Pheroid® technology in the transdermal delivery of selected non-steroidal anti-inflammatory drugs

D Kilian

11985070

BPharm, MSc (Pharmaceutics)

Thesis submitted in fulfilment of the requirements for the degree *Philosophiae Doctor* in Pharmaceutics at the Potchefstroom Campus of the North-West University

Promoter: Prof J du Plessis

Co-promoter: Dr M Gerber

November 2015

Abstract

Drug delivery through the skin still remains an area that enjoys active research with much of the research focusing on physical or chemical methods to reversibly alter the skin's permeability to compounds because of the skin's very low permeability. Vesicular carriers are one of the many chemical approaches used in order to deliver drugs into and sometimes through the skin. A review article offers an overview of various vesicles that have been investigated during dermal and transdermal drug delivery research, with special emphasis on a relatively new carrier, namely the Pheroid™. Based on the review of previous work that was done it was found that it is still unclear whether an API requires certain physicochemical characteristics in order for the Pheroid™ to enhance its permeation or not. It was also observed that the type of formulation had a significant impact on the API's transdermal delivery behaviour.

To aid in this investigation of the optimal physicochemical properties for the transdermal delivery of selected non-steroidal anti-inflammatory drugs (NSAIDs) dispersed in the Pheroid™ delivery system, a novel flux-independent mathematical model was derived and tested. The model is based on restrictions made to Lipinski's rule of five. The results suggested that the same molecular size and log P (octanol-water partition coefficient) ranges that had determined the skin permeability of an API having been dissolved in PBS, also determined its permeability when dissolved in Pheroid™, and that these ranges were consistent with the previously described restrictions that should be placed on Lipinski's rule of five, to account for the formidable resistance offered by the skin's stratum corneum. The model gave reasonable approximations of the experimentally observed concentrations. The results suggested that certain restrictions placed on Lipinski's rule of five could be used to accurately model transdermal delivery.

The ability of Bayesian networks to determine the correct probabilistic dependencies between skin permeability and the physicochemical properties of selected drugs dissolved in PBS at pH 7.4 and dispersed in a lipid-based drug delivery system was also investigated. The networks identified a probabilistic dependence between pKa and skin permeability for drugs dissolved in PBS (pH 7.4), which was not observed for the same drugs dispersed in the lipid micelles of the Pheroid™ drug delivery system. For both dissolved and dispersed drugs the same probabilistic dependencies existed between topological polar surface area (TPSA), melting point (MP) and cumulative amount of drug dissolved in PBS (pH 7.4) (CPBS), which permeated the skin after 12 h, in comparison to the cumulative amount of drug dispersed in

Pheroid™ (CPher). Although both networks shared a causal relationship between permeability and molecular size descriptors, the network determined from the dissolved drugs displayed a probabilistic dependence between molecular weight (MW) and permeability, while the dispersed drugs displayed dependence between molecular volume (MV) and permeability. Both networks identified similar physicochemical regions for optimal transdermal delivery, i.e. low MP, small TPSA to molecular size ratio and, in the case of the dissolved drugs, a pKa value close to the pH of the buffer solution. Regions for poor transdermal delivery were also identified, i.e. high MP and a TPSA to molecular size ratio close to 0.5. The results suggest that Bayesian networks determined from online bioinformatics and cheminformatics databases can be viable classification tools in early drug discovery and development, and can aid in the identification of suitable drug candidates and formulation strategies.

Exploratory data analysis of the dependencies between skin permeability, MW and log P was also investigated as they remain the most frequently used physicochemical properties in models that predict skin permeability. The results suggest that, in general, MW and log P are poorly correlated to log Kp (permeability coefficient). However, after employing several exploratory data analysis techniques, regions within the dataset of statistically significant dependencies were identified. As an example of the possible applicability of the information extracted from the exploratory data analyses, a multiple linear regression model was constructed, bounded by the ranges of dependence. This model gave reasonable approximations to log Kp values obtained from skin permeability studies of selected NSAIDs administered from a buffer solution and a lipid-based drug delivery system. Knowing the ranges within which molecular weight and log P are statistically related to log Kp can supplement existing methods of screening, risk analysis or early drug development decision making to add confidence to predictions made regarding skin permeability.

Keywords: Transdermal drug delivery, Pheroid™, exploratory data analysis, physicochemical properties, vesicular carriers, permeability, skin, NSAID, mathematical model, Lipinski's rule

Uittreksel

Geneesmiddelaflewering deur die vel bly vandag nog 'n onderwerp wat aktief nagevors word, met spesifieke fokus op fisiese en chemiese metodes wat veldeurlaatbaarheid omkeerbaar verhoog, as gevolg van die lae deurlaatbaarheid van die stratum korneum. Vesikeldraers is een van die vele chemiese benaderings wat gebruik word om geneesmiddels in en soms deur die vel af te lewer. 'n Oorsigartikel beskryf verskeie vesikels wat al nagevors is tydens dermale en transdermale geneesmiddelaflewering, spesifiek ingestel op 'n relatiewe nuwe draer genaamd Pheroid™. Die artikel is gebaseer op 'n oorsig van vorige werk wat gedoen is en gevind het dat dit steeds onduidelik is of 'n geneesmiddel sekere fisiese en/of chemiese eienskappe moet hê vir die Pheroid™ om deurlaatbaarheid te verhoog. Daar is ook bevind dat die tipe formulering 'n beduidende impak op die geneesmiddel se transdermale aflewering het.

'n Nuwe fluks onafhanklike wiskundige model is ontwikkel en getoets om die optimale fisiese en chemiese eienskappe van geselekteerde nie-steroïedale anti-inflammatoriese middels (NSAIMs) in die Pheroid™ vir transdermale aflewering te bepaal. Die model is gebaseer op beperkings wat geplaas is op Lipinski se reël van vyf. Die resultate impliseer dat dieselfde molekulêre grootte- en log P- (oktanol-water verdelingskoëffisiënt) eienskappe, wat die vel se deurlaatbaarheid vir 'n geneesmiddel opgelos in PBS bepaal het, ook die deurlaatbaarheid van die geneesmiddel bepaal wanneer dit in die Pheroid™ afleweringssisteem opgelos is, en dat die areas konstant bly met vorige beskrewe beperkings wat op Lipinski se reël van vyf geplaas moet word om die formidabele weerstand wat deur die stratum korneum gebied word in berekening te bring. Die model het aanvaarbare approksimasies van die konsentrasies wat eksperimenteel waargeneem is weergegee. Die resultate impliseer dat sekere beperkings wat op Lipinski se reël van vyf geplaas word, gebruik kan word om akkuraat transdermale aflewering te modelleer.

Die vermoë van Bayesiaannetwerke om die korrekte probabilistiese afhanklikhede tussen veldeurlaatbaarheid en die fisiese en chemiese eienskappe van geselekteerde geneesmiddels opgelos in PBS (pH 7.4) en gedispergeer in 'n lipied-basis geneesmiddelafleweringssisteem te bepaal, is ook ondersoek. Die netwerke het 'n probabilistiese afhanklikheid tussen pKa en veldeurlaatbaarheid vir geneesmiddels opgelos in PBS (pH 7.4) geïdentifiseer, wat nie gesien is vir dieselfde geneesmiddel wat gedispergeer is in lipied miselle of die Pheroid™ geneesmiddelafleweringssisteem nie. Vir beide die opgeloste en gedispergeerde geneesmiddels bestaan dieselfde probabilistiese afhanklikhede tussen die topologiese polêre

oppervlakte, smeltpunt en kumulatiewe hoeveelheid geneesmiddel opgelos in PBS (pH 7.4) wat die vel na 12 uur deurdring het en die kumulatiewe hoeveelheid geneesmiddel gedispergeer in die Pheroid™ afleweringssisteem. Alhoewel beide netwerke 'n oorsaaklike verhouding gedeel het tussen deurlaatbaarheid en molekulêre grootte, het die netwerk van die opgeloste geneesmiddels 'n probabilistiese afhanklikheid tussen molekulêre volume en deurlaatbaarheid getoon, terwyl die gedispergeerde geneesmiddels 'n afhanklikheid tussen molekulêre volume en deurlaatbaarheid getoon het. Beide netwerke het soortgelyke fisiese en chemiese eienskappe vir optimale transdermale aflewering, soos 'n lae smeltpunt, klein topologiese polêre oppervlakte tot molekulêre grootteverhouding en in die geval van opgeloste geneesmiddels 'n pKa-waarde naby aan die pH van die bufferoplossing geïdentifiseer. Geneesmiddeleienskappe vir swak transdermale aflewering, sluit in hoë smeltpunte en 'n topologiese polêre oppervlakte tot molekulêre grootteverhouding naby aan 0.5 is ook geïdentifiseer. Die resultate impliseer dat Bayesiaannetwerke, beide bio-informatika en cheminformatika databasisse, wesenlike klassifikasie netwerke kan wees in vroeë geneesmiddelontdekking en -ontwikkeling en van hulp kan wees in die identifikasie van geskikte geneesmiddelkandidate en formuleringstrategieë.

Verkennende data-analise van die afhanklikhede tussen veldeurlaatbaarheid, molekulêre massa en log P-waarde is ook ondersoek omrede hierdie fisiese en chemiese eienskappe tans nog die eienskappe is wat die algemeenste gebruik word in modelle wat veldeurlaatbaarheid voorspel. Die resultate impliseer dat molekulêre massa en log P oor die algemeen slegs korreleer met log Kp (deurlaatbaarheidskoëffisiënt). Nadat verskeie verkennende data-analisetegnieke egter toegepas is, is statisties betekenisvolle eienskappe van afhanklikhede binne die datastel geïdentifiseer. As 'n voorbeeld van die moontlike toepaslikheid van die inligting wat onttrek is van die verkennende data-analise, is 'n multilineêre regressiemodel geskep, gebind deur die eienskappe van afhanklikheid. Die model het redelike approksimasies tot log Kp-waardes getoon, wat veldeurlaatbaarheidstudies van geselekteerde NSAIMs, toegedien vanaf 'n bufferoplossing, en 'n lipied-basis geneesmiddelafleweringssisteem insluit. Die data rakende die eienskappe waarin molekulêre massa en log P statisties tot log Kp verband hou, kan aangewend word vir sifting, risiko-analise en vroeë geneesmiddelontwikkeling, wat voorspellings rakende veldeurlaatbaarheid komplementeer.

Sleutelwoorde: Transdermale geneesmiddelaflewering, Pheroid™, verkennende data-analise, fisies-chemiese eienskappe, vesikulêre draers, deurlaatbaarheid, vel, nie-steroïedale anti-inflammatoriese middels, wiskundige model, Lipinski se reël

Acknowledgments

Prof. Jeanetta du Plessis, my promoter, thank you for never giving up on me, for all the encouragement and support throughout the years. You certainly have gone above and beyond what would have been expected of a promoter in order to enable me complete this work. You are an inspiration and an asset to the research community.

Dr Minja Gerber, my co-supervisor, for all your valuable inputs during the writing of this document.

Prof Jan du Preez for assisting with the drug analysis and I am thankful to **Dr. Righard Lemmer** for his kind support with the modelling of results in order to establish new suggested models.

Dr. Yasser Shahzad and Dr. Lizelle Fox for assistance with the literature review article.

My sincere appreciation goes to the **National Research Foundation of South Africa (NRF)** as this work was carried out with their financial support (Grants no. IFRR81178 and CPRR13091742482) and to the **Centre of Excellence for Pharmaceutical Sciences (Pharmacen)** of the North-West University, Potchefstroom Campus, South Africa.

Disclaimer: Any opinions, findings and conclusions, or recommendations expressed in this material are those of the authors and therefore the NRF does not accept any liability in regard thereto.

Prof. Jonathan Hadgraft for the financial support and guidance during the year I spent in the U.K. at the London School of Pharmacy.

To all my **friends and colleagues** at the North West University, I would like to say thank you for all the good times we had over the many years we spent on campus.

I would also like to thank my parents **Louis and Wilma Kilian** for their unwavering support and encouragement over the years.

Without the support and understanding of my loving wife **Juanita** this work would also not have been possible**.**

Preface

This thesis is submitted in an article format and written according to the requirements of the NWU manual for postgraduate studies and conforms to the requirements preferred by the appropriate journals. The thesis is written according to UK English spelling, the article Chapters are written according to each journals Guide to Authors.

Chapter 2:

Article 1: Vesicular carriers for skin drug delivery: The Pheroid™ technology

Article published in Current Pharmaceutical Design.

This review publication was written in order to fulfil the requirement of the NWU that a complete literature overview should be included. No separate literature overview was thus included in this thesis as this review was seen as fulfilment of the above requirement.

Chapter 3:

Article 2: A flux-independent model assisted investigation into the optimal skin permeation properties of selected NSAIDs in a lipid based carrier system.

Article for publication in the Journal of Pharmaceutical Sciences

Chapter 4:

Article 3: A cheminformatics and Bayesian network approach to investigating the probabilistic dependencies in transdermal drug delivery.

Article for publication in Artificial Intelligence in Medicine

Chapter 5:

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Article for publication in Pharmaceutical Statistics

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Chapter 1: Introduction and problem statement

The utilisation of the skin as a means of delivering active pharmaceutical ingredients (APIs) into the systemic circulation of the human body to elicit a pharmaceutical response, has been extensively researched for decades and still remains a very dynamic research topic, as new ways of trying to breach the skin's barrier function are constantly developed, or improved upon. The main physiological features of the skin, as well as some of the physiochemical characteristics of APIs are briefly discussed in Chapter 1, whilst some reported methods, used in transdermal drug delivery, are also reviewed.

The skin comprises the largest organ of the body and acts as a protective barrier, whilst also fulfilling sensory and immunological functions (Foldvari, 2000:417). The skin of an average human weighs approximately 4 kg and has a surface area of about 1.8 m^2 (Bronaugh & Collier, 1993:98). The topical application of drugs to the skin for the treatment of skin disorders is known as dermal drug delivery. Dermal drug delivery has the advantage that high concentrations of the applied drugs are localised at the site of action, which reduces systemic side effects associated with the applied drugs. Transdermal drug delivery employs the skin as an alternative route for delivering drugs for a systemic effect on the body. The advantage of delivering a drug intended for systemic effects through the skin rather than through the oral route is that it bypasses the gastro-intestinal route with all its variables, such as pH, gastro-intestinal motility and food intake. It also circumvents the hepatic first-pass effect, whilst it can deliver constantly controlled drug concentrations, which would minimise drug plasma level variations and hence reduce the side effects of drugs with a narrow therapeutic window (Honeywell-Nguyen & Bouwstra, 2005:67). It is difficult to predict the permeability of a drug or a compound through the skin because of the complex nature of the mechanisms and the structures in the skin that make up the delivery pathway of the drug in order for it to be absorbed systemically (Jepps *et al.*, 2013:153).

The skin's barrier function is entirely and quite remarkably achieved by the highly hydrophobic, outermost 10 - 20 µm of the skin, the stratum corneum, which is a compositionally and morphologically unique bio-membrane (Naik *et al.,* 2000:318).

Different vesicle delivery systems exist, each with its own unique characteristics that make it more or less suitable to act as carrier for certain APIs, including ethosomes, liposomes, niosomes, phyto-vesicles, dendrimers, nano-emulsions and transfersomes.

