At each visit the Investigator will assist the study monitor in terms of reviewing and verifying those records associated with the study.

10.2.3 *Recording of volunteer data*

Drug administration times and blood sampling times will be recorded as appropriate to each volunteer. Demographic and background information and results relevant to the volunteer's medical history, physical examination, vital signs and clinical laboratory data will also be recorded in the appropriate volunteer's forms.

The production of suitable volunteer record forms (CRF) will be the responsibility of the Investigator. The forms will be forwarded to the Study Monitor (Sponsor) for approval prior to the start of study. The actual clock time of all doses, blood samples and meals will be recorded directly into the case record form. Any deviations will be noted. Data reported on the CRF, that are derived from source documents, should be consistent with the source documents or the discrepancies should be explained.

The results of the haematology, biochemistry and urine tests will be supplied, together with the normal range of such values. All values outside of the normal range will be highlighted.

10.3 *Quality Assurance*

The quality of the clinical part of this study will be controlled by the Quality Assurance Department of BIOCEN Clinic in accordance with the Standard Operating Procedures. The analytics and statistics will also be quality assured in a similar manner by the analytical centre performing the analyses. BIOCEN Clinic Quality Assurance unit will also check the study final report according to GCP. The analytical final report will be written and quality assured in accordance with GLP.

10.4 *Pharmacokinetic Analysis*

Pharmacokinetic parameters as described in Section 2.1 will be derived from the relevant blood concentration data.

10.5 *Statistical Analysis*

10.5.1 *Procedure*

The bioavailability of each formulation after first dosing (Day 1 of each period) will be measured with calculations of C_{max} and AUC values. The data collected will be analysed and commented upon
Any volunteer who fails to complete all periods of the study must be replaced.
All evaluable volunteers will be included in the statistical analysis.

Outliers are defined as volunteers having discordant values of one or more pharmacokinetic parameters when compared with other values; e.g. a volunteer differs notably from the rest of the volunteers for the Test product response versus the Reference product response. For outlier identification, statistical tests will be performed. When one or more outliers are identified, scientific evidence or explanations have to be provided to justify the exclusion of the subjects data from statistical analysis.

10.5.2 *Personnel*

Pharmacokinetic and statistical analysis will be performed by Prof. H.S. Steyn, of The Statistical Consultant Services at the Potchefstroom University for CHE.

11. PHARMACY SUPPLIES

The Sponsor will supply the necessary test and reference samples. A sample of the active raw materials for analytical purposes shall also be provided. On receipt of the medication, a drug receipt form shall be completed as well as a drug inventory form.

The Investigator or nominated delegate will dispense the study medication only to volunteers included in the study by following the procedures set out in the study protocol. To use the study medication for any other purpose is not allowed.

11.1 *Test and Reference Materials*

Reference: Cyclizine50 mg
Test A: Cyclizine125 mg/ml.

11.2 *Packaging and Labeling*

Supplies will be provided by the Investigator for each volunteer (plus a minimum of 6 extra in case volunteer replacements are necessary). The sponsor will label the medication as listed on page 12. Each volunteer dose will be labelled by BIOCEN Clinic.

11.3 *Identification of test and reference samples*

Test and reference samples will be identified as to their nature, origin, lot or batch number and expiry date. Prior to initiating the study the Sponsor will conduct drug identification tests and assay drug content for both test and reference products. The results of these tests will be provided to the Investigator by the Sponsor prior to study commencement. The certificate of analysis and results of dissolution tests will accompany the pharmacy supplies.

11.4 *Receipt and return of supplies*

Upon receipt of the pharmacy supplies, the Investigator (or his deputy) will conduct an inventory, complete and sign the Drugs Received Form. The Investigator will confirm receipt of study medication in writing, including all follow up supplies. At the end of the study, a final drug study inventory will be performed and a Drug Returned Form completed and signed by the Investigator or his nominated deputy. Should any supplies be missing an indication must be given and an explanation provided. The inventory will show clearly the details of materials received, when they were dispensed and to which volunteer. The inventory will also indicate the quantity and description of all investigational drugs on hand at any time during the course of the study. Drug supplies (study medication including reference) which have not been used are to be returned to the Sponsor after completion of the study. All supplies must be accounted for at the end of the study. A drug inventory is to be filled in for this purpose.

11.5 *Storage of Pharmacy Supplies*

The study medication will be supplied by the Sponsor. The medication will be secured in the BIOCEN Clinic Pharmacy and stored as specified by the Sponsor in accordance with regulatory requirements.

11.6 *Access to Pharmacy Supplies*

The Investigator will ensure that all study drugs are retained in a suitable secure facility accessible only to those individuals authorised by the Investigator.

12 ETHICS

12.1 *General*

The study will be performed in accordance with the ICH guidelines on Good Clinical Practice (GCP). The conditions will be in compliance with the Helsinki Declaration (as reviewed at the 42nd World Medical Assembly in South Africa 1996) and the recommendations of the World Health Organisation (W.H.O. Technical Reports Series No.403 - 'Principles for the Clinical Evaluation of Drugs' and Technical Report Series No. 563 - 'Guidelines for Evaluation of Drugs for Use in Man')

12.2 *Institutional Review Board (IRB)*

The protocol for the study and the written informed consent form will be submitted to the Ethics Committee of the School of Pharmacy, Potchefstroom University and the Medicines Control Council for review. Approval of the protocol and consent forms must be received before starting the study. Any major change to these documents will require approval from the IRB, including amendments to the protocol which must be signed by the Investigator and Sponsor

12.3 *Written Informed Consent*

Volunteers will receive a full explanation of the nature, purpose, procedures and risks of the study from the Investigator. The information supplied to the volunteer will be both verbal and written. The written informed consent form will contain information relating to the objectives of the study, the procedures during the study and the risks involved in undergoing such a trial, with special reference to possible side effects of the medication; important paragraphs will be initialed by the volunteer. Volunteers will then personally sign and date the informed consent form.

12.4 *Investigator's Responsibilities*

The Investigator will ensure that a physician is responsible for the care of the volunteer participants during the course of the study. If the physician is not present in the clinical centre he will leave instructions for the staff and a telephone number where he may be contacted. After completion of the study, the physician will be responsible for the medical follow up of the volunteers. In the event of any volunteer having any medical abnormality during the post-dosing period, the volunteer will remain under the supervision of the physician until normal health has returned. In the case of the abnormality relating to a laboratory value or values, the physician will remain in supervision of the volunteer until the repeat value returns to the normal range. Should any volunteer refuse to comply with the instructions of the physician then the latter is released from his responsibility. In this event the Study Monitor (Sponsor) will be notified immediately.

12.5 *Notification of General Practitioner*

A letter is sent to each volunteer's family doctor notifying him/her of the study and asking for immediate notification if the doctor has any objections. A stamped addressed envelope is enclosed for this purpose.

12.6 *Confidentiality*

All data generated from medical examination and laboratory tests during the course of the study will be regarded as confidential as outlined in the conditions of the Data Protection Act. Similarly all other information relating to the study and provided by the volunteers in the course of the study will be regarded as confidential. No disclosures regarding the state of health of any or all of the volunteers may be made to a third party other than relevant regulatory authorities and Sponsor without the agreement of the volunteer concerned. All data (whether medical, laboratory or pharmacokinetic) generated in the course of the study will remain the property of the Sponsor. No publication or dissemination of the data will be permitted without prior written agreement with the Sponsor.

12.7 *Compensation*

Volunteers must understand that the study will be performed for research purposes only and that no therapeutic benefit may be expected as a consequence of their involvement. In addition, each volunteer must understand that participation in the study may involve risks which are currently unforeseeable (although minor, transient bruising or haematoma may occur near sites of venipuncture). The Sponsor accepts, in terms of EC Guidelines 111/3976/88-EN (Final), "Good Clinical Practice for trials on medicinal products in the European Community", paragraph 2.3 (j), to provide adequate compensation / treatment for volunteers in the event of trial related injury or death, and to provide indemnity

(legal and financial cover) for the Investigator, except for claims resulting from malpractice and /or negligence.

12.8 *Payment to volunteers*

Payment will be made to volunteers for the time and inconvenience of the study at a level to be decided by the Investigator. The precise level of payment will be subject to the approval of the IRB and relevant regulatory bodies. Volunteers who fail to complete the study will be paid at the discretion of the Investigator.

12.9 *Obligations of Volunteers*

Each volunteer should adhere to the instructions given in the informed consent form and is obliged to notify the Investigator if unable to follow the procedures or if he suffers from any adverse event. In particular, he should notify the Investigator before taking any additional medication of any kind during the course of the study. Each volunteer will be given a card giving details of how the Investigator may be contacted (both within and outside of office hours) and will be requested to notify the Investigator of any suspected adverse event.

13 DOCUMENTATION

13.1 *Before the start of the study*

The following study documentation shall be provided before the start of the study:

By the Investigator to the Sponsor

- Curricula vitarum of the Investigators and study personnel involved in clinical and scientific procedures of the study
- Signed copy of the Protocol/Protocol Amendments
- Copy of the written informed consent form
- Examples of all case record forms and related forms
- Documented approval of IRB
- Documented approval of Regulatory Authority (when requested by Sponsor)

By the Sponsor to the Investigator

- Investigator's brochure and/or other relevant toxicological, pharmacological and clinical information
- Certificate of analysis of the test and reference study medication including in-vitro dissolution data
- Instructions for handling and storage of investigative products
- Photocopy of insurance policy or letter giving details of insurance policy.

Additionally, a study agreement, including details of financial arrangements, will be signed by both parties.

13.2 *During the study*

The following forms will be completed during the study:

- Personal medical history record
- Physical examination record with ECG (pre-trial).
- Haematology, clinical chemistry and virology data (pre and post-trial).
- Written informed consent record
- Drug accountability record/drug dispensing record
- Other case record forms for the documentation of all relevant activities and observations during the study
- QC Checklists/QA Reports
- Other case record forms for the documentation of all relevant activities and observations during the study

13.3 *After the study*

A final report will be issued by the Investigator including a clinical and an analytical report and an evaluation of all pharmacokinetic and safety data. Information regarding analytical methodology and validation will be supplied by the Investigator. The names of all personnel involved at various periods of the study will be provided. The date and place of study performance will also be reported.