An evaluation of previous work done, using the Pheroid™ delivery system, has revealed that many variables exist among the different dosage forms in which the APIs and Pheroid™ technology had been incorporated and investigated (Kilian *et al.,* 2015:2758*).* The ability to determine which APIs could potentially be favourable candidates for transdermal delivery, when using the Pheroid™ delivery system, is therefore not an easy task.

Oral dosage forms of drugs mostly have gastric side effects, due to the drugs being subjected to the first-pass hepatic liver metabolism. As an alternative dosage form, proven enhanced transdermal drug delivery of APIs could thus offer better patient compliance by eliminating undesirable side-effects, whilst a lower amount of API in the dosage form, with the same therapeutic effects as the oral dosage form, could lead to lower production costs and a lower cost of the therapy to the patient, since less of the expensive API is used in the formulation. This in turn could increase the availability of drugs to poorer countries and aid organisations globally.

The purpose of this study was to use a range of non-steroidal anti-inflammatory drugs (NSAIDs), namely ibuprofen, ketoprofen, acetylsalicylic acid, piroxicam, meloxicam, indomethacin, acetaminophen, diclofenac sodium and mefenamic acid as model drugs and incorporate them into basic Pheroid™ solutions. Other variables found in currently available dosage forms where the Pheroid™ delivery system has been incorporated before are thus eliminated, in order to solely consider the physiochemical properties of each API in an attempt to identify a typical set of physiochemical characteristics that would qualify an API as a favourable candidate for use with the Pheroid™ drug delivery system.

The specific objectives of this study were to

- conduct a literature review on all previous transdermal delivery studies that were done using the Pheroid™ as a delivery system;
- carry out transdermal diffusion experiments using NSAIDs as model drugs;
- investigate the optimal physicochemical properties for transdermal delivery of selected NSAIDs administered with the Pheroid™ delivery system;
- investigate the potential of Bayesian networks to accurately model the probabilistic dependencies between physicochemical properties and skin permeability of the selected drugs; and
- investigate the probabilistic dependencies between molecular weight, log P (octanol-water partition coefficient) and log Kp (permeability coefficient) making use of exploratory data analysis.

Chapter 2 is in the form of an article entitled “Vesicular carriers for skin drug delivery: The Pheroid™ technology”, which has been published in the Current Pharmaceutical Design journal and will discuss the objectives described above. A second article entitled “A flux-independent model-assisted investigation into the optimal skin permeation properties of selected NSAIDs in a lipid-based carrier system” is presented in Chapter 3. In this article the optimal physicochemical properties for transdermal delivery of selected NSAIDs administered with the Pheroid™ delivery system are investigated. In a third article entitled “A cheminformatics and Bayesian network approach to investigating the probabilistic dependencies in transdermal drug delivery” (see Chapter 4), the potential of Bayesian networks to accurately model the probabilistic dependencies between physicochemical properties and skin permeability of selected drugs is investigated. In addition the ability of Bayesian networks to assist in early drug discovery and development decision making was investigated. In a fourth article entitled “Exploratory data analysis of the dependencies between skin permeability, molecular weight and log P” that can be found in Chapter 5, an exploratory data analysis of the probabilistic dependencies between molecular weight, log P and log Kp was investigated. Final conclusions and future prospects are discussed in Chapter 6.

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Chapter 2: Article published in Current Pharmaceutical Design

Chapter 2 is written in article format and was published in the journal Current Pharmaceutical Design in 2015 (doi: 10.2174/1381612821666150428125812). The complete author's guide is given in Annexure B.

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2758 *Current Pharmaceutical Design*, 2015, *21*, 2758-2770

Vesicular Carriers for Skin Drug Delivery: The Pheroid™ Technology

Dewald Kilian, Yasser Shahzad, Lizelle Fox, Minja Gerber and Jeanetta Du Plessis*

Centre of Excellence for Pharmaceutical Sciences, Faculty of Health Sciences, North-West University, Potchefstroom, South Africa

Jeanetta Du Plessis

Abstract: The skin remains an attractive area for drug delivery. The skin, however, often limits the ingress of drugs, because of its very low permeability. Much research, focusing on employing a variety of physical and chemical methods, aimed at reversibly altering skin permeability in favour of compounds, has been reported. Of the many chemical approaches that exist, one comprises the use of vesicular carriers for delivering drugs into and possibly through the skin. This review offers an overview of various vesicles that have been investigated during dermal and transdermal drug delivery research in recent years, with special emphasis on a relatively new carrier, namely the Pheroid™. The progress made to date by our research group with regards to the use of the Pheroid™ as transdermal delivery vector, is also discussed in detail.

Keywords: Elasticity, permeability, pheroid™, skin, mechanism of action, transdermal, vesicular carriers.

INTRODUCTION

Controlling the drug release and its site-specific delivery by carriers is not the only challenge in therapeutic research. Many physicochemical constraints of active pharmaceutical ingredients (APIs) negatively impact on their ability to cross biological membranes, therefore limiting their efficacy [1]. The development of vesicular carriers has attracted considerable attention during the last two decades, and vesicles are sought to address the problems associated with targeted drug delivery. The ultimate goal of vesicular carriers is to produce therapeutically efficient formulations for various clinical situations. For this reason, carriers are expected to meet a number of prerequisites, such as 1) biocompatibility and biodegradability, 2) high drug loading, 3) site-specific drug delivery to avoid any undesirable side effects, and 4) the controlled release of the drug at the target site [2]. To date, a limited number of vesicular carriers have successfully achieved these requirements, and have been further tested for dermal and transdermal, parenteral, ocular, oral and pulmonary drug delivery. This review aims at discussing the vesicular carriers intended for dermal and transdermal drug delivery. To augment this, the application and mechanisms of action of various vesicular carriers in dermal and transdermal drug delivery are discussed first. Afterwards, the versatility and effectiveness of the novel patented Pheroid™ delivery system in delivering APIs through the dermal route is extensively discussed. The Pheroid is a colloidal delivery system comprised of essential and plant fatty acids emulsified in nitrous oxide saturated water [3]. Next, an overview of the progress made to date by our research group in this domain will be given. Finally, this review concludes with a summary.

SKIN AS A ROUTE OF DRUG DELIVERY

The skin, having a large surface area of approximately 2 m^2 [4], has increasingly been considered as a potential drug delivery alternative. Topical application of drugs for local targeting of various skin disease conditions is known as dermal drug delivery, while transdermal drug delivery comprises the systemic transport of drugs through the skin. Drug delivery through the skin offers various advantages, including avoidance of hepatic metabolism, the elimination of gastrointestinal disturbances associated with oral delivery, non-invasive needless drug delivery, whilst a constant and controlled release of the drug can be achieved [5, 6].

The skin, being a protective layer of the body, offers a barrier to most compounds requiring delivery into and through it [7]. The outermost layer of the skin, the stratum corneum (SC), represents the effective barrier that limits the ingress of chemicals. The structure of the SC has classically been described as a brick and mortar model [8], with the corneocytes acting as the bricks and the intercellular lipid lamellae as the mortar. It has been suggested that the highly organised intercellular lipids comprise the main component of the barrier properties of the SC [9, 10]. Main pathways of drug permeation include transepidermal-, transappendageal- and transfollicular routes (Fig. **1**) [11]. The transepidermal pathway consists of intercellular and intracellular routes. The intercellular route is considered a continuous, but tortuous pathway through the intercellular lipids. Contrary, the transcellular pathway starts through the keratinocytes and then crosses through the intercellular lipids. To pass through the transcellular pathway, drugs need to successively partition and diffuse through the keratinocytes and intercellular lipid layers [12]. The transappendageal and transfollicular routes play a minor role in the transport of compounds across the skin, because both of these pathways cover only 0.1% of the total skin surface area [13].

Application of materials on the skin surface creates a concentration gradient, which drives molecules into and across the skin through the diffusion process. This concentration gradient directly depends upon skin permeability and the application area on the skin surface. When molecules diffuse across the skin under free molecular motion, skin permeability is proportional to the diffusivity and the partition coefficient of the permeant [7], as calculated by the following equation. The diffusion path length also influences the penetration ability of a permeant.

$$Skin\ Permeability = \frac{Diffusion\ coefficient \times Partition\ coefficient}{Path\ length}$$

Generally, the effective solute transport across the skin depends upon the relative extent of the trans-barrier water and permeant flows [15]. Any solute being administered epicutaneously is therefore pushed across the skin, under the influence of concentration gradient. In the process, the solute attracts water towards the skin surface, whilst maintaining its inward thrust through the intrinsic osmotic pressure difference. Conversely, for a perfectly permeable

*Address correspondence to this author at the Centre of Excellence for Pharmaceutical Sciences, Faculty of Health Sciences, North-West University, Potchefstroom, South Africa; Tel: +27 (0)18 299 2274; Fax: +27 (0)87 231 5432; E-mail: Jeanetta.DuPlessis@nwu.ac.za

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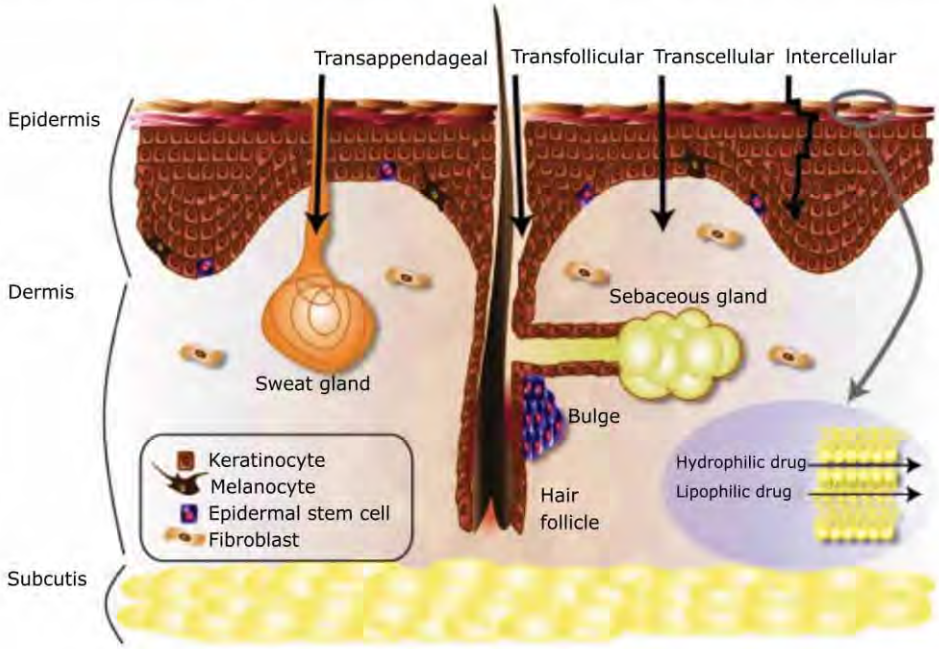

Fig. (1). Representation of a cross-section of the skin and various possible routes of drug penetration [14].

solute, transport across the skin barrier does not require an osmotically driven trans-barrier water flow [16].

The application area on the skin surface also dictates how much of a permeant can pass through. Since the amount of permeant that can pass through the skin also depends upon the permeant solubility in the skin, limited solubility can restrict the maximum achievable flux. This can be countered by either changing the application area, or by enhancing skin permeability through the use of chemical enhancers, or through physical energy means. So far, various physical and chemical methods have been investigated in an attempt to compromise the SC barrier to enable the effective transport of compounds across the skin [17]. These approaches are discussed in detail in various recent publications [18-21].

VESICLES FOR DERMAL AND TRANSDERMAL DRUG DELIVERY

Vesicles are spherical, often nano-sized, lipid-based carriers, within which a drug can be encapsulated in either the aqueous core, or inside of the lipid bilayer. Usually, these vesicles are formulated using physiological lipids, such as phospholipids and cholesterol, and thus, they are well tolerated, non-toxic and biodegradable. Various vesicular carriers that are used in dermal and transdermal drug delivery are discussed next. These vesicles include liposomes, niosomes, transfersomes, ethosomes, and a relatively new type of vesicle, namely Pheroid.

Liposomes

Structure, Methods of Preparation and Properties

Liposomes are phospholipid-based mono- or multi-concentric bilayer vesicles that contain a central aqueous compartment, and are generally in the size range of 20 nm to several micrometres [22]. Phospholipids are amphiphilic and spontaneously form bilayers upon hydration. Liposomes can be categorised into various classes, based upon their lipid bilayers, size and method of preparation. Examples are small unilamellar vesicles (SUV), large unilamellar vesicles (LUV), multilamellar vesicles (MUV), oligolamellar vesicles (OUV) and multi-vesicular vesicles (MVV) [23, 24].

Liposomes can be prepared through a variety of methods, depending upon the target size and their properties. Various formulation methods of liposomes have recently been reviewed by other authors [25]. Generally, all methods of preparation and subsequent drug loading involve four basic steps [25], namely:

1. Drying of the lipids and removal of the organic solvent.
2. Hydration of the dried lipid film to form liposomes.
3. Purifying the resultant liposomes.
4. Analysing the final product.

Liposomes are the most common and the most studied nanocarriers for drug delivery purposes, because of their unique characteristics [26]. They are biocompatible, biodegradable, non-toxic, and have an excellent capability of targeting the desired site of action, while also having easily tunable surface properties.

Mechanisms of Action

Various mechanisms of action of liposomes have been proposed and are reported in detail in previous publications [27, 28]. The subject of liposomes, however, remains controversial with regards to their ability to enhance topical and transdermal drug delivery. A brief description of the reported mechanisms is discussed in the next few paragraphs.

Firstly, the free drug mechanism, as proposed by Ganesan *et al.* [29], comprises that the drug penetrates the skin after its release from the vesicles on the skin surface. Enhancement of the topical delivery of estradiol, when employing the proposed mechanism was, however, found negligible during another study [30].

Secondly, Kato *et al.* [31] described the penetration enhancement properties of lecithin-based liposomes by compromising the skin barrier properties. Similar findings are also reported by other researchers [30, 32-34]. Contrary, other studies also report on the

negative effects that liposomes have on drug penetration through the skin [35-37].

Thirdly, liposomes may possibly interact with the skin and fuse with the lipid bilayers to become part of the membrane [38], and therefore enhance drug partitioning in the SC [30, 39].

Finally, early reports by Mezei and Gulasekharam [40, 41] propose that the intact vesicle itself may penetrate the skin. Similarly, Foldvari *et al*. [42] suggest that intact liposomes could reach the dermis. Lasch *et al*. [43] further suggest that only intact SUVs are able to penetrate the SC. Contrary, Du Plessis *et al*. [44] report that relatively larger sized liposomes were found to have enhanced the drug deposition in the skin, compared to smaller sized vesicles. Such enhanced drug deposition was believed to be indicative of the intact vesicles not having penetrated the skin.

In summary, it still remains enigmatic as to what extent liposomes are able to penetrate the skin. From the mixed reports it appears that small intact liposomes may penetrate the SC to some extent, but not beyond it. The penetration enhancing effect also relies upon the composition of the liposomes.

Liposomes as Dermal and Transdermal Delivery Vectors

Mezei and Gulasekharam [40] had initiated research on liposomes as a potential drug carrier in skin. Their work showed that triamcinolone loaded liposome-based lotion formulations could increase the drug deposition in the skin layers by three- to four-fold, with a minimal increase in systemic drug concentrations, compared with standard drug solutions. Later, Touituo *et al.* [45] also demonstrated a higher skin deposition of caffeine from liposomal formulations. These initial studies promised the suitability of liposomes as topical delivery vectors for localised effects. Some studies also highlighted that liposomes could target the appendages (hair follicles and associated sebaceous glands). Investigation of the deposition of gamma interferon into human, hairless rat and hamster skin revealed the highest accumulation in the hamster skin, having the highest follicular density among the three skin models [46]. Subsequently, the follicular pathway was proposed as a route of drug deposition into the skin. Contrary, El Maghraby *et al.* [47] demonstrated that the follicular pathway in transdermal delivery from liposomes played a negligible role. The discrepancies among the reported outcomes could translate into different approaches being adopted in formulating liposomes, for example the use of different lipid components in the vesicles.

Surprisingly, some earlier reports had also claimed transdermal permeation of drugs from liposomes. The research outcomes by Ganesan *et al*. [29] on rat skin and by Artmann *et al*. [48] on piglet skin clearly demonstrated enhanced percutaneous absorption of test drugs and antibodies, respectively. The reason behind these conflicting outcomes may have resulted from different mechanisms operating concurrently, such as the drug having diffused percutaneously from the localised reservoir, thus eliciting transdermal permeation.

Niosomes

Structure, Method of Preparation and Properties

Niosomes are surfactant vesicles that are similar to liposomes in structure and properties. They consist of non-ionic surfactants, either with or without cholesterol or lipids, and are cost effective, compared with conventional liposomes [49]. Niosomes share the same general preparation methodology like other lipid vesicles, and can be prepared as uni- and multi-lamellar vesicles. Several other methods have, however, also been reported [49]. Proniosome is another term routinely used for the dried, non-ionic surfactant vesicles, coated with a water-soluble carrier such as sorbitol and maltodextrin, which are converted into niosomes upon hydration. Proniosomes address the instability disadvantages associated with niosomes, such as aggregation, fusion and leaking, and can enhance the entrapment efficiency of drugs [50].

Mechanisms of Action

The mechanism of niosome action generally resembles that of the liposomes, as reported by several researchers [32, 51-53].

Niosomes as Dermal and Transdermal Delivery Vectors

In recent years, niosomes have been extensively studied as dermal and transdermal delivery carriers for various drugs. In this context, Fang *et al*. [54] compared the skin permeation of enoxacin from niosomes and conventional liposomes. Results revealed superior permeation and skin retention of drugs from niosomal formulations, compared with their liposomal counterparts. Such enhanced skin permeation was attributed to the penetration enhancement effect of the niosomal vesicles, coupled with their fusion with the skin lipids. In another study, Agarwal *et al*. [55] demonstrated that the antipsoriatic activity of dithranol loaded niosomes was superior to that of the conventional cream formulation, and a two-fold increase in flux was recorded across mouse abdominal skin, compared with the control. Lakshmi *et al*. [56] also studied the antipsoriatic activity of methotrexate loaded niosomes. The study protocol was double-blind placebo controlled, involving healthy human volunteers and psoriatic patients. The antipsoriatic activity of the niosomal formulation was three times higher than that of the marketed gel formulation. These studies confirmed superior dermal targeting of niosomes, compared with the conventional formulations. A recent *ex vivo* permeation study of tenoxicam loaded proniosomes revealed higher flux rates, compared with the carbopol gel formulation [57]. This enhancement was believed to have been as a result of the fusion of vesicles with the skin surface and the subsequent transfer of the tenoxicam directly on the skin surface for faster permeation.

Although all of these studies had revealed an enhanced permeation of the drugs being loaded into niosomes, the extent to which these niosomes could penetrate the skin could not be established. Junyaprasert *et al*. studied the *in vitro* permeation of ellagic acid (EA) from niosomes made of Span 60 and Tween 60 [58]. They applied confocal laser scanning microscopy (CLSM) to investigate the skin distribution of these vesicles. On analysing the receptor media, EA was found only when a niosomal formulation was used, compared with EA solutions. This meant that EA was able to cross the skin only when niosomes had been used. The CLSM photographs (data not shown) revealed deeper distribution of EA from niosomes, compared with using the solution formulations, which confirmed the enhanced penetration of niosomes across the human skin.

In summary, non-ionic surfactant vesicles have shown promise in targeting deeper skin layers, and could possibly be employed as dermal and transdermal delivery vectors.

Transfersomes

Structure, Method of Preparation and Properties

Transfersomes comprise of a unique class of liposomal vesicles that are highly elastic and ultra-deformable. These vesicles had been introduced by Cevc and Blume in 1992 [59] and were claimed to have penetrated deep into the skin layers, thus reaching the systemic circulation. Transfersomes are composed of a mixture of phospholipids and surfactants. Phospholipids form bilayers, while elasticity is provided by a high mobility surfactant or an edge activator. Commonly used surfactants include bile salts (sodium cholate, sodium deoxycholate) and non-ionic surfactants (e.g. Span 60, Span 80, Tween 60, and Tween 80). Upon mechanical stress, the edge activators are displaced to relocate to zones with higher stress, whereas the amphipathic lipids form bilayers with smaller curvature. Such rearrangements are responsible for eliminating the membrane's elastic energy, resulting in the formation of highly deformable vesicles, with elasticities of more than five-fold that of the conventional liposomes [60].

Mechanism of Action

Transfersomes have the ability to squeeze into pores that are much smaller than the vesicle size. This property makes them interesting drug vectors for skin penetration. According to Cevc [16], transfersomes should be applied under non-occlusive conditions, such that the osmotic gradient on the skin surface allows for them to penetrate deep into the viable epidermis, while maintaining their intactness.

Transfersomes as Dermal and Transdermal Delivery Vectors

The basic elastic properties of transfersomes have been exploited in delivering drugs topically and transdermally. Cevc *et al.* [61] report on the unfragmented penetration of fluorescence marked transfersomes across murine skin. They also demonstrated that the sizes of the vesicles had not changed during penetration across intact skin, which further confirmed that the elasticity of the vesicles had been sufficient for crossing the much smaller SC pores. In another study, Cevc and Blume had demonstrated a higher potency of hydrocortisone when entrapped in elastic vesicles, compared with traditional cream formulation that contained five-fold more of the drug [62]. They also showed a four-fold longer duration of action of dexamethasone when encapsulated in transfersome, compared with the commercial cream formulation. Transfersomes, therefore, demonstrated the ability to improve therapeutic benefits, arguably because of better targeting and higher drug deposition in the skin. Similar results were also reported by Trotta *et al.* [63]. They prepared transfersomes for topical targeting of methotrexate, using full thickness pig ear skin as model membrane. The elastic vesicles showed better permeation and higher skin retention, compared with aqueous solution and conventional liposomes. Other studies also showed successful transdermal delivery of insulin [64], interferon and interleukin [65] from ultra-deformable vesicles.

In a very recent study, Zhang *et al.* [66] demonstrated the transdermal penetration of cinnamic acid loaded transfersomes across the skin of Sprague-Dawley rats. They reported a higher flux of cinnamic acid from transfersomes, compared with the conventional liposomes. More interestingly, *in vivo* dermal microdialysis investigations revealed a higher concentration of cinnamic acid retained in the skin by conventional liposomes (3.21 ± 0.25 μg/mL), compared with transfersomes (0.59 ± 0.02 μg/mL), suggesting that transfersomes had penetrated the skin and reached the systemic circulation.

In summary, transfersomes appear to have the ability to cross intact skin, and are the vesicles of choice for targeting deeper skin layers and for transdermal drug delivery.

Ethosomes

Structure, Method of Preparation and Properties

Ethosomes are nano-sized soft vesicles, mainly composed of phospholipids with a high concentration of ethanol (20 – 50%) and water. Their size is highly tunable and depends on the ethanol content in the formulation. The higher the ethanol content, the smaller the vesicle size. Ethosomes are patented nano-carriers, as reported by Professor Elka Touitou [67, 68], and they are claimed to penetrate deeper skin layers, whilst being capable of even reaching the systemic circulation. Unlike liposomes, the phospholipids in ethosomes are less densely packed and fluidic, because of the high ethanol content, thus making these vesicles softer and the vesicular membrane highly permeable to cations [69]. The presence of high concentrations of ethanol can dramatically enhance the solubility of hydrophobic drugs in the ethosomes, and they are thus able to payload a higher amount of drugs to the target sites.

Mechanism of Action

Ethosomes are known to be efficient permeation enhancers, owing to their ability to fluidise the SC lipids. The presence of ethanol not only makes the vesicles softer, but also disturbs the lipid organisation of the SC, thereby, exerting a penetration enhancement effect. These softer vesicles can then easily penetrate across the SC and may result in enhanced transdermal drug delivery [70].

Ethosomes as Dermal and Transdermal Delivery Vectors

Ethosomes have been successfully used as vectors for dermal and transdermal drug delivery. Godin *et al.* [71] investigated the targeting of minoxidil loaded ethosomes of hair follicles of hairless rats *in vivo*. Results indicated that a higher amount of drug could be delivered to the pilosebaceous units in comparison with conventional liposomes. In another study, Horwitz *et al.* [72] loaded ethosomes with acyclovir to target herpetic infection in a double-blind, randomised clinical trial. The results showed quick improvement in the disease condition, using an ethosomal formulation, compared with a marketed cream product. This significantly improved pharmacodynamic profile endorsed ethosomes as potential dermal delivery vectors.

Touitou *et al.* also found enhanced transdermal delivery of testosterone from ethosomes, both *in vitro* and *in vivo*, compared with marketed patch formulations [73]. Subsequently, Dayan and Touitou [74] compared ethosomes and conventional liposomes for the delivery of trihexyphenidyl HCl across nude mouse skin. The resultant flux of the drug from ethosomes was significantly higher than that from conventional liposomes and hydroalcoholic solutions. Skin retention of the drug was also higher for ethosomes, compared with liposomes and hydroalcoholic solutions.

Surface charge of such colloidal vesicles is an important determinant of interaction with the target site. Considering the net negative charge of the skin surface, Meng *et al.* [75] deliberately modified the ethosome surface charge, using a cationic surfactant, namely cetyltrimethylammonium bromide. These surfactant-modified ethosomes were loaded with testosterone propionate and subjected to *in vitro* and *in vivo* skin permeation. Results indicated a significantly higher drug flux across mouse skin, compared with conventional liposomes. The *in vivo* pharmacokinetic profiles in rats also revealed higher blood plasma levels of testosterone from the ethosomal formulations, compared with liposomes.

These reports emphasised the possibility of ethosomes being suitable for use in dermal and transdermal drug delivery.

Pheroid™

Structure, Methods of Preparation and Properties

The novel Pheroid colloidal delivery system, formerly known as Emzaloid®, comprises of stable, lipid-based, submicron- and micron sized structures, or vesicles, that are homogenously distributed within a dispersion medium [76]. These vesicles are liposome like structures, consisting of a hydrophilic core and a bilayer membrane [3]. Numerous registered patents on the Pheroid technology describe the use of the Pheroid delivery system in enhancing the efficacy and in promoting the absorption of biological, dermatological and oral medicines [76]. One of the advantages of the Pheroid system is that this stable structure (within a system) can be manipulated with regards to its function, morphology, structure and size [3, 76]. Several Pheroid types can be formulated to have diameters ranging between 200 nm and 2 μm, depending on the administration route, delivery rate, and the amount and size of the API [76].

A Pheroid mainly comprises of ethylated and pegylated polyunsaturated fatty acids, which include the omega-3 and omega-6 fatty acids (excluding arachidonic acid) [76]. Slightly modified linolenic acid and linoleic acid, emulsified in nitrous oxide saturated water, are inherent components of the Pheroid and are present in all formulations [76, 77]. Numerous additional long chain unsaturated fatty acids can be added to support the unsaturated fatty acids, depending on the precise indication and pathology of the Pheroid [76]. The Pheroid is a skin-friendly carrier system, as the fatty acids present in Pheroid are oriented in the *cis*-formation, making it compatible with the fatty acids found in human cells. At present, all

topical Pheroid formulations contain tocopherol, or tocopherol-based molecules, to serve as both an anti-oxidant and an emulsion stabiliser [76]. Fig. (**2**) offers a schematic representation of the general composition of the Pheroid.

The Pheroid has some features similar to other colloidal dosage forms. In general, the Pheroid contains a lipid bilayer, like the liposomes, although it does not contain phospholipids or cholesterol. A Pheroid is formed by a self-assembly process, comparable with that of low-energy emulsions and micro-emulsions. The Pheroid contains two liquid phases (such as emulsions), as well as a dispersed gas phase (i.e. nitrous oxide). The nitrous oxide is associated with the fatty acid dispersed phase and contributes towards the self-assembly process of the Pheroid, its stability, as well as the miscibility of the fatty acids in the dispersion medium. The Pheroid is a versatile carrier system, having some of the reservoir characteristics of polymeric microspheres and can also form natural depots, similar to macromolecular microspheres [76].

Most lipid-based delivery systems contain cholesterol in the lipid bilayer, resulting in rigid vesicular structures [76]. In contrast, the Pheroid contains no cholesterol in its lipid bilayer [3] and it is sterically stabilised by electro-chemical interaction, allowing for its highly elastic vesicular structure [76]. Another advantage of the Pheroid delivery system is that hydrophobic compounds can be entrapped within its elastic lipid membrane, whereas hydrophilic compounds can reside in the central hydrophilic aqueous core [3]. Fig. (**2**) indicates the phase in which either lipophilic or hydrophilic drugs would be incorporated into the Pheroid system.

Mechanism of Action

It is believed that the fluidity of the Pheroid membrane plays a part in the dermal and transdermal delivery of APIs. However, the uptake of the Pheroid may also be influenced by the cell's mechanism of uptake, as well as by the formulation of the Pheroid. The mechanism by which the Pheroid is taken up by cells is hypothesised to be facilitated by the fatty acid membrane-binding proteins usually present within lipid rafts in the cell membrane. Thereafter, the Pheroid is either metabolised within the peroxisomes, or inside the cells' mitochondria (depending on the composition of the Pheroid), resulting in the release of the entrapped API [76].

Pheroid as Transdermal Delivery Vector

The Pheroid has proven a successful transdermal and dermal delivery vehicle of some compounds, but not for all the APIs and formulations tested. For the purpose of this review, the focus will only be on transdermal delivery and not on dermal delivery. Furthermore, the different studies performed are divided into two groups, i.e., enhanced or reduced transdermal delivery with the Pheroid.

Improved Transdermal Drug Delivery with Pheroid

The Pheroid system had enhanced the transdermal delivery of certain APIs, of which selected physicochemical properties are summarised in Table **1**.

The permeation enhancement abilities of Emzaloid® (now known as Pheroid™), compared with the heat separated epidermal layer of human abdominal skin were compared with two lamellar gel phase systems (referred to as commercial systems A and B), with the use of two APIs, i.e. 5-fluorouracil (5FU) (1.0% w/w) and idoxuridine (1.0% w/w). Compared with a control group (API dissolved in HPLC grade water), Emzaloid® had the highest enhancement ratio (ER = 20.33) for 5FU, followed by commercial product B (ER = 5.00) and commercial product A (ER = 4.67). Emzaloid® also had the highest enhancement ratio (ER = 3.50) for idoxuridine, compared with the control, followed by commercial product B (ER = 1.75) and commercial product A (ER = 1.31) [78].