13.4 *Location of Data.*

All original volunteer case record forms and the study archive file will be maintained in the permanent files of the Investigator at BIOCEN, Potchefstroom University, Potchefstroom and will be available for inspection by the Sponsor or other authorised party at any time. All volunteer source data will be kept at the clinical site in Potchefstroom. All documents and data will be retained for a period of 15 years. Residual plasma samples will be kept for 2 months after issue of the final report.

13.5 *Publication*

The Investigator may not publish the data collected during the course of this study in any form, except with the written permission of the Sponsor. Any data published will not reveal the identity of any study volunteers and confidentiality will be maintained. Any member of the research team, including those employed by the Sponsor, who makes a real contribution to the preparation of publications may be included amongst the authors of the paper(s) prepared.

14 REFERENCES

Goodman and Gillman. The Pharmacological Basis of Therapeutics,

15 APPENDICES

A Randomisation code
B Volunteer informed consent form
C Adverse event form
D Metropolitan Life Insurance Co height and weight table
E Curriculum Vitarum of Investigators.
F ICH Guidelines on GCP (Step 4) and Declaration of Helsinki

APPENDIX 7: LETTER OF APPROVAL FOR HUMAN STUDIES

Potchefstroomse Universiteit
vir Christelike Hoër Onderwys

Potchefstroom University
for Christian Higher Education

Private bag X6001 Potchefstroom 2520
Tel (018) 299 1111 Fax (018) 299 2799
http://www.puk.ac.za

Ethics Committee
Tel (018) 2992256
Fax (018) 2994245
E-Mail ethicbt@puknet.puk.ac.za

Prof DG Muller
Box 36
Potchefstroom University for Christian Higher Education
POTCHEFSTROOM
2520
SOUTH AFRICA

29 August 2002

Dear Prof Muller

APPROVAL FOR EXPERIMENTATION WITH HUMANS

I have pleasure to inform you that your project titled *"Determination of the pharmacokinetics of cyclizine HCl after single dose intravenous, intranasal and oral administration to healthy volunteers"* was approved by the Ethics Committee of this university under the number **02M14**.

This approval is subject to the acceptance/registration of the intranasal dosage form of cyclizine by the Medicines Control Council and the trial may only commence after approval by the MCC has been received.

Please quote this number in all correspondence and remember that it is expected from all researchers working under the approval of this Committee to report on the ethical aspects of their projects once a year during June on the prescribed form.

Approval of the Ethics Committee is valid for a maximum period of five years (according to decision by Senate of 4 November 1992, art. 9.13.2). For continuation of projects after expiry of this period, renewed approval must be obtained.

Yours sincerely

PROF JC BREYTENBACH
Secretary: Ethics committee

APPENDIX 8:

SOUTH AFRICA : CLINICAL TRIAL APPLICATION

SECTION 1 – CHECK-LIST OF REQUIRED DOCUMENTATION

1.6.

1.7. To be completed by Applicants for all Clinical Trials

1.8.	COVER SHEET

Study Title: Determination of the pharmacokinetics of cyclizine HCl after single dose intranasal and oral administration to healthy human volunteers.

Protocol No:

Version No: Date of Protocol:

Study Drug: Cyclizine HCl

MCC Ref number (if applicable):

MCC Ref number(s) of comparator drug(s) (if applicable):

MCC Ref number(s) of concomitant drug(s) (if applicable):

Date(s) MCC approval of previous protocol(s):

Sponsor: Potchefstroom University for CHE

Applicant: Bohloko NS

Contact Person: Prof. DG Muller
Address: Pharmaceutics Dept, Potchefstroom University for CHE, Box 36, Potchefstroom 2520
Telephone Number: 018 299 2272 Fax Number: 018 2992248
Cell Number:
E-mail address: fmsdgm@puknet.puk.ac.za

To be completed by MCC

Date original application received:

Tracking No:

Proposed Clinical Trials Committee Meeting Date if applicable:

Signature: Date:

ACKNOWLEDGEMENT OF RECEIPT OF CTA (Contact details to be completed by the applicant). Whole cover sheet to be faxed to applicant once details in block above are completed.

Contact Details: Name : NS Bohloko Fax No.: 018 2992248

Receipt of new application is hereby acknowledged. Date:

Signature (of MCC recipient): Name:

CHECKLIST

Applicant's

MCC

check list double-check

- ☐ COVERING LETTER ☐
- ☐ FULLY COMPLETED APPLICATION (SECTIONS 1–3) ☐
- ☐ PROTOCOL (INCLUDING RELEVANT QUESTIONNAIRES ETC.) ☐
- ☐ PATIENT INFORMATION LEAFLET(S) AND INFORMED CONSENT(S) ☐
- ☐ INVESTIGATORS BROCHURE AND / OR ALL PACKAGE INSERT(s) ☐
- ☐ INVESTIGATOR'S CV(s) IN MCC FORMAT ☐
- ☐ SIGNED DECLARATION(s) BY INVESTIGATOR(s) ☐
- ☐ REGIONAL MONITOR'S CV AND DECLARATION ☐
- ☐ CERTIFICATE(S) OF ANALYSIS (May be submitted with ethics approval letter) ☐
- ☐ INSURANCE CERTIFICATE ☐

AND IF NECESSARY:

- ☐ LETTER ENDORSING GENERIC INSURANCE CERTIFICATE ☐
- ☐ ETHICS APPROVAL

OR

☐ COPY OF LETTER APPLYING FOR ETHICS COMMITTEE APPROVAL

☐ COPY/IES OF RECRUITMENT ADVERTISMENT(s) (IF APPLICABLE)

☐ FINANCIAL DECLARATION (SPONSOR AND NATIONAL PI)

Electronic versions of the application form (Sections 1 –3), the protocol, the investigator's brochure and/or other relevant documents:

☐ LABELLED DISKETTE/CD-ROM (MSWORD OR RICH TEXT FORMAT)

List of files submitted on diskette/CD-ROM:

NB: DO NOT SUBMIT THE APPLICATION IF DOCUMENTATION IS INCOMPLETE: IT WILL NOT BE PROCESSED

Declaration by applicant:

We, the undersigned have submitted all requested and required documentation, and have disclosed all information which may influence the approval of this application.

We, the undersigned, agree to ensure that if the above-said clinical trial is approved, it will be conducted according to the submitted protocol and South African legal, ethical and regulatory requirements.

_____ 23rd March 2003

Applicant (local contact) Date

_____ 23 rd March 2003

National Principal Investigator / Date

National Co-ordinator /

Other (state designation)

SECTION 2 – ADMINISTRATIVE AND SUPPLEMENTARY DETAILS

Title:

Protocol Number/identification:

Date of protocol (initial/final):

Part 1: CONTACT DETAILS (NAME/ADDRESS/TEL/CELL/FAX/E-MAIL)

1.1 Applicant: (as in Section 1) Bohloko NS Pharmaceutics Dept, PU for CHE, Box 36, Potchefstroom 2520; 018 2992257, 0835328818; 0182992248; fmsnsb@puknet.puk.ac.za

1.2 Sponsor: (as in Section 1) PU for CHE

1.3 If no sponsor – person or organisation initiating, managing, and / or funding the clinical trial:

1.4 Local Contact Person for correspondence: Prof DG Muller

1.5 National Principal Investigator/Coordinator: (or equivalent person) Prof DG Muller

1.6 International Principal Investigator: (if applicable)

1.7 Regional Monitor: (as in Section 1)

Part 2: DETAILS OF INVESTIGATIONAL PRODUCT(s)

2.1 Name(s) and details of investigational product(s) to be used in trial: [Formulation(s) and strength(s) (e.g. 10 mg/ml–10ml amp.)] Include MCC registration number and date of registration if applicable. Cyclizine HCl (valoid) 50mg tab and gel intranasal 125mg/ml.

2.2 Name(s) and details (as above) of comparator product(s) and MCC registration number(s) and date(s) of registration if applicable: [Ensure package inserts or complete pharmacological information been included (Section 1).] (valoid) 50mg tab. 2.3 Name(s) and details (as above) of concomitant medication(s) including rescue medications which are required in the protocol, and MCC registration number(s) if applicable: [Ensure package inserts or complete pharmacological information has been included with application (Section 1).]

Estimated Quantity of Trial Material (each drug detailed separately) for which exemption will be required:10ml gel / 12 patients, 12 x 50mg tabs,

2.5 If any of the above drugs are available in South Africa, give an explanation for not using what is available in South Africa:
2.6 Details of receiving of drugs from supplier, storage, dispensing, packaging of drugs:

2.7 Date MCC registration applied for – or envisaged date of application for trial medication. Explain if registration is not envisaged:

2.8 Registration status of entity, for the indication to be tested in this trial, in other countries: (i.e. Country: date registered / date applied for / date registration refused / date registration withdrawn by applicant / date registration cancelled by regulatory authority) [Attach as an appendix if necessary.]

Part 3: DETAILS OF TRIALIST(s) AND SITE(s)

3.1 Details of Investigator(s): [designation, title: (i.e. principal investigators / investigators) Include Name/Address/Tel/Cell/Fax/E-Mail] Prof DG Muller, Biocen Clinic, Pharmaceutics Dept, PU for CHE, Box 36, Potchefstroom 2520; 018 2992272, 018 299 2248, fmsdgm@puknet.puk.ac.za

3.2 Current work-load of Investigator(s): (Number of studies currently undertaken by trialist(s) as principal and/or co- or sub-investigator, and the total number of patients represented by these studies. Time-commitments of researcher(s) in relation to clinical trial work *and* non-trial work.)

Recommended format for response:

Investigator (Name and designation):	Prof DG Muller		
Total number of current studies (all stages) on specified date	Number	Date	
Total number of patients / participants for which responsible on specified date	Number 12	Date	
ESTIMATED TIME PER WEEK	[168 hours denominator]	Hours	%
1.1.1.1.1 Clinical trials	Clinical work (patient contact)		
	Administrative work		
Organisation (Practice / university / employer)	Clinical work		
	Administrative work		
1.1.1.1.2 Teaching	Preparation / evaluation		
	Lectures / tutorials		
Writing up work for publication / presentation			
Reading / sourcing information (e.g. internet searches)			
Other (specify)			

3.3 Details of Site(s) (Name of site, physical address, contact details, contact person, etc.)

3.4 Capacity of Site(s): (Number of staff, names, qualifications, experience -- including study co-ordinators, site facilities, emergency facilities, other relevant infrastructure)