Further studies by Van Dyk [79] were performed by dissolving or entrapping 5FU (1.0% w/v) in either HPLC grade water (control), phosphate buffer solution (PBS, control), water-based Pheroid solution, or PBS-based Pheroid solution. It was found that the Pheroid delivery system had enhanced the transdermal penetration of 5FU over the heat separated epidermal layer of the skin (over 12 hours). The water-based Pheroid formulation was found to be far more superior in enhancing the average flux of 5FU (0.891 ± 1.577 $\mu g/cm^2.h$) than the PBS-based Pheroid (0.412 ± 0.824 $\mu g/cm^2.h$), followed by PBS and water with average flux values for 5FU of 0.122 ± 0.210 $\mu g/cm^2.h$ and 0.075 ± 0.103 $\mu g/cm^2.h$, respectively. Additionally, it was concluded from this study that a 0.5% 5FU concentration in water-based Pheroid formulation could be used instead of a 1.0% concentration, since no statistically significant differences were found between a 1.0% and 0.5% formulation [79].

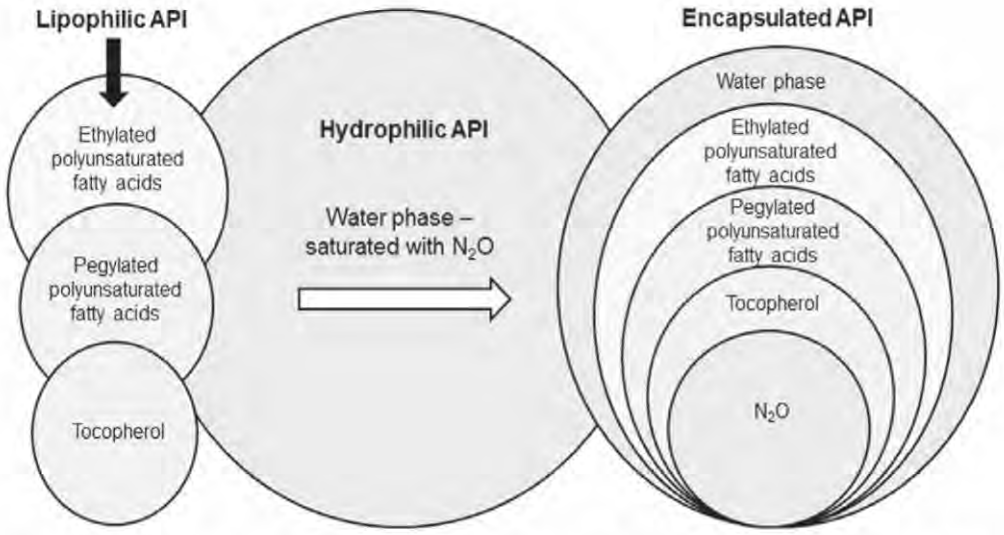

Fig. (2). Schematic representation of the hydrophilic and lipophilic components present in the Pheroid, as well as co-formulation with either lipophilic or hydrophilic APIs to produce a Pheroid vesicle containing the encapsulated API.

Table 1. Physicochemical characteristics of APIs of which their transdermal permeation was enhanced by the Pheroid™.

Compound	Molecular weight (g/mol)	Melting point (°C)	Log D* (pH 7.4)	Log P	Aqueous solubility	Reference
Acyclovir	225.20	256.5 – 257.0	0.02 (0.2 M PBS)	-4.27	2.50 mg/mL in water at 25°C	[82-84]
Alpha-lipoic acid	206.33	59.0 – 62.0	-0.78	-	8.60 mg/mL in PBS, pH 7.4 at 32°C	[85, 86]
Azelaic acid	188.22	106.5	0.11	-	2.84 mg/mL in PBS, pH 7.4 at 32°C	[87, 88]
L-carnitine L-tartrate	472.49	170.0	1.35	-	16.63 mg/mL in PBS, pH 7.4 at 32°C	[89, 90]
5-fluorouracil**	130.08	282.0 – 283.0	-0.98	-	0.56 mg/mL in PBS, pH 7.4 at 32°C	[80, 88, 91]
Idoxuridine	354.10	180.0	-	-0.39	Slightly soluble in water	[78]
Isoniazid**	137.14	171.4	-	-0.64	140 mg/ml in water at 25°C	[88, 92]
Ketoconazole**	531.43	146.0	-	3.73	40.00 µg/mL in water at 25°C	[83, 93, 94]
Lidocaine HCl**	288.80	75.0 – 79.0	-	3.40	579.80 mg/mL in PBS, pH 8	[84, 94, 95]
Prilocaine HCl**	256.80	168.0 – 171.0	-	2.09	388.86 mg/mL in PBS, pH 8	[84, 94, 96]
Rifampicin**	822.95	-	-	3.71	Slightly soluble in water, 1400.00 mg/L in water at 25°C	[84, 94]
Zinc	219.50	237.0	-	1.22	43.00 g/100 mL in water	[88, 94, 97]

*Log D: *n*-octanol-PBS (pH 7.4) partition coefficient, unless specified otherwise; **Depending on the formulation type, these APIs either experienced improved, or delayed (or no) permeation enhancement (Refer to Section 3.5.3.2.).

Studies by Vermaas [80], however, did not result in the same enhancement of the permeation of 5FU (at a concentration of 0.5% w/v) by the Pheroid delivery system in semi-solid formulations(refer to Section 3.5.3.2). As a result, more studies were performed to determine the effect of the Pheroid delivery system on the permeation of 5FU from lotions with different concentrations of this API. Different concentrations of 5FU (i.e. 0.5, 1.0, 2.0 and 4.0% w/w) lotions were formulated with and without the addition of Pheroid. The total amount of 5FU that had diffused over a period of 12 hours across dermatomed human abdominal skin (400 µm thickness) was the highest for the 4% w/w Pheroid lotion (57.60 µg/cm^2), followed by the 0.5% w/w Pheroid lotion (31.60 µg/cm^2), 2.0% w/w Pheroid lotion (28.50 µg/cm^2), 1.0% w/w Pheroid lotion (16.20 µg/cm^2), 4.0% w/w lotion (15.90 µg/cm^2), 1.0% w/w lotion (11.90 µg/cm^2), 2.0% w/w lotion (9.71 µg/cm^2) and finally the 0.5% w/w lotion (4.63 µg/cm^2). These outcomes indicated that, generally, higher total amounts of 5FU had diffused from the Pheroid lotions (with different concentrations of 5FU) than from those lotions without Pheroid [81].

The Pheroid delivery system was found to have improved the flux of the peptide drug, arginine vasopressin (150.0 µg/mL), compared with the passive control cells, in which the drug was dissolved in Hepes buffer, pH 5.5. The *in vitro* permeation profiles (across the epidermal layer of the skin) of arginine vasopressin showed a biphasic character, with most of the permeation having occurred during the first 2 hours of the study. These flux values obtained within the first 2 hours were higher for arginine vasopressin in Pheroid (0.495 ± 0.188 µg/cm^2.h), than for arginine vasopressin in Hepes buffer (pH 5.5) (0.037 ± 0.065 µg/cm^2.h). Flux values obtained for the 2 – 12 hours' time interval showed a similar pattern, i.e. arginine vasopressin in Pheroid (0.038 ± 0.050 µg/cm^2.h) had a higher flux than when arginine vasopressin had been dissolved in Hepes buffer (pH 5.5) (0.018 ± 0.028 µg/cm^2.h). In further diffusion studies, bestatin HCl (300 µg/mL, a selective aminopeptidase inhibitor) was included in the donor solution to prevent the degradation of the active by skin enzymes, which in turn may have also had an enhancing effect on the permeation of arginine vasopressin. A comparison of the flux values obtained during the first 2 hours of the study showed that the flux obtained when arginine vasopressin and bestatin HCl had been dissolved in Pheroid was higher (0.218 ± 0.186 µg/cm^2.h) than when arginine vasopressin and bestatin had been dissolved in Hepes buffer (pH 5.5) (0.158 ± 0.169 µg/cm^2.h). The flux values obtained during the 2 – 12 hours' time interval, however, showed that the flux values were higher when arginine vasopressin and bestatin had been dissolved in Hepes buffer (pH 5.5) (0.062 ± 0.063 µg/cm^2.h), than when dissolved in Pheroid (0.058 ± 0.051 µg/cm^2.h) [98].

The Pheroid delivery system also proved to be advantageous to the transdermal delivery (across full thickness abdominal skin, only fat layer removed) of two other peptide drugs, i.e. tumour necrosis factor-alpha (TNF-α) and transforming growth factor-beta1 (TGF-β1). The effects of the Pheroid were compared with the outcomes of a study during which these actives had been dissolved in PBS (pH 7.4). The aminopeptidase inhibitor, bestatin HCl, was again added to prevent any skin related degradation. TNF-α and TGF-β1were entrapped within Pheroid vesicles at concentrations of 500.0 pg/mL and 1000.0 pg/mL, respectively. The average diffused concentration of TNF-α after 12 hours with Pheroid was 88.48 ± 0.98 pg/cm^2, only slightly higher than the average concentration of 82.66 ± 1.44 pg/cm^2 obtained with PBS (pH 7.4). The average diffused concentration of TGF-β1 after 6 hours was much higher with Pheroid (302.44 pg/cm^2) than with PBS (pH 7.4) (148.28 pg/cm^2) [99].

The Pheroid delivery system furthermore proved to be more effective in the delivery of two anti-tubercular drugs, i.e. isoniazid (INH) (5.0 mg/mL) and rifampicin (RH) (10.0 mg/mL), compared with the PBS (pH 5.5) control study outcomes. Permeation profiles obtained after *in vitro* diffusion studies across the epidermal layer of human abdominal skin for INH showed a biphasic character,

whereas the permeation profiles for RH were triphasic. The average flux of INH during the 0 – 2 hours interval was 0.290 ± 0.650 μg/cm^2.h and 2.640 ± 2.310 μg/cm^2.h for PBS (pH 5.5) and Pheroid, respectively. For the 2 – 12 hours interval, the average INH flux was 0.300 ± 0.150 μg/cm^2.h for PBS (pH 5.5) and 1.140 ± 0.890 μg/cm^2.h for Pheroid. No flux was observed for RH in PBS (pH 5.5), whereas the average flux of RH with Pheroid was 1.870 ± 2.080 μg/cm^2.h, 0.66 ± 0.90 μg/cm^2.h and 0.230 ± 0.410 μg/cm^2.h for the 0.0 – 1.5 hours, 1.5 – 4.0 hours and 4.0 – 12.0 hours intervals, respectively [100].

A preliminary study showed that the Pheroid delivery system had improved the transdermal diffusion of acyclovir (5.0% w/v), but not that of ketoconazole (2.0% w/v) (refer to Section 3.5.3.3), when Franz cell skin diffusion studies were conducted on the epidermal layer of human abdominal skin. The permeation profile of acyclovir showed a biphasic character. During the 0 – 2 hours interval, the Pheroid delivery system had increased the average flux of acyclovir (ER of 1.12), compared with the PBS control. The flux of the Pheroid during the 2 – 12 hours interval was 1.63 order higher than that of the control [101].

Following the above mentioned study, acyclovir (5.0% w/w) and ketoconazole (2.0% w/w) were formulated into semi-solid products (creams and emulgels), with and without (control) the Pheroid delivery system. It was found that the Pheroid cream had the highest (2970.03 μg/cm^2) average cumulative amount of acyclovir that had permeated full-thickness human abdominal skin over a period of 12 hours, followed by the cream (2172.20 μg/cm^2), the emulgel (482.38 μg/cm^2) and the Pheroid emulgel (413.56 μg/cm^2). The concentrations of ketoconazole that had diffused through the skin after 12 hours had the following ranking order: Pheroid emulgel (447.55 μg/cm^2) > cream (38.86 μg/cm^2) > emulgel (25.68 μg/cm^2) > Pheroid cream (0.00 μg/cm^2). From these results it was concluded that the Pheroid formulae had increased the transdermal permeation of these APIs, although the type of formulation (i.e. emulgel or cream) was also a determining factor [102].

The transdermal delivery (across the epidermal layer of human abdominal skin) of two ionisable amide types of local anaesthetics (i.e. lidocaine HCl and prilocaine HCl, both at a concentration of 2.5% w/v) with the Pheroid was compared with a commercial cream. The vasoconstrictor, adrenaline, was added to the formulations in a concentration of 1: 100 relative to the APIs to localise the anaesthetic effect. The permeation profiles obtained with the Pheroid vesicles showed a biphasic character, in which rapid permeation was observed during the first 2 hours, where after it plateaued between 3 - 12 hours. The average flux of lidocaine HCl during the first 2 hours was higher for Pheroid (617.120 μg/cm^2.h) than for the commercial product (73.810 μg/cm^2.h). This was the same for prilocaine HCl, i.e. the flux for the Pheroid was 699.490 μg/cm^2.h, compared with 53.930 μg/cm^2.h for the commercial product. A comparison of the flux values obtained during the 3 – 12 hours interval showed that Pheroid (106.420 μg/cm^2.h) was still slightly superior to the commercial cream (99.430 μg/cm^2.h) in improving the permeation of lidocaine HCl. Flux values for prilocaine HCl during this time interval was marginally higher for the Pheroid (118.890 μg/cm^2.h) than for the commercial cream (101.500 μg/cm^2.h). The addition of adrenaline to the formulations had no significant effect [103].

In another study, it was found that the average amount per area of L-carnitine L-tartrate (2% w/v) that had diffused across full thickness human abdominal skin was 1.6 times better with the Pheroid after 8 hours, than with PBS (pH 7.4) [89].

Creams containing 20.0% of azelaic acid (with and without Pheroid), as well as gels containing 15.0% of azelaic acid (with and without Pheroid) were formulated. A comparison of the 12 hours average cumulative concentrations (across full thickness abdominal skin) of azelaic acid showed that more azelaic acid (38.48 μg/cm^2) had diffused from the Pheroid creams than from the non-Pheroid creams (3.23 μg/cm^2). In contrast, the non-Pheroid gel formulations (24.51 μg/cm^2) showed better diffusion values than the Pheroid gels (11.03 μg/cm^2) [87].

A study showed that zinc acetate (1.2%) was able to diffuse through full thickness abdominal skin from Pheroid and non-Pheroid creams and emulgels; although no flux values were obtained. Placebo formulations (without zinc) were also tested to determine any possible endogenous zinc diffusion. When comparing the average concentration of zinc that had permeated through the skin after 6 hours, it was found that the Pheroid emulgels (0.12 μg/cm^2) and non-Pheroid emulgels (0.05 μg/cm^2) had transferred insignificant zinc concentrations transdermally, compared with the placebos. In contrast, the Pheroid creams (9.23 μg/cm^2) and non-Pheroid creams (8.71 μg/cm^2) had delivered significant amounts of zinc transdermally. These results showed that the incorporation of Pheroid into the formulations had a positive effect on the diffusion of zinc across the skin [97].

Alpha-lipoic acid (1.0% w/w) was used as the active in a Pheroid cream, a Pheroid gel, and a non-Pheroid cream and gel. It was determined that the active did not penetrate through full thickness skin from the non-Pheroid cream, nor the gel over a 12 hour period. The results, however, indicated that the Pheroid had improved alpha-lipoic acid's transdermal delivery across the skin. For the Pheroid cream a steady state flux of 58.010 ± 6.630 μg/cm^2.h was observed after the first 2 hours of the diffusion study, where after the cumulative concentration of the active remained the same for the subsequent 3 – 6 hours interval. This suggested that the entire amount of alpha-lipoic acid had diffused through the skin from the Pheroid cream. The Pheroid gel had an average flux of 22.180 ± 3.330 μg/cm^2.h during the 4 - 12 hours interval [86].

Reduced or No Enhancement in Transdermal Drug Delivery with Pheroid

In this section those studies during which the Pheroid had either shown no effect or a reduced effect on the transdermal delivery of certain APIs are discussed. The physicochemical properties of these APIs are summarised in Table **2**. Those APIs that had shown improved transdermal delivery during initial studies are listed in Table **1** only.

Previous results (discussed above, [78, 79]) showed that Pheroid had improved the transdermal penetration of 5FU. Subsequently, six formulations, each containing 0.5% of 5FU were prepared, i.e. a non-Pheroid cream, -emulgel and -lotion, and a Pheroid cream, -emulgel and -lotion. A 0.5% 5FU water solution, a 0.5% 5FU Pheroid solution and a 5% 5FU commercial product (ointment) were also tested. A direct comparison with the commercial product was made by testing two solutions, i.e. a 5.0% 5FU non-Pheroid and a 5.0% 5FU Pheroid solution during Franz cell skin diffusion studies. The water solution containing the 5.0% 5FU had achieved the highest average cumulative concentration (11.158 μg/cm^2) after 12 hours across full thickness human abdominal skin. The comparison of the 0.5% formulations and solutions showed that the emulgel had achieved the highest average cumulative concentration (10.86 μg/cm^2), followed by the Pheroid lotion (10.01 μg/cm^2), the 0.5% water solution (9.63 μg/cm^2), the lotion (9.06 μg/cm^2), the Pheroid emulgel (8.97 μg/cm^2), the cream (7.59 μg/cm^2), the Pheroid cream (7.07 μg/cm^2) and the 0.5% Pheroid solution (6.18 μg/cm^2). The 5.0% 5FU containing Pheroid solution and the commercial product had the lowest average cumulative concentrations, i.e. 2.58 μg/cm^2 and 2.51 μg/cm^2, respectively [80].

Following the successful outcomes of previous studies [100] on INH (1.0% w/w) and RH (0.5% w/w) (see Section 3.5.3.1), four spray formulations were formulated for these APIs, i.e. a non-Pheroid lotion and emulgel, and a Pheroid lotion and emulgel. Both INH and RH had failed to permeate through full thickness skin (over 12 hours) from all of these formulations. The reason for the

Table 2. Physicochemical characteristics of APIs of which their transdermal permeation was not enhanced by the Pheroid.

Compound	Molecular weight (g/mol)	Melting point (°C)	Log D* (pH 7.4)	Log P	Aqueous solubility	Reference
Adapalene	412.50	247.8	-	8.51	0.14 µg/mL in water	[84]
Cyclizine	226.40	285.0	3.11	3.55	<0.1 mg/mL in water at 25°C	[85, 88, 104]
Diclofenac hydroxyethyl pyrrolidine	411.30	101.0 – 104.0	1.90	-	7.10 µg/mL in PBS, pH 7.4 at 32°C	[105]
Diclofenac diethylamine	369.38	145.0 – 148.0	1.60	4.50	4.30 µg/mL in PBS, pH 7.4 at 32°C	[105, 106]
Diclofenac sodium	318.10	280.0	1.70	4.50	6.20 µg/mL in PBS, pH 7.4 at 32°C	[105]
Flurbiprofen	244.30	112.0	3.41, pH 5 at 32°C	3.70	0.06 mg/mL in water, pH 4.75 at 32°C 0.07 mg/mL in phosphate buffer solution, pH 4.69 at 32°C	[107-113]
Ibuprofen	206.00	75.0 – 77.0	3.11 at pH 5 0.39 at pH 7.4	4.24	0.10 (± 25.48) mg/mL in water at 37°C	[112-114]
Lamivudine	229.30	162.0	-0.83 at pH 7.4 -1.19 at pH 5 -1.15 at pH 7	-	188.02 mg/mL in PBS, pH 7.4 at 32°C 144.78 ± 29.61mg/mL in PBS, pH 5 at 32°C 100.16 ± 4.74 mg/mL in PBS, pH 7	[115, 116]
Niacinamide (nicotinamide)	122.13	128.0 – 131.0	-0.32	-	212.92 mg/mL in PBS, pH 7.4 at 32°C	[87, 94, 117, 118]
Salicylic acid	138.12	159.0	0.33	2.26	4.07 mg/mL in PBS, pH 7.4 at 32°C	[112, 117, 119]
Scopolamine	303.35	59.0	1.77	0.80	100.00 mg/mL in water	[88, 120]
Sodium ascorbyl phosphate	322.05	260.0	-	-0.01	6.14 mg/mL in PBS, pH 5.5 at 37°C	[121]
Stavudine	224.20	159.0 – 160.0	-0.85 at pH 7	-	104.75 mg/mL in PBS, pH 7.4 at 32°C	[88, 94, 122]
Tretinoin	300.40	182.0	-	6.30	0.13 mg/L in water	[94, 123-125]
Vitamin A acetate	328.49	57.0 – 58.0	-	7.39	Insoluble in water	[88, 97]
Zalcitabine	211.22	217.0 – 218.0	-1.50 at pH 5 -1.78 at pH 7	-	114.36 ± 9.36 mg/mL in PBS, pH 5 at 32°C 91.57 ± 3.79 mg/mL in PBS, pH 7 at 32°C	[88, 116]

*Log D: *n*-octanol-PBS (pH 7.4) partition coefficient, unless specified otherwise

inability of the APIs to permeate the skin was believed to have been as a result of the poor release of the APIs from all formulations [126].

A preliminary study showed that the Pheroid had decreased the flux of ketoconazole (5.0% m/v) across the epidermal layer of human skin, when compared with the control PBS group. The average flux (over 12 hours) of ketoprofen was 0.023 ± 0.010 µg/cm^2.h and 0.0006 ± 0.0005 µg/cm^2.h for PBS and the Pheroid, respectively. In addition to the unfavourable physicochemical properties of the API (low aqueous solubility, high molecular weight and a high partition coefficient), this decrease in flux was also ascribed to the crystallisation of the drug within the Pheroid delivery system, which had possibly inhibited the diffusion of ketoconazole through the skin [101].

Following the initial experiments performed by Kruger [103] to determine the effect of the Pheroid on the transdermal delivery of prilocaine HCl and lidocaine HCl (refer to Section 3.5.3.1), four semi-solid formulations (i.e. a non-Pheroid emulgel, a Pheroid emulgel, a hydrogel and a commercial product) and two solutions (PBS at pH 8, and a solution containing Pheroid) were further tested. The formulations and solutions all contained prilocaine HCl and lidocaine HCl at a concentration of 2.5% w/v. The average flux

values after 12 hours (across dermatomed skin, 400 μm) obtained for prilocaine HCl from the various formulations was the highest for the hydrogel (96.490 ± 25.800 μg/cm^2.h), followed by the commercial product (60.960 ± 12.800 μg/cm^2.h), the PBS (pH 8) solution (53.600 ± 5.800 μg/cm^2.h), the Pheroid solution (44.750 ± 14.000 μg/cm^2.h), the emulgel (33.29 ± 11.80 μg/cm^2.h), and lastly the Pheroid emulgel (30.690 ± 7.500 μg/cm^2.h). The average flux values attained for lidocaine HCl was the highest for the commercial product (108.980 ± 23.800 μg/cm^2.h), followed by the PBS (pH 8) solution (68.950 ± 7.900 μg/cm^2.h), Pheroid™ solution (54.140 ± 12.000 μg/cm^2.h), hydrogel (29.470 ± 7.200 μg/cm^2.h), emulgel (25.830 ± 3.200 μg/cm^2.h) and lastly, the Pheroid emulgel (16.040 ± 4.900 μg/cm^2.h). These results indicated that the Pheroid was not superior to the other formulations in enhancing the permeation of these two APIs [96].

In another study to establish the effectiveness of the Pheroid delivery system, the transdermal penetration of the active ingredient, sodium ascorbyl phosphate (vitamin C) across dermatomed skin was investigated. Different formulations, with varying concentrations of the API (i.e. 1.0, 2.0. and 3.0%), with and without the Pheroid, were examined. Furthermore, a lower polarity, 2.0% sodium ascorbyl phosphate cream, containing more paraffin liquid (with and without Pheroid), and a higher polarity, 2% sodium ascorbyl phosphate cream, containing less paraffin liquid (with and without Pheroid) were investigated. The average intrinsic concentration of vitamin C that had diffused the skin after 12 hours was determined by placing placebo formulations into the donor compartments of the Franz cells. The average placebo effect was then deducted from the results obtained for the vitamin C containing formulations. Results showed that the 2% non-Pheroid cream had the highest average concentration (3.76 μg/cm^2) of diffused vitamin C over a period of 12 hours, followed by the 1% non-Pheroid formulation (3.56 μg/cm^2). It appeared that those formulations with lower concentrations of the API had achieved higher average concentrations than those formulations containing higher amounts of the active. It was also found that the non-Pheroid creams had diffused better than their counterpart Pheroid creams, whilst the more polar formulations had penetrated the skin more efficiently than the less polar creams [121].

A gel, an emulgel and a Pheroid emulgel, each containing ibuprofen (5.0% w/w), were formulated (at pH 5 and pH 7.4) for the purpose of establishing the influence of the Pheroid delivery system, as well as that of pH on the transdermal permeation of the active. A comparison of these results with a commercial product (gel) revealed that all of the formulations had exhibited an increased diffusion of ibuprofen. The emulgel (pH 5) had the highest average cumulative amount of diffused ibuprofen over a 12 hour period, followed by the Pheroid emulgel (pH 5), the gel (pH 5), the emulgel (pH 7.4), the Pheroid emulgel (pH 7.4), the commercial product, and finally the gel (pH 7.4). These results showed that the pH of the formulation had significantly impacted on the permeation of the active [114].

An anti-inflammatory drug, flurbiprofen (1.0%), was used as a representative compound for investigating the extent to which essential fatty acids (EFAs) would enhance its permeation across dermatomed skin, with and without incorporation into a delivery system. Cream-based formulations, containing evening primrose oil, vitamin F and Pheroid were compared with a control cream. It was found that the evening primrose oil (94.14 ± 25.78 μg/cm^2) formulation had resulted in the highest average cumulative concentration of diffused flurbiprofen after 12 hours, followed by the control (91.53 ± 7.24 μg/cm^2), and by the vitamin F (81.80 ± 20.15 μg/cm²) and Pheroid (45.32 ± 14.26 μg/cm²) formulations [111].

Three diclofenac salts (i.e. diclofenac hydroxyethyl pyrrolidine, diclofenac diethylamine and diclofenac sodium, each at 1.0% w/w), none of these possessing favourable physicochemical characteristics for transdermal diffusion, were formulated into Pheroid and non-Pheroid preparations. They were also compared with their corresponding, commercially available counterparts. No diffusion of the diclofenac salts were observed from any of these formulations during the 12 hours of skin diffusion studies [105].

Cyclizine (5.0%) and scopolamine (1.0%) solutions, each with and without Pheroid, were tested over a period of 12 hours and was found that the cyclizine did not penetrate full thickness skin from any of the preparations, whereas scopolamine did (from both solutions). The scopolamine solution with Pheroid (6.49 μg/cm²) had achieved a lower average cumulative concentration of the API than the solution without Pheroid (14.01 μg.cm²). Following these results, scopolamine was formulated into an emulgel with and without Pheroid. Results from the 12 hours skin diffusion study (using only the epidermis) indicated that the emulgel (2.65 μg/cm²) had generated the highest average concentration of diffused active, compared with the Pheroid emulgel (0.02 μg/cm²) [120].

Pheroid had not improved the transdermal penetration (through human epidermis) of saturated solutions of zalcitabine, lamivudine and synthesised *N*-acyl lamivudine esters, when compared to a control group (i.e. actives dissolved in PBS, pH 5). For the PBS (pH 5) solutions it was found that lamivudine (4.289 μmol/cm^2.h) had resulted in a higher median flux value than zalcitabine (0.442 μmol/cm^2.h) over 24 hours. Zalcitabine (0.015 μmol/cm^2.h), however, showed a slightly higher median flux value when entrapped in the Pheroid, than lamivudine (0.011 μmol/cm^2.h). Lamivudine in PBS (pH 5) and in the Pheroid had a higher median flux value than its derivatives [116].

Lamivudine and its synthesised methoxypolyethylene glycol carbamates and carbonate (0.2 M) derivatives were also tested using Franz diffusion cells and human epidermis over a period of 24 hours. It was found that the parent drug itself had permeated the skin better than its derivatives in both PBS (pH 7.4) (4.23 ± 2.98 μmol/cm^2/h) and Pheroid (0.20 ±0.22 μmol/cm^2/h). Overall, it was found that the Pheroid had inhibited skin permeation, compared with the PBS (pH 7.4) solutions, except for the 2'3'-dideoxy-3'-thiacytidin-6'yl-O-(methoxypolyethylene glycol) carbonate derivative [115].

Six stavudine-5'-esters were each prepared with and without Pheroid (saturated solutions) and their transdermal penetration investigated. A comparison of the stavudine solutions showed that the average flux values achieved by the PBS (pH 7) solutions over the 24 hours period were slightly higher (6.520 ± 1.402 μmol/cm^2.h) than those of the Pheroid solutions (6.120 ± 2.857 μmol/cm^2.h). The flux values of the stavudine PBS (pH 7) solutions were also much higher than those of the PBS (pH 7) solutions containing the stavudine derivatives, ranging between 0.060 and 0.230 μmol/cm^2.h. Among the Pheroid solutions (ranging between 0.750 and 6.870 μmol/cm^2.h), however, the butyryl (6.870 ± 3.776 μmol/cm^2.h) and propionyl esters (6.640 ± 8.429 μmol/cm^2.h) had achieved the highest fluxes [122].

The impact of the Pheroid technology was further investigated by formulating and comparing castor oil, vitamin F and Pheroid creams containing tretinoin (0.025% w/w) and adapalene (0.1% w/w). No retinoids were detected in any of the receptor compartments of the Franz cells after 12 hours (with full thickness human abdominal skin) for any of the formulations tested. It was, however, suggested that a low percentage of unionised molecules, the lipophilicity of the retinoids and inadequate concentration gradients had negatively affected the diffusion results. It was also believed that due to the suboptimal release of these actives from the cream formulations, decreased concentrations of retinoids had been available for diffusion (as established through membrane studies) [127].

Skin diffusion studies (6 hours) were performed on creams and emulgels (with and without Pheroid) containing vitamin A acetate (0.5%) across full thickness abdominal skin. These formulations were compared to a commercial product containing vitamin A ace-

tate. Results indicated that vitamin A acetate had not penetrated through the skin from any of the developed formulations or the commercial product [97].