Part 4: PARTICIPANTS (SUBJECTS)

4.1 Number of participants in South Africa:12

4.2 Total worldwide:12

4.3 Total enrollment in each SA centre: (if competitive enrollment, state minimum and maximum number per site.)

4.4 Volunteer base from which South African participants will be drawn: PU for CHE

4.5 Retrospective data indicating potential of each site to recruit required number of patients within envisaged duration of trial. (SA Guidelines 2000, Item 3.3, p15) [May be attached. Label clearly as 'Section 2 Item 4.5']

Part 5: OTHER DETAILS

5.1 If the trial is to be conducted in SA and not in the host country of the applicant / sponsor, provide an explanation:N/A

5.2 Estimated duration of trial: +/- 2 Months

5.3 Name other Regulatory Authorities to which applications to do this trial have been submitted, but approval has not yet been granted. Include date(s) of application:N/A

5.4 Name other Regulatory Authorities which have approved this trial, date(s) of approval and number of sites per country: PU for CHE ethics committee

5.5 If applicable, name other Regulatory Authorities or Ethics Committees which have rejected this trial and give reasons for rejection:N/A

5.6 If applicable, details of and reasons for this trial having been halted at any stage by other Regulatory Authorities:N/A

5.7 Details if this trial is being undertaken in SADC, any other country in Africa, or any country where there is no regulatory control of clinical trials:N/A

5.8 Previous studies using this agent which have been approved by MCC: :N/A

1.1.2 MCC approval number:

Study title:
Protocol number:

Date of approval:
National PI / Principal Investigator:
Date(s) Progress report(s):

1.1.2.1 Date Final report:

5.9 If any substudies are proposed as part of this protocol, indicate whether or not they will also be done in South Africa. If not, please explain.

1.1.2.1.1 Part 6: ETHICS

6.1 Ethics Committee responsible for each site, date of approval or date of application: PU for CHE ethics committee

6.2 Attach copy of response(s) made by, and/or conditions required by ethics committee(s) if available. Ensure that date of EC response is legible.

6.3 State which Good Clinical Practice (GCP) guidelines are being followed. (Particular reference to the South African guidelines required):

6.4 Details of capacity building component of the trial, if any:

6.5 Details of the training of investigators, monitors, study co-ordinators in terms of carrying out this trial and in terms of GCP:

6.6 Detailed safety and monitoring plan for each site: [May be attached. Label as 'Section 2 Item 6.6']

6.7 Details of trial insurance certificate: (e.g. title, protocol, dates, policy #, amount)

6.8 Details of possible conflict of interest of any person(s)/organisation(s) who/which will be involved in the trial:

6.9 Remuneration to be received in SA Rands: (Investigators) (Trial participants) (Others) Indicate broad breakdown of costs to be covered by this amount – if applicable. [Note: the CTC recommends a *mini*mum compensation of R50.00 per visit for participants travel and incidental expenses.]

Reviewer's comments on Section 2:

SECTION 3 – APPLICANT'S REPORT / PRESENTATION
[Please use Black 12 point Arial Font, using MSWord or rich text format (rtf) for electronic version]

1. Title: Determination of the pharmacokinetics of cyclizine HCl after single dose of intranasal and oral administration to healthy human volunteers

CTC Reviewer's comment:

2. Protocol Number/identification:

3. Rationale for study summarised: (Why should this trial be done at all?) Include statement about South African contribution, if any, to the development of this protocol.Improved absorption and hence bioavailability of cyclizine HCl through prolongation of the retention time in the nasal mucosa and direct delivery of the unchanged drug into the systemic circulation will be achieved. Employment of the intranasal route for drug administration will broaden the technological options for the design of intranasal delivery systems and give an insight to the mechanisms of nasal absorption of drugs and eventually the bioavailability which are currently the most challenging aspects.

CTC Reviewer's comment:

4. Background information (summarised – essential points that apply to this trial) [1-2 sentences max for each point]:
Disease / problem:
South African context (e.g. local epidemiology)

- To compare the AUC, Cmax and Tmax of cyclizine HCl after intravenous, intranasal and oral administration
- To try to enhance the intranasal drug delivery of cyclizine using various absorption enhancers
- To develop a formulation in order to optimize intranasal delivery of cyclizine
- To assess the *in-vivo* correlation between rats and humans

Properties of Drug / Entity; hypotheses about mechanism of action, etc.
Mechanism of action

The mechanism by which cyclizine exerts its anti-emetic and anti-motion sickness is not precisely known but may be related to its central anticholinergic actions thus it tends to diminish vestibular stimulation and depresses the labyrinth function. An action on the medullary chemoreceptor trigger zone may be involved in the anti-emetic effects (USP DI, 1998).

Like all antihistamines, cyclizine competitively inhibits the H_1-receptors of the smooth muscles to histamine stimulation. Within the vascular tree, it also inhibits both

vasoconstrictor effects of histamine and to a degree the more rapid vasodilator effects that are mediated by H_1-receptors on the endothelial cells (Hardma, 1996).

Effects on capillary permeability: It strongly blocks the action of histamine that results in the increase in capillary permeability and the formation of oedema and wheal.