The impact of the Pheroid delivery system on the transdermal delivery (across full thickness skin) of niacinamide (3.0%) from cream and emulgel (each with and without Pheroid) formulations was also investigated. The average cumulative concentration of nicotinamide after 12 hours was the highest for the emulgel (29.07 µg/mL), followed by the Pheroid emulgel (23.60 µg/mL). The Pheroid cream (21.41 µg/mL), however, had a higher average cumulative concentration of nicotinamide after 12 hours than its counterpart, i.e. the cream formulation (13.91 µg/mL) [118].

In another study, a Pheroid gel was compared to a gel formulation without Pheroid, both containing salicylic acid (2.0% w/w) and niacinamide (3.0% w/w). Cream formulations containing salicylic acid at a concentration of 2.0% w/w and niacinamide at a concentration of 4.0% w/w were also compared [117]. It is worth mentioning that the formulations tested during this study differed from the above mentioned study [118]. Full thickness human abdominal skin of Caucasian female patients was used to perform the 12 hours diffusion studies. The gel (2.040 µg/cm^2.h) and cream (2.699 µg/cm^2.h) formulations of niacinamide depicted flux values approximately double those of the corresponding flux values of the Pheroid gel (1.316 µg/cm^2.h) and Pheroid cream (1.010 µg/cm^2.h). For salicylic acid, the gel (31.952 µg/cm^2.h) showed a higher flux value than the Pheroid gel (20.853 µg/cm^2.h), the cream (11.335 µg/cm^2.h) and the Pheroid cream (10.282 µg/cm^2.h) [117].

In a further study, different cream and gel formulations, containing niacinamide (4.0%), were formulated with and without Pheroid. Following 12 hours of Franz cell diffusion studies on full thickness female abdominal skin, no flux values were obtained for these formulations. The average cumulative concentration of niacinamide after 12 hours, however, was highest for the cream formulation (65.31 µg/cm^2), followed by the Pheroid cream (16.15 µg/cm^2), the gel (7.70 µg/cm^2) and lastly the Pheroid gel (3.50 µg/cm^2) [87].

These studies with niacinamide showed that the type of formulation could significantly impact the transdermal delivery of an API and requires careful consideration during the formulation process.

SUMMARY AND FUTURE PERSPECTIVES

Vesicular carriers have shown high potential as vectors for transdermal drug delivery, due to their versatility, small size, control over drug entrapment and release, and their ability to penetrate the skin to some extent. Many attempts have been made for enhancing transdermal drug delivery by employing classical vesicular systems (liposomes, transfersomes, ethosomes and niosomes), while some studies also reported on the mechanisms of penetration. Much of the available reported test outcomes to date are, however, somewhat speculative, while different research groups have different opinions regarding the mechanism and extent of vesicular penetration into the skin. No general mechanism therefore currently exists that accurately describes the vesicular action, and there are many variables, such as composition and methods of preparation, that result in vesicles with diverse properties in terms of size, shape, charge, fluidity, elasticity and entrapment of APIs.

The Pheroid is a relatively new vesicular delivery system that is very adaptable, effective and cost-effective, and is it composed of pharmaceutically safe constituents, based on the innate molecules in the body [76]. Pheroid, like other vesicular counterparts, are also capable of entrapping, protecting and delivering both hydrophobic and hydrophilic drug molecules across a series of biological membranes [3], such as human skin.

Ideally, for an API to permeate the skin, it should have a melting point below 200°C and an aqueous solubility of more than 1 mg/mL [128]. The API should also have a log P value between 1 and 3 [129] and should be small in size, since molecules larger than 500 Dalton would not diffuse through the skin [128, 130]. A comparison of the physicochemical characteristics of all of the APIs tested failed to establish any pattern within the two groups, i.e. APIs undergoing enhanced transdermal permeation with Pheroid (Table **1**) and APIs of which their transdermal permeation had been reduced by the Pheroid technology (Table **2**). It is therefore still unclear whether or not an API requires certain physicochemical characteristics in order for the Pheroid to enhance its permeation. It was also observed that the type of formulation significantly impacted the API's transdermal delivery behaviour.

Further studies could be directed towards the short- and long term stability of Pheroid. More efforts should be focused on optimising the Pheroid preparation technique, whilst a need exists for establishing a correlation between the properties of the API and the Pheroid, to assist in designing successful transdermal delivery products. Formulation stability and future investments could be crucial factors for successfully transforming laboratory scale studies into viable commercial products.

CONFLICT OF INTEREST

None is associated with this work.

ACKNOWLEDGEMENTS

The authors would like to express their sincere gratitude towards the National Research Foundation (NRF) of South Africa and the Centre of Excellence for Pharmaceutical Sciences (North-West University, Potchefstroom, South Africa) for the financial support. Any opinions, findings and conclusions, or recommendations expressed in this material are those of the authors and therefore the NRF does not accept any liability in regard thereto.

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Received: February 25, 2015 **Accepted: April 9, 2015**

Chapter 3: Article for publication in the Journal of Pharmaceutical Sciences

Chapter 3 is written in article format for the purpose of publication in the Journal of Pharmaceutical Sciences. The complete author's guide is given in Annexure C. Please note that Chapter 3 is written in US English and not UK English and that all formatting is done according to the guide to authors. The text has also been justified to ease reading.

Investigation of the optimal skin permeation properties of selected NSAIDs using a novel flux-independent model

Dewald Kilian, Hendrik J.R. Lemmer, Minja Gerber, Jan L. du Preez, Jeanetta du Plessis*

Centre of Excellence for Pharmaceutical Sciences (Pharmacen), North-West University, Private Bag X6001, Potchefstroom 2520, South Africa

* Corresponding author. Tel.: +2718 299-2274; Fax: +2718 299-2225. E-mail address: Jeanetta.duPlessis@nwu.ac.za (J du Plessis).

Abstract

The optimal physicochemical properties for the transdermal delivery of selected non-steroidal anti-inflammatory drugs (NSAIDs), dispersed in the Pheroid™ delivery system, were investigated. To aid in this investigation, a novel flux-independent mathematical model, based on restrictions made to Lipinski's rule of five, was derived and tested. As a control, the selected NSAIDs were dissolved in phosphate buffer solution (PBS), and the cumulative amounts that had diffused after a 12 hour period, were compared to those obtained with the Pheroid™ delivery system. The results suggested that the same molecular size and octanol-water partition coefficient (log P) ranges determined the extent of skin permeation of the selected NSAIDs from both PBS and the Pheroid™. In half the cases where permeation was observed, the NSAIDs dispersed in the Pheroid™ displayed lower cumulative transport compared to those dissolved in PBS. Ibuprofen displayed the best skin permeation from both the PBS donor and the Pheroid™, with a lower cumulative concentration after 12 h from the Pheroid™. The mathematical model developed for this study consistently returned reasonable approximations of the observed concentrations, and could possibly be used to model the changes in skin permeability of a decomposing system.

Keywords: NSAID, transdermal delivery, physicochemical properties, mathematical model, Lipinski's rule, Pheroid™

INTRODUCTION

Over the past 50 years, *in vitro* skin permeability studies had become a major research topic in various fields, ranging from pharmaceutical sciences (Hadgraft & Lane, 2005) to cosmetics (Shen *et al*., 2014) and risk assessment (Fitzpatrick *et al*., 2004). The transdermal delivery of active pharmaceutical ingredients (APIs) offers an attractive alternative to other routes of administration. Advantages include the elimination of gastric side-effects, occasionally associated with oral dosage forms, which could in turn increase patient compliance. Transdermal delivery also bypasses the first-pass metabolic effect, potentially lowering the amount of API needed to elicit the same therapeutic response obtained through other routes of administration. This may decrease the cost of the formulation and potentially increase the availability of drugs to aid organizations and poorer countries.

One of the most important factors that determine an API's ability to permeate the skin is its physiochemical properties. When selecting potential candidates for transdermal drug delivery, important physiochemical attributes to consider include the molecular weight, aqueous solubility, melting point and oil/water partition coefficient (Roy, 1997). Some of the reported ideal properties for satisfactory skin permeation are a low molecular weight, i.e. preferably less than 600 Da (Daltons), good solubility in oil and water, and a low melting point (Barry, 2001).

In general, approximately 1 mg of an API can be delivered across a 1 cm^2 area of skin in 24 h under the most ideal circumstances. This amount may be reduced by ten- to hundred-fold or even more, if it has a melting point above 150°C and a molecular weight higher than 500 Da. Considering the dosage requirements of various drugs, this would imply that less than 1% may qualify as potential candidates for transdermal delivery (Ghosh & Pfitser, 1997).

Lipinski's rule of five (Lipinski *et al.*, 2001) is frequently used by pharmaceutical chemists during the drug discovery process, to identify leads with favorable physicochemical properties for oral drug delivery. The rule of five states that the optimal physicochemical selection criteria are: (1) no more than 5 H-bond donors, (2) at most 10 H-bond acceptors, (3) a maximum molecular weight of 500 Da and (4) a log P value not higher than 5. Because the stratum corneum forms a more formidable barrier to drug permeation than the intestinal epithelium, some studies caution against the direct use of the rule of five to identify drug potential candidates for transdermal delivery (Choy, 2011). Most authors, however, agree that the rule of five indeed applies to transdermal delivery, as long as some restrictions are placed on the physicochemical properties' selection criteria. These restrictions include the addition of a lower boundary for the log P values, i.e. log $P \geq 0$, with optimal values at around 2 to 3, as well as a lowering of the upper boundary of the molecular weight to between 400 and 500 Da, with ideal values below 400 Da and optimal values at around 150 to 220 Da (Bos & Meinardi, 2000; Choy, 2011; Magnusson *et al*., 2004; Wiedersberg & Guy, 2014). Potts and Guy (1992) determined from quantitative structure-permeability relationships (QSPR) that the main physicochemical properties that determine transdermal permeability are the log P and the molecular size. This model of Potts and Guy had consistently proven to be the most accurate QSPR model for determining the permeability constant, K_p (Lian *et al*., 2008).

Previous studies that aimed at relating the efficacy of the Pheroid™, as a transdermal delivery system, to the ideal physicochemical properties of APIs being administered, were inconclusive. This was attributed to variables that had been created by the excipients in the different formulations and the different dosage forms themselves, into which the Pheroid™ had been formulated (Vermaas, 2010).

In this study, an attempt was made to establish which physiochemical characteristics an API must possess to make it a suitable candidate for transdermal delivery with the Pheroid™ drug delivery system. This was done by eliminating dosage forms as a variable and by simply incorporating a range of APIs into a basic Pheroid™ dispersion. To aid in this investigation, a novel, flux-independent mathematical model had been derived. This model was based on the restricted Lipinski's rule of five,

as discussed above, whilst molecular size and log P were also considered the two main determinants of skin permeation.

Current mathematical models of skin permeation are concerned with estimating the value of K_p, since the rate at which a solute in the donor solution of the diffusion cell permeates the skin can be expressed as Eq. (1):

$$\frac{dC}{dt} = -K_p A(C_r - C_d)\,, \tag{1}$$

where A is the surface area of the skin, C_d is the concentration of the solute in the donor solution at equilibrium permeation and C_r is the solute concentration in the receptor solution. Separating the variables and solving Eq. (1) gives:

$$C_r = C_d + (C_r - C_d)e^{-K_p A t} \tag{2}$$

Assuming that $C_r = 0$ at $t = 0$, the above equation simplifies to:

$$C_r = C_d - C_d e^{-K_p A t} \tag{3}$$

Therefore, if the skin's surface area and the concentration of solute in the donor solution are known, all that is needed to determine the concentration in the receptor solution at time t is the permeability constant, K_p. Mathematical models to calculate K_p have attracted much interest, as is evident from existing studies on skin permeation. These models include QSPR (Potts & Guy, 1992; Moss *et al*., 2002), artificial neural networks (Chen *et al*., 1993), random walks (Frasch, 2002) and mechanistic models (Mitragotri, 2002). With the flux-independent model presented in this work, the prediction of cross-section concentration values, specifically after 12 h, directly from molecular size and log P values was attempted.

THEORETICAL DEVELOPMENT

As mentioned in the introduction, molecular size and log P have been shown to be the main determinants of transdermal permeability (Potts & Guy, 1992; Lian *et al*., 2008). Since different

polymorphic forms of the drugs were not investigated in this study, the melting point was omitted from our model. Unlike Eq. (1), a model relating the physicochemical properties to the mean cumulative drug concentration in a flux-independent manner cannot be autonomous. From our dataset (Table 2) and the restrictions placed on Lipinski's rule of five for skin permeation, we would expect the model that describes the cumulative concentration after 12 h as a function of molecular weight to initially increase proportionally. To this end we can initialize the model with Eq. (4):

$$\frac{\partial C}{\partial MW} = kC \tag{4}$$

Where C is the cumulative concentration after 12 h, k is the proportionality constant and MW is the molecular weight. In this paper, we use molecular volume as a measure of molecular size. However, the strong linear correlation between molecular weight and volume (Fig. 1) suggests that the two variables are interchangeable. The researcher is therefore free to choose which ever one they feel more comfortable with. After the initial proportional increase, the function must pass an inflection point and decelerate towards the optimum molecular volume, MV_{opt}, after which it must again asymptotically approach zero, following a path almost symmetric, but opposite in slope, to the one from 0 to MV_{opt}. This is based on the observation that the restricted upper boundary for molecular weight is roughly 400 Da and the optimal weight is somewhere between 150 to 220 Da, taking into account that $\lim_{MW\to 0} C(MW)$ and $\lim_{MW\to 500} C(MW)$ must both approach zero asymptotically, we can approximate the distance from the weight of an arbitrarily small molecule (close to the lower boundary) to the optimal weight to be roughly the same as from the optimal weight to the upper boundary (400 Da). Using the linear relationship between molecular weight and volume, we can substitute weight for volume and incorporate the findings discussed above into Eq. (4) by replacing k with the term $-k\,(MV - MV_{opt})$:

$$\frac{\partial C}{\partial MV} = -kC\left(MV - MV_{opt}\right) \tag{5}$$

Where k is again a constant and $|MV - MV_{opt}|$ becomes smaller as the molecular volume approaches MV_{opt}, then increases again as it exceeds MV_{opt}. The term $-k\,(MV - MV_{opt})$ ensures a positive change

in concentration while MV < MV_{opt} and a negative change for MV > MV_{opt}. Separating the variables and solving using u-substitution gives:

$$\int_{-\infty}^{\infty} \frac{\partial C}{C} = -k \int_{-\infty}^{\infty} u \partial u \tag{6}$$