Effects on exocrine glands: Cyclizine does not inhibit the secretion of gastric fluids but suppresses histamine evoked salivary, lachrymal and other exocrimal secretions with variable responses (Hardma, 1996).

Clinical applications and dosage

Cyclizine is an H_1-receptor antagonist with central nervous system depressant, anticholinergic, anti-emetic, antispasmodic and local anaesthesia effects. It is mainly used as an anti-emetic and anti-motion sickness due to its prominent anticholinergic activity and actions on the vomiting centre. It is therefore indicated for the following:

- Prevention and treatment of motion sickness
- Treatment of irradiation sickness
- Control of post-operative and drug induced vomiting
- Treatment of nausea and vomiting
- Symptomatic treatment of vertigo caused by Mernier's disease and other labyrinth disturbances (Martindale, 1992).

The different clinical uses and doses for each disease state are presented in the table 1 below. The dose largely depends on the indication presented. Usually the intravenous and oral dose range between 25mg to 300mg and the rectal dose is between 100mg and 300mg.

Table 1: Clinical presentations and recommended dose

Indication	IV/ Oral/rectal dose Adult	IV/ Oral/rectal dose Paediatric
Motion sickness	100 mg tid supp, 50mg tid po/im	50mg tid supp 12.5 mg tid po/im
Post–operative nausea	50mg im before end of operation	12.5mg im before end of operation
Nausea and Vomiting/Labyrinth	100 mg tid supp, 50mg tid po/im	50mg tid supp 12.5 mg tid po/im

disorders		

1.1.2.2 Pharmacokinetics

There is limited information on the pharmacokinetics of antihistamines and cyclizine in particular.

Pharmacodynamics

Absorption

Cyclizine is well absorbed following oral or parenteral administration. Peak plasma concentrations after a single oral dosing of 50mg occurs after 2 to 3 hours and last for 4 to 6 hours following oral administration. Usually symptomatic relief begins after 15 to 30 minutes post oral administration (Drugs, 1988_g).

Metabolism

The drug is extensively metabolised by N-demethylation in the liver to form an inactive metabolite, norcyclizine that is widely distributed throughout the tissues especially the kidneys, liver, lungs, and spleen. In plasma, norcyclizine is 60 % protein bound. Due mainly to the drug's extensive hepatic extraction, its bioavailability after oral administration is reported to be low (Clarke, 1986).

Distribution

The distribution of cyclizine has not been well characterised but available literature indicate that high concentrations of both the drug and the inactive metabolite (norcyclizine) are found in the kidneys, liver, lungs and spleen (Clarke, 1986).

Elimination

The metabolic fate of cyclizine is not clearly stated however, reports indicate that the drug undergoes an extensive hepatic metabolism and is excreted as an inactive metabolite (norcyclizine) in urine.

Half-life

- There is no documented information on the pharmacokinetics of cyclizine however, studies indicate a biological half-life of about 13 hours (Walker, 1995)
- Dryness of mouth, nose and throat
- Tachycardia

- Loss of appetite
- Nervousness
- Restlessness
- Trouble in sleep/skin rash
- Input stomach (USP DI, 1998)

Contraindications

The use of cyclizine is contraindicated in patients with the following diseases or disorders:

- Acute asthma
- Glaucoma
- Urinary retention
- Prostrate hypertrophy
- Chronic pulmonary disease
- Shortness/difficulty of breathing
- For geriatrics, no documented information is available on the relationship of age to effects of cyclizine but caution should taken as regards usage by geriatrics. This group of patients tends to exhibit sensitivity to anticholinergics, which are pharmacologically related to cyclizine and therefore constipation, dryness of mouth, and urinary retention may occur (USP DI, 1998).

Drug Interaction

3. Concurrent use of cyclizine with:

- Anticholinergics and other drugs with anticholinergic activity potentiates the anticholinergic side effects.
- Central nervous system depressants potentiates the sedative effect of these medications.

4. Prior administration of cyclizine may reduce the anti-emetic response to apomorphine in the treatment of poisoning (USP DI, 1998).

References

AHFS *Drug Information;* 1988_g : 2

Clarke E G C. *Isolation and identification of drugs in pharmaceuticals, body fluids and post-mortem materials;* 1986 2nd Edition : 497-498

Hardma J G, Limbird L E. *Goodman and Gillman's Pharmacological basis of therapeutics 9th Edition;* 1996 : 584, 585, 586-7

USP DI *Drug Information for Health Care Professionals 18th Edition;* 1998 : 1118
Wade A. *The Extra Martindale Pharmacopoeia;*1992 30th Edition : 1279

Pre-clinical findings: (e.g. laboratory / animal / toxicity / mutagenicity)
Clinical findings (e.g. phases; PK; PD; dose-finding; ADRs, NNT/NNH, other)
Systematic review(s) and/or citations per year-group on a Medline search

CTC Reviewer's comment:

5. Objectives of study (clearly listed and justified)

CTC Reviewer's comment:

6. Study design (clearly described and each component justified)
[includes phase, use of placebo, dosages, randomisation, blinding, duration, etc.]

CTC Reviewer's comment:

Each volunteer will be informed of the advantages and disadvantages of the trial. Each volunteer will have to sign an informed consent form before admission to the trial. A thorough medical examination will be performed on each volunteer as well as a series of pathological tests to make sure that the volunteer is healthy and normal. It will be expected from the volunteers to report to the clinic 12 hours before commencement of the trial.

A single blind three phase cross-over study involving 12 healthy subjects will be performed. At the end of the experimental period each patient would have received a dose via the intravenous, intranasal and oral route. Blood samples will be taken at predetermined time periods after administration of the dose. After a washout period of one week (t ½ = 13 hours) the volunteers will receive another dose via another route using a randomised schedule.

Subjects will undergo the following treatments:
Oral administration of 100mg of cyclizine
Intranasal administration of 125mg/ml cyclizine i.e 25mg/25ul

Blood will then be drawn from each subject post drug treatment at the following time intervals:

0,15,30,45,60,120,180,240,360,480,600,720 min,24hrs, 48 hrs,72 hrs. The samples will be centrifuged under cooled conditions and the plasma stored at – 70° C until analysed.

7. Participants: (number of participants; ability to enroll required number within stated time)

CTC Reviewer's comment:
12 human subjects
8. Eligibility and enrollment: (Inclusion and exclusion criteria listed and justified)

CTC Reviewer's comment:

9. Treatment modalities and regimens, drug accountability [clearly explained and justified for all participant groups/arms e.g. in terms of route of administration, dose, etc. Drug accountability clearly described.]

CTC Reviewer's comment:

10. Outcome measurements/variables (each clearly stated and justified)

CTC Reviewer's comment:
AUC, Tmax, Cmax
11. Adverse events (prevention, definitions – including causality assignment, recording, reporting, time-lines, action to be taken, all clearly described)

CTC Reviewer's comment:

12. Statistical measures:
Determination of sample size correct, clear and justified (with and/or without stratification)
Statistical method(s) and analysis of quantitative measures appropriate, clear and justified
Statistical method(s) and analysis of qualitative measures appropriate, clear and justified
Data processing (how, where, when, who) clearly described and justified. If a SA person will be involved in data processing, please identify that person
Interim analysis envisaged or not (justify) and stopping rules if applicable (explain)

CTC Reviewer's comment:
PU for CHE statistics dept through Prof Steyn will conduct the stats analysis
13. Ethical Issues: justification of 'Section 2 part 6' including:

- Explanation of which GCP guidelines are or are not being followed – with particular reference to the South African guidelines
- Comment on choice of investigators (refer to point C of Introduction, page 2 SA Clinical Trials Guidelines 2000)

- Comment on need for, appropriateness of, and relevance of GCP training / updating / for staff involved in this trial
- Comment on capacity building element of trial
- Comment on resources of sites and sponsor
- Comment on monitors and monitoring plan
- Indicate how additional staff (monitors, pharmacists, nursing staff, etc.) will maintain patient confidentiality, follow the protocol, and abide by ethical and regulatory requirements
- Comment on insurance and indemnity measures
- Comment on Patient Information Leaflet and Informed Consent (NB: inclusion of ABPI guidelines; appropriate level of education/English; possible benefits / risks clear; ensuring patient rights; contact names and numbers, as well as MCC details, included)
- Comment on availability and completeness of separate PILs and informed consent forms for any proposed archiving of blood specimens for later research or for genetics research.
- Comment on ethics of the publication policy
- Comment on treatment and/or management of participants and their disease condition(s) after completion of trial
- Comment on ethics committee capacity to monitor site if not a local ethics committee
- Provide an explanation if minimum recommended compensation for participants is not being provided.

CTC Reviewer's comment:

1.1.2.2.1

1.1.2.2.2 14. Other relevant information not included above

E.g. Are references adequate and dates of references current?
Are there discrepancies between protocol and IB or package inserts? Are there specific explanation(s) for these discrepancies?
Are the explanations for not following the SA 'GCP guidelines' acceptable?
Other comments on this trial.

CTC Reviewer's comment:

For office use:

CTC Reviewer's questions and concerns to be considered and/or forwarded to applicant:

CTC Reviewer's recommendation:

Declaration of conflict of interests by CTC reviewer:

CTC recommendation (date): 1A, 1B, 2, 3, 4, 5

MCC decision (date):

APPENDIX 9: CBF measurements in the various solutions at pH6.8

Table 1: Effect of DMEM cell culture on CBF

Time	CBF					Ave	SD
0	13.2	13.2	13.1	13.1	13	13.2	0.083666
5	13.1	13	13.2	13.1	13.2	13.2	0.083666
10	13	13.2	13	13.1	13.2	13.1	0.1
15	13.2	13.2	13.1	13.2	13	13.14	0.089443
30	13.1	13	13	13.1	13.2	13.08	0.083666
45	13.2	13.2	13.2	13.1	13	13.14	0.089443
60	13.2	13.2	13	13.1	13.2	13.14	0.089443
90	13.1	13.1	13.2	13.2	13	13.12	0.083666
120	13.2	13	13.2	13.1	13.2	13.14	0.089443
150	13.2	13	13.1	12.9	13	13.04	0.114002