U-substitution was used solely to avoid misunderstanding how the term (MV – MV_{opt}) should be integrated. This term is a variable, since there exists no such function f (MV) = (MV – MV_{opt}). After integration, (MV – MV_{opt}) can be substituted back to give:

$$|C| = e^{\frac{-k(MV - MV_{opt})^2}{2}} . e^I \tag{7}$$

We note here that the constant, e^I, can be assigned the value of C_{max}, the concentration obtained from the optimal molecular volume, since MV = MV_{opt} if dC/dMV = 0, so the term in the exponent of Eq. (7) becomes zero, and e^I = C (MV_{opt}). Simplifying, we arrive at first partial model:

$$C(MV) = C_{max} e^{\frac{-k(MV - MV_{opt})^2}{2}} \tag{8}$$

The partial model relating cumulative concentration values to log P values works for both positive and negative log P values. However, for notational simplicity, we will first only look at the positive log P values. The same rationale follows for the negative log P values, but with the appropriate switching of the terms describing the changes in the function. To model the change in concentration relative to changes in log P, we recall that the lower boundary for log P is zero, from where the concentration should increase to an optimal value corresponding to log P values of between 2 to 3. After which it should decrease again and asymptotically approach a concentration of zero at around a log P value of 5. Based on our observed data (Table 2) and the restrictions places on Lipinski's rule of five given in the introduction, the initial part of our model should exhibit a proportional increase. We can therefore again start with a model similar to Eq. (4), and replace the constant, k, with a new term containing two functions, - (k_1 log P - k_2 log P^{-1}):

$$\frac{\partial C}{\partial log P} = -C \left(k_1 \log P - \frac{k_2}{\log P} \right) \tag{9}$$

The first, k_1 log P, models proportional increase, while the second, k_2 log P^{-1}, models decrease in an inversely proportional manner to the increase in log P. For small values of log P we have that k_2 log $P^{-1} >> k_1$ log P, and the concentration increases proportionally. As the values of log P increase, the system reaches an inflection point at log P = $\pm(-k_2/k_1)^{1/2}$, when $\partial^2 C/\partial$ log $P^2 = 0$. This is an imaginary value, so care must be taken when selecting values for the constants, especially k_2. One way to deal with this is to place a restriction on k_2, e.g. $k_2 \in \mathbb{Z}$. After this point, the rate of change in C decelerates until the system reaches an unstable equilibrium point at log P = $\pm(k_2/k_1)^{1/2}$. When log P increases past $\pm(k_2/k_1)^{1/2}$, k_1 log P becomes increasingly larger than k_2 log P^{-1}, and the function asymptotically decreases to zero. However, unlike Eq. (8), it does not necessarily do so in a manner that is symmetric to its increase. This enables us to model an asymmetric relationship, since the distance from 0 to log P = $\pm(k_2/k_1)^{1/2}$ need not be the same as from log P = $\pm(k_2/k_1)^{1/2}$ to log P = 5. Separating the variables and solving Eq. (9) gives:

$$|C| = e^{\frac{-k_1 \log P^2}{2}} . e^{\ln(\log P)^{k_2}} . e^{I} \tag{10}$$

Setting the constant $e^I = A$ and simplifying, we arrive at the second partial model:

$$C(\log P) = A log P^{k_2} e^{\frac{-k_1 \log P^2}{2}} \tag{11}$$

Combining Eq. (8) and (11), the final form of the model can be expressed as:

$$C(MV, \log P) = \left(C_{max} e^{\frac{-k(MV - MV_{opt})^2}{2}}, A\ log P^{k_2} e^{\frac{-k_1 \log P^2}{2}} \right) \tag{12}$$

Since this model was designed to function partially it does not have a potential function. However, it does give us a system of coordinates, an ordered n-tuple, (MV, log P, C), which offers the researcher some flexibility. The constants used in the model presented here are not fixed, and can be customized for specific datasets. This does mean that some data points are initially needed to estimate the constants, but these can be obtained from a pilot study, previous studies or from the literature. It is possible that, with a large enough dataset, general constants for this model can be estimated.

158 # 5. Materials and Methods

159 ## 5.1. Materials

160 All of the NSAIDs, used in this study, were donated by Adcock Ingram (South Africa). Analytical
161 grade methanol, ethanol and phosphoric acid, PEG-400, as well as sodium chloride, disodium
162 orthophosphate dehydrate, sodium dihydrogen orthophosphate dehydrate and dipotassium hydrogen
163 orthophosphate anhydrous were purchased from Merck Laboratory Supplies (Midrand, South Africa).
164 Double distilled deionized water was prepared with a Milli-Q water purification system (Millipore,
165 Milford, USA). HPLC grade water was used throughout the study.

166 Vitamin F ethyl ester was obtained from Chemimpo (Johannesburg, South Africa), Cremophor®
167 RH40 from BASF (Midrand, South Africa) and dl-α tocopherol from DSM (Basel, Switzerland). The
168 Pheroid™ used was prepared in-house by The Centre of Excellence for Pharmaceutical Sciences
169 (Pharmacen) at the North-West University (Potchefstroom Campus, South Africa).

170 ## 5.2. Apparatus

171 The high performance liquid chromatograpic (HPLC) system used for the analysis was an Agilent
172 1100 series, equipped with a variable wavelength ultraviolet (UV) detector, isocratic pump,
173 autosampler, and ChemStation (Rev. A.09.01 (1206)) data acquisition and analysis software. All
174 analyses were performed using HPLC grade water and reactants. The temperature of the columns was
175 kept at 25°C throughout the analysis.

176 ### 5.2.1. Chromatographic conditions

177 All HPLC analyses were done, using a Phenomenex™ Luna 5 µ C_{18} (250 x 4.60 mm) column at a
178 flow rate of 1 ml/min. All methods had previously been validated by the Analytical Technology
179 Laboratory at the North-West University (Potchefstroom Campus).

180

5.2.2. Preparation of standard solutions

The API (10 mg) was accurately weighed and transferred into a 100 ml volumetric flask and made up to volume with PBS (pH of 7.4) to produce a 100.0 µg/ml stock solution. Dilutions with concentrations of 1.0; 2.5; 10.0; 25.0 and 100.0 µg/ml were prepared from the 100.0 µg/ml stock solution.

5.2.3. HPLC data analysis

The HPLC data analysis was done, based upon the linear regression model being generated from the series of prepared standard solutions, as described above.

5.2.4. Solubility studies

The solubility of each API included in this study, was determined in PBS (pH 7.4). All of the solubility determinations were done in triplicate. The Pheroid™ was prepared with PBS (pH 7.4) as dispersion medium.

The solubility samples were left to stir in a water bath at 32°C for 24 hours. An excess of solute was present at all times to ensure a saturated solution. Each sample was then filtered through a 0.22 µm Millipore filter at 32°C, to remove any crystals, or solids from the saturated solution. The first 2 ml of solute was discarded, since the filter also had to be saturated with the active and with the solute. The filtrate was then diluted and HPLC analysis was performed to determine the solubility.

Each sample peak area value was placed in a straight line equation that had been obtained from assaying five standard solutions with known concentrations on HPLC. The equation was solved and the concentration calculated from the HPLC generated peak area to determine the unknown solubility of each sample.

5.2.5. Preparation of Pheroid™ vesicles

The Pheroid™ vesicles were prepared in-house at Pharmacen (North-West University, Potchefstroom Campus), by first mixing vitamin F ethyl ester (2.8% (w/v)), Cremophor® EL (1% (w/v)) and D-

205 tocopherol (0.2% (w/v)). The mixture was heated to 75°C to complete the oil phase of the vesicles.
206 Purified water that had been saturated with N_2O was also heated and maintained at 75°C. The
207 vesicles were further prepared by adding the heated oil mixture to the N_2O saturated 75°C water
208 (96%). The mixture was then homogenized, using a Heidolf Diax 600 homogenizer. The
209 homogenous oil in water emulsion was then transferred and split into a number of amber glass bottles
210 and each API added to a separate bottle. These emulsions were continuously mechanically shaken,
211 until room temperature was reached. The bottles, now containing Pheroid™ vesicles, were placed in
212 a fridge and kept at 5°C (Du Plessis *et al*., 2010:182).

213 **5.3. Diffusion studies**

214 **5.3.1. Preparation of skin**

215 Prior approval for the project, *In vitro* transdermal delivery of drugs through human skin, had been
216 granted by the North-West University Ethics Committee (reference number NWU-00114-11-A5).
217 Caucasian, female skin from informed consenting patients was obtained after cosmetic surgery. The
218 permeability properties of skin samples, originating from different anatomical locations of the donors,
219 differ from each other, as a result of variations in the thicknesses of the stratum corneum and varying
220 follicle densities. To minimize such variations among different skin, only abdominal skin was used.
221 The full thickness skin was collected immediately after surgical removal and was prepared within 24
222 hours post-surgery.

223 The skin was rinsed with deionized water and blotted dry with clean tissue paper. To remove any
224 residual fat from the subcutaneous fat layer and surface sebaceous lipids, the skin was carefully wiped
225 once with an ethanol moistened cotton swab.

226 A skin layer having a thickness of 400 µm, including the stratum corneum, the viable epidermis and
227 the upper dermis, with a width of 2.5 cm, was cut using a dermatome (Zimmer Inc., Warsaw, IN,
228 USA). The prepared skin was placed dermal side down on filter paper. It was then wrapped in
229 aluminum foil and frozen at -20°C, until use. All skin samples were used within one month after

preparation. An hour prior to commencing with the permeation experiments, the skin was removed from the freezer and thawed at room temperature and cut into circular pieces, 15 mm in diameter.

5.3.2. Permeation experiments

Vertically mounted Franz diffusion cells, each with a donor capacity of 1.0 ml and receptor capacity of 2.0 ml, were used. Preceding the permeation experiments, the integrity of the skin to be used was tested by measuring the electrical resistance across it. The prepared skin was placed in between the donor and receptor compartments with the epidermal side facing the upper donor compartment. Both the donor and receptor compartments were filled with a 0.9% aqueous sodium chloride solution that had been degassed in an ultrasonic water bath for 15 minutes. The Franz cells were then placed in a 37 ± 1°C water bath and left to equilibrate for 30 minutes. After the 30 minutes, electrical resistance of the skin was measured, using a Tinsley LCR Databridge Model 6401 (Tinsley Precision Instruments, Croydon, United Kingdom) at 1 kHz, with a maximum voltage of 300 mV root-mean-square (rms) in the parallel equivalent circuit mode, by employing an alternating current (Fasano *et al*., 2002:732). After completion of the resistance measurements, the aqueous sodium chloride solution was removed and those cells with a resistance of less than 10 kΩ were rejected. The effective diffusion area was calculated as 1.13 cm^2.

The PBS (pH 7.4) was degassed on an ultrasonic bath, prior to filling the receptor compartment. Care was taken to ensure that no air bubbles were trapped in the compartment or under the skin, as this would decrease the effective diffusion area.

Each bottle, containing the prepared Pheroid™ and API, was removed from the fridge and placed in a water bath at 32°C to warm, during constant mechanical shaking. This was done in order to ensure that any API that might have crystallized out during storage in the fridge would dissolve again to be encapsulated by the Pheroid™ vesicles. As observed during the study by Slabbert *et al.* (2011:214), it was found that the API may be added to the Pheroid™ system after, or during the manufacturing process, as this had no effect on the entrapment efficacy of the carrier vesicle. The freshly prepared

donor solution, containing the API, referred to as the control, was also placed in a water bath at 32°C to allow it to reach the same temperature.

The contents of the receptor compartments were continuously stirred at 500 rpm, by using a small magnetic bar and magnetic stirrer plate, and the temperature maintained at 37°C by the water bath. This way the stratum corneum surface temperature was maintained at approximately 32°C, hence simulating human skin temperature.

The donor compartments were filled with 1 ml of Pheroid™ (experimental cells, n = 8), or 1 ml of the PBS (pH 7.4) solution (control cells, n = 7), each containing a different API. The donor compartments were then covered with Parafilm™ to prevent evaporation from the donor vehicles during the 12 hour long experiments.

The Pheroid™ delivery system had been prepared by using the same dissolution medium as the control (PBS), as a basis for the experimental Pheroid™ delivery system. This was then compared to the control in the transdermal diffusion studies for each API.

5.3.3. Sample collection

During sample collection, the entire receptor phase of each cell was removed at 20, 40, 60, 80 and 100 minutes, and at 2, 4, 6, 8, 10 and 12 hours. After extraction, the receptor phase was immediately replaced with an equal volume of fresh PBS (pH 7.4), also maintained at 37°C, to maintain sink conditions. The concentration of the API that had penetrated the stratum corneum was recovered in the receptor compartment and after sampling analyzed by HPLC.

5.4. Model fitting

All statistical analyses and mathematical modeling were performed, using MATLAB R2013a (MATLAB, The MathWorks Inc., MA., USA) mathematical software. To assess how well the derived models fitted the experimentally observed data, the following three measures were used.

Firstly, the coefficient of determination, r^2, which gives an indication of the ratio of explained variation to total variation, was used.

Secondly, studentized deleted residuals were used to identify possible outliers. If we let e_i = (observed concentration – predicted concentration) denote the residual for observation i, and let h_i denote the leverage value, calculated as:

$$h_i = \frac{1}{n} + \frac{(x_i - \bar{x})^2}{SS_{xx}}$$

Where:

$$SS_{xx} = \sum_{i=1}^{n} (x_i - \bar{x})^2$$

The unexplained error in the model, using the sum of squared forecast errors (*SSE*), could be determined:

$$SSE = \sum_{i=1}^{n} (y_i - \hat{y})^2$$

and the standard error of the model as:

$$s = \sqrt{\frac{SSE}{n - (k+1)}}$$

Subsequently, by using the standard error of the residual e_i, and the standard error of the deleted residual s_{di}, for observation i, the studentized deleted residual, d_i/s_{di}, could be calculated as:

$$\frac{d_i}{s_{d_i}} = e_i \left[\frac{n - (k+2)}{SSE(1 - h_i) - e_i^2} \right]^{1/2}$$

This enabled the researchers to *t*-test whether or not the i^{th} observation was influential, by comparing the value of d_i/s_{di} to $t_{0.025}$ with (n – (k + 2)) degrees of freedom.

Thirdly, Cook's distance was used to determine the presence of influential observations in the dataset. It was calculated for observation *i*, using:

$$CD_i = \frac{e_i^2}{(k+1)s^2}\left[\frac{h_i}{(1-h_i)^2}\right]$$

Cook's distance, which is sensitive to both outliers and leverage points, also enabled the research team to statistically test the fit of the used data. This was done by comparing the CD_i value to F-critical values with (k + 1) degrees of freedom in the numerator and (n – (k + 1)) degrees of freedom in the denominator. Another known indication of an influential observation is the rule of thumb, CD_i > 1, which was also tested for.

6. Calculations

The data used to derive this model, had been collected from within the research group (Table 2). The rationale behind using this dataset, rather than the datasets compiled by Flynn (Flynn, 1990), or Lian *et al*. (Lian *et al*., 2008), stemmed from the fact that, with this data, particular aspects of the experimental setup were kept relatively constant, e.g. the conditions under which the log P experiments were performed, the type of buffer used and the pH, as well as the laboratory and analytical conditions. Since data obtained from biological studies are inherently prone to large variations, maintaining these variables enabled the researchers to more reliably relate differences in cumulative permeation to the physicochemical properties of the drugs. The data in Table 2 was consistent with the relationship between skin permeability and the optimal physicochemical properties, as described in the introduction, with some minor exceptions. Firstly, the optimal molecular weight in this dataset was 288 Da, up from the 220 Da, mentioned above. Secondly, the restriction that log P had to be greater or equal to zero, would suggest that no skin permeation would occur for drugs with negative log P values, yet the used dataset indicated that below zero, log P behaved in a manner similar to above zero. The difference was that the cumulative diffused concentrations of these drugs were much smaller. This was incorporated into the proposed model.