Table 2: Effect of cyclizine HCl solution on CBF

TIME	CBF±SD			
	0.3384mg/ml	0.9948mg/ml	1.66mg/ml	3.642mg/ml
0	11.26±0.08	11.3±0.05	11.4±0.22	11.2±0.25
5	11.1±0.07	11.1±0.23	11.1±0.52	11.2±0.46
10	11.56±0.2	11.3±0.23	11.8±0.68	11.6±0.12
15	11.66±0.27	11.7±0.15	11.7±.0.6	11.7±0.09
30	11.22±0.25	11.7±0.28	11±0.04	11.1±0.05
45	11.74±0.08	12±0.26	11.3±0.25	11.7±0.02
60	11.64±0.09	11.6±0.87	11.8±0.26	11.7±0.01
90	11.78±0.58	11.7±0.23	11.8±0.58	11.8±0.036
120	11.26±0.52	11.2±.0.36	11.3±0.41	11.2±.0.87

Table 3: Effect of HPMC on CBF

Time	CBF+SD				
	0.0625%w/v	0.125%w/v	0.25%w/v	0.5%w/v	1%w/v
0	12.4±0.20	12.4±0.56	12±0.23	12±0.59	11.2±0.26
5	12.1±0.36	12.3±0.86	12.4±0.34	12.1±0.05	11±0.21
10	12.7±0.15	12.9±.0.03	12.6±0.31	12.6±0.69	11.4±0.12
15	12.5±0.68	12.5±0.25	12.9±0.15	12.7±0.20	11.6±0.14
30	12.3±0.24	12.5±0.24	12.6±0.87	12.7±0.52	11.1±0.18
45	12.8±0.25	12.8±0.69	12.9±0.14	12.9±0.12	11.9±.0.18
60	12.5±0.14	12.5±.0.42	12.3±0.69	12.5±0.13	11.4±0.25
90	12.4±.0158	12.7±0.14	12.3±0.98	12.4±0.14	11.9±.0.24
120	12±0.65	12.2±0.75	12.6±0.12	12.4±0.30	11.3±0.23
180	11.9±0.78	12.3±0.69	12.2±0.36	12±0.26	11.0±.0.22

Table 4: Effect of Carbopol 934P on CBF

TIME	CBF+SD				
	0.0625%w/v	0.125%w/v	0.25%w/v	0.5%w/v	1%w/v
0	12.6±0.05	12.8±0.12	12.7±0.52	12.7±0.09	11.8±0.23
5	12.7±0.04	12.6±0.14	12.9±0.025	12.6±0.07	11.7±0.17
10	12.7±0.1	12.6±0.13	12.7±0.23	12.6± 0.26	11.6±0.04
15	12.7±0.12	12.6±0.15	12.7±0.13	12.5± 0.28	11.5±0.15
30	13±0.06	12.9±0.18	13.1±0.09	13.2± 0.18	10.9±0.08
45	13.3±0.08	13.3±0.22	13.3±0.18	13±0.17	11.2±0.48
60	14.1±0.59	14±0.26	14.1±0.44	14.1±0.48	11±0.09
90	13±0.058	13.6±0.28	13.8±0.12	13.8±0.15	11.9±0.18
120	12.9±0.056	12.7±0.30	13±0.3	13.2±0.04	11.2±0.15
180	13±0.058	13.7±0.042	12.9±.011	13.7±0.54	11.0±0.03

Table 5: Effect of Na-CMC on CBF

TIME	CBF+SD				
	0.0625 %w/v	0.125 %w/v	0.25% w/v	0.5%w/v	1%w/v
0	9.7±0.02	10.4±0.25	10.4±0.85	10.3±0.25	10.1±0.054
5	10.8±0.05	11±0.24	10.8±0.54	10.7±0.24	10.9±0.012
10	12.8±0.09	12.8±0.012	13.1±0.056	12.8±0.13	9.6±0.035
15	12.6±0.080.	12.5±0.16	12.5±0.12	12.6±0.57	9.8±0.089
30	10.4±0.02	10.3±0.35	10.8±0.18	10.6±0.058	10.6±0.098
45	10.6±0.16	10.7±0.125	10.7±0.17	10.7±0.098	10.7±0.047
60	9.6±0.22	9.7±0.21	9.7±0.09	9.5±0.48	9. 6±0.056
90	12.5±0.17	12.8±0.068	12.8±0.08	11±0.054	10.7±0.04
120	12±0.19	13.6±0.08	13.4±0.52	10.9±0.24	9.7±0.12
180	11.8±.0.21	12±0.09	12.4±0.012	11.9±0.15	11±0.135

Table 6: Effect of TMC 36.3%DQ on CBF

TIME	CBF+SD				
	0.0625%w/v	0.125%w/v	0.25%w/v	0.5%w/v	1%w/v
0	10.4±.0.025	7.6±.0.058	6.5±.0.18	4.3±.0.016	
5	10.7±.0.28	7.5±.0.089	6.1±.0.14	4.5±.0.014	
10	10.1±.0.12	7.6±.0.078	6.2±.0.16	4.6±.0.25	
15	9.5±.0.16	7.7±.0.09	6.1±.0.09	4.6±.0.22	
30	9.7±.0.05	7.6±.0.15	6.3±.0.080		
45		6.9±.0.16	6.0±.0.14		
60		7.2±.0.15			
90					
120					