For these models, the cumulative diffused amounts were expressed in μmol/mL quantities, instead of μg/mL, to facilitate comparison between the observed values.

For the model based on molecular volume, a value of $k = 0.002$ was selected and from Table 2, values of $MV_{opt} = 247.26$ Å^3 and $C_{max} = 1.4284$ μmol/mL for the APIs administered with the PBS donor, and $MV_{opt} = 229.37$ Å^3 and $C_{max} = 1.0073$ μmol/mL for the APIs administered with Pheroid™. The graphs of these values, when incorporated into Eq. (8), are presented in Figure 2. To determine how well this model, based on molecular volume (Eq. (8)), fitted the observed data in Table 2, the measures, as described in the methods section, were employed. The r^2 values for the correlation between the calculated concentrations and the experimental concentrations were 0.926 and 0.892, for the APIs administered with Pheroid™ and from the PBS donor, respectively. Additional model fitting parameters are presented in Table 3. By comparing the studentized deleted residuals to *t*-critical values with 9 degrees of freedom, and testing at the $\alpha = 0.05$ level of significance, the rejection region was established as $|d_i s_{di}| > t_{0.025} = 2.262$. For the drugs that had been administered without Pheroid™, scopolamine's d_i/s_{di} value was higher in absolute value than the *t*-critical value, $|-2.989| > 2.262$. Similarly, for the drugs being administered with Pheroid™, isoniazid's d_i/s_{di} value was higher than $t_{0.025}$, $2.8373 > 2.262$. Statistical evidence hence seemed to indicate that these two values were outliers. Removal of these values increased the above r^2 values to 0.96 and 0.938, respectively. To investigate whether there were any influential observations in the dataset used, the CD_i values were analyzed. In a comparison of the CD_i values to the F-critical value with 2 degrees of freedom in the numerator and 9 degrees of freedom in the denominator, whilst testing at the $\alpha = 0.05$ level of significance, a rejection region of $CD_i > F_9^2 = 4.26$ was found. Since none of the CD_i values exceeded 1, or the F-critical value, it was concluded that there was insufficient statistical evidence to support the existence of influential observations in the used dataset.

This model, based on log P, could be applied to both positive and negative log P values. For drugs with positive log P values, dissolved in PBS, values of $A = 0.5$, $k_1 = 1.2$ and $k_2 = 5$ were selected, while $A = 0.5$, $k_1 = 3.7$ and $k_2 = 10$ were selected for those drugs having negative log P values. For drugs with positive log P values, administered with Pheroid™, values of $A = 0.5$, $k_1 = 1$ and $k_2 = 3.9$

were selected, while $A = 0.5$, $k_1 = 1.5$ and $k_2 = 2$ were selected for those with negative log P values. The resulting graphs of predicted and observed data (Table 2) are presented in Figure 3. To assess the fit of the observed data to the model (Eq. (11)), the same measures were used, as described above. The r^2 values were 0.956 and 0.962, for the drugs administered with Pheroid™ and PBS, respectively. The additional model fitting parameters are presented in Table 4. Since the degrees of freedom and the significance level of the studentized deleted residual remained the same, the rejection region of $|d_i s_{di}| > t_{0.025} = 2.262$ remained unchanged. Among the drugs administered with and without Pheroid™, only the lidocaine HCl administered without Pheroid™ had a d_i/s_{di} value higher than the critical value of the rejection region, 2.8322 > 2.262. However, removal of the data entry did not improve the r^2 value. To investigate whether this, or any other observation in the used dataset, could be classified as influential, the CD_i values were analyzed. Using the same criteria than with molecular volume, it was again found that there was inadequate statistical evidence to confirm the existence of influential observations in the used dataset.

Noting that the final form of the model (Eq. (12)) was an ordered n-tuple, it was intuitive that a certain concentration value would be associated with a specific set of molecular volume and log P coordinates. A goodness of fit test on the concentration values returned by Eq. (8) and (11) resulted in an r^2 value of 0.999 and an adjusted r^2 value of 0.9899. To help visualize this system, a diagrammatic representation is presented in Figure 4. To understand this, consider the following example, based on Figure 4: A drug (hollow circle) has a certain known molecular volume, say MV_1, Eq. (8) can be used to calculate the expected cumulative concentration, C_1, for that drug after 12 h. The calculated concentration, C_1, can then be substituted into Eq. (11) and solved for log P to calculate an approximate log P value for that drug. Suppose another drug (filled circle) exists, of which the log P value is known, say $\log P_2$. Eq. (11) can be used to calculate an expected cumulative concentration, C_2, after 12 h and, if so wished, use that concentration value and Eq. (8) to find an approximate molecular volume, MV_2, for that drug. Another possible use for this model is to check for consistency between experimentally determined concentrations, and those predicted from either molecular volume, or log P, or both. Because both these models would return the same concentration

value, a discrepancy would warrant further investigation into the importance of the property that returned the poor approximation.

7. Results

The individual models, Eq. 8 and 11, were applied to the NSAIDs, used in this study (Table 5), and the results of the partial models are presented in Figures 5 and 6. The values of the constants used in Figure 5 were, $k = 0.02$, $C_{max} = 0.8264$ μmol/mL and $MV_{opt} = 211.86$ Å^3 for the NSAIDs administered without Pheroid™, and $k = 0.02$, $C_{max} = 0.6224$ and $MV_{opt} = 211.86$ Å^3 for the NSAIDs administered with Pheroid™. The result was a much narrower function, with some loss in the goodness of fit in relation to the Pheroid™ group. The r^2 values were 0.681 and 0.893 for the NSAIDs administered with Pheroid™ and PBS, respectively. Additional model fitting parameters are presented in Table 6. Using a 5% level of significance and noting that the test statistic was based on 5 degrees of freedom, the rejection region was constructed as $|d_i s_{di}| > t_{0.025} = 2.571$. In both the samples of drugs administered with and without Pheroid™, the data points, associated with ketoprofen, had $d_i s_{di}$ values > 2.571. Removal of these values resulted in an increase in the r^2 values reported above, to 0.956 and 0.962, respectively. In testing for the presence of influential observations, the rejection region was constructed as $CD_i > F_6^2 = 5.14$ at the 5% level of significance. Since none of the CD_i values were higher than 1, nor higher than the F-critical value, it was concluded that there was insufficient statistical evidence to indicate the existence of influential observations in the used dataset.

For the model based on the log P values, the constants used in Figure 6 were $A = 1.6$, $k_1 = 2$ and $k_2 = 5$ for the NSAIDs dissolved in PBS, and $A = 1.5$, $k_1 = 2.6$ and $k_2 = 5$ for the NSAIDs administered with Pheroid™. Since only one NSAID, acetylsalicylic acid, had a negative log P value, the behavior of the function in that region could not be determined. Without the ability to reliably approximate values for the constants, the negative log P region was omitted. With this model, there was a pronounced loss in goodness of fit, with r^2 values of 0.53 and 0.361, for those NSAIDs administered with and without Pheroid™, respectively. From the additional information in Table 6, a very large discrepancy between the observed and calculated concentrations of piroxicam was observed. Testing at the 5%

level of significance, with 4 degrees of freedom, the rejection region could be constructed as $|d_i s_{di}| > t_{0.025} = 2.776$. None of the $d_i s_{di}$ values exceeded the *t*-critical value. However, the $d_i s_{di}$ value of piroxicam could not be determined as a real value, because the large difference between the observed and calculated concentrations had led to a negative value in the square root. This value was therefore not compared to the *t*-critical value. Because of the apparent visual difference, the concentration values of piroxicam were removed and the effect on the r^2 values investigated. The result was an increase in the r^2 values, reported above, to 0.974 and 0.753, respectively. Using a rejection region of $CD_i > F_5^2 = 5.79$ at the 5% level of significance, it was found that there was adequate statistical evidence to conclude that the used dataset did not contain influential observations. However, the effect of piroxicam's observed concentration value on the r^2 values could not be denied, and could be regarded as an influential observation.

From the experimental data (Table 5), it was identified that no diffusion was observed for indomethacin and acetylsalicylic acid, neither from the PBS donor, nor from the Pheroid™ delivery system, over the 12 h time duration of the diffusion experiments. The cumulative amount of NSAIDs that did manage to permeate the skin over the 12 h period, are presented in Figure 7, as functions of time.

For indomethacin, its high molecular weight (357.80 g/mol) and high log P value (3.8), may have been determining factors in its inability to permeate the skin. This was supported by the proposed model (Figures 5 and 6). Acetylsalicylic acid had a molecular weight and volume, much lower than the optimal values, as described by the restricted rule of five, which may explain why it failed to permeate the skin (Table 6). This observation was consistent with the predictions made from the model (Figure 5). In a previous study, it had been shown that a log P value close to 2 was ideal for transdermal delivery of an NSAID (Swart *et al.* 2005:78). This was consistent with the proposed model, which had predicted the optimal log P value for the NSAIDs in this study to be somewhere between 1 and 2. The log P value of ibuprofen, the NSAID that had displayed the best skin permeation, was within this region. Ibuprofen has very favorable physiochemical characteristics for

421 transdermal diffusion, in that it has a molecular weight and a log P value in the predicted "sweet
422 spots" of this model.

423 The Pheroid™ delivery system was unable to further increase the cumulative amount of ibuprofen
424 being delivered from it as a donor system. Ibuprofen, diclofenac sodium and mefenamic acid showed
425 a decrease in the amount of the drug that had permeated the skin, when administered with Pheroid™,
426 compared to administration with PBS (Figure 7, A, C and D). The Pheroid™ delivery system could
427 only outperform the PBS donor solution for three of the NSAIDs, i.e. ketoprofen, acetaminophen and
428 piroxicam, as illustrated by B, E and F, respectively (Figure 7). Ketoprofen, with a molecular weight
429 and log P value close to the optimal values, as predicted by the model (Eq. 12), showed satisfactory
430 transdermal delivery from both the PBS and the Pheroid™ delivery systems (Table 4).
431 Acetaminophen had the lowest molecular weight of all of tested the NSAIDs (151.20 g/mol), and a
432 low log P of 0.64. The Pheroid™ delivery system was able to increase the cumulative amount of
433 acetaminophen delivered over 12 h from 14.6 μg/cm^2 from the PBS solution to 15.9 μg/ml. The skin
434 permeability of piroxicam, although accurately modeled by the molecular volume model, could not be
435 accurately modeled by the log P model. It is possible that its molecular weight, 331.35 g/mol, or
436 some other variable, had a more decisive influence on its permeability.

437 ## 8. Conclusion

438 In this study, the physicochemical properties of selected NSAIDs that would be ideal for transdermal
439 delivery, using the Pheroid™ delivery system, were investigated. A new flux-independent
440 mathematical model was also derived and tested, based upon restrictions placed on Lipinski's rule of
441 five. The purpose of this model was to assist in testing and visualizing the optimal molecular size and
442 log P ranges associated with the highest cumulative amount of NSAID transported across the skin,
443 over a 12 h period.

444 The results suggested that the same molecular size and log P ranges that had determined the skin
445 permeability of an API having been dissolved in PBS, also determined its permeability when
446 dissolved in Pheroid™, and that these ranges were consistent with the previously described

447 restrictions that should be placed on Lipinski's rule of five, to account for the formidable resistance
448 offered by the skin's stratum corneum.

449 The model gave reasonable approximations of the experimentally observed concentrations. The
450 results suggested that the restrictions placed on Lipinski's rule of five, as discussed in the
451 introduction, could be used to accurately model transdermal delivery. Future studies could be
452 conducted to investigate how this derived permeation model scales time, possibly as a function of the
453 permeability constant. This could enable the prediction of changes in skin permeation of a chemical
454 entity, as it changes structure over time, e.g. as a result of decomposition. With these flux-dependent
455 models, a mathematical model relating H-bonding to skin permeation can be included, completing the
456 association with the rule of five.

457 **Acknowledgements**

458 This work was carried out with the financial support of the National Research Foundation of South
459 Africa (NRF) (Grants no. IFRR81178 and CPRR13091742482) and The Centre of Excellence for
460 Pharmaceutical Sciences (Pharmacen) of the North-West University, Potchefstroom Campus, South
461 Africa.

462 **Disclaimer**

463 Any opinion, findings and conclusions, or recommendations expressed in this material are those of the
464 authors and therefore the NRF does not accept any liability in regard thereto.

465

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522

523 **Tables**

524 **Table 1:** Chromatographic conditions and mobile phase composition for analysis of each API

Drug	Mobile phase (acetonitrile/water)	Retention time (min)	Wave length (nm)	Stop time (min)
Ibuprofen	65/35	6.0	225	8
Ketoprofen	65/35	2.5	260	5
Acetylsalicylic acid	35/65	4.2	228	8
Piroxicam	65/35	2.9	340	5
Meloxicam	65/35	3.5	350	6
Indomethacin	65/35	5.2	230	8
Acetaminophen	15/85	3.4	249	5
Diclofenac sodium	65/35	3.9	277	5
Mefenamic acid	65/35	7.8	350	10

525

526

Table 2: The APIs used to derive the model, with their respective physicochemical properties and cumulative diffused concentrations (mean ± STD) after 12 h

Drug	Molecular weight (g/mol)	Molecular volume ($Å^3$)	Log P	Concentration after 12 h (µmol/mL) in PBS	Concentration after 12 h (µmol/mL) in Pheroid™
5-Fluorouracil	130.08	93.60	-0.980	0.017	0.0529
Acyclovir	225.00	183.96	0.018	0.0074 ± 0.0045	0.0101 ± 0.0052
Ketoconazole	531.00	450.75	3.730	0.0012 ± 0.0008	0.0004 ± 0.0004
Scopolamine	303.35	277.00	0.800	0.0462 ± 0.0099	0.0214 ± 0.0068
Arginine vasopressin	1084.23	945.01	-4.800	0.0081 ± 0.0083	0.0078 ± 0.0125
Lidocaine HCl	288.00	247.26	2.440	1.4284 ± 0.1642	0.9145 ± 0.2505
Prilocaine HCl	256.80	229.37	2.110	1.2772 ± 0.1365	1.0073 ± 0.3285
Isoniazid	137.14	120.44	-0.640	0.0498 ± 0.0345	0.2252 ± 0.1498
Rifampicin	822.95	758.41	3.714	0.0000	0.0079 ± 0.0084
Zalcitabine	211.22	183.91	-1.150	0.2674 ± 0.2963	0.2278 ± 0.2155
Lamivudine	229.25	186.04	-1.780	0.4858 ± 0.4547	0.1391 ± 0.0698

Table 3: Predicted concentrations (from Eq. 8), studentized deleted residuals (d_i/s_{di}) and Cook's distance (CD_i) of the APIs used to derive the model

Drug	Predicted concentration	d_i/s_{di}	CD_i
API in PBS donor			
5-Fluorouracil	0.0000	0.0669	0.0005
Acyclovir	0.0260	-0.0713	0.0004
Ketoconazole	0.0000	0.0046	0.0000
Scopolamine	0.5898	-2.9890	0.2498
Arginine vasopressin	0.0000	0.0451	0.0016
Lidocaine HCl	1.4284	0.0000	0.0000
Prilocaine HCl	1.0372	0.9637	0.0551
Isoniazid	0.0000	0.1948	0.0038
Rifampicin	0.0000	0.0000	0.0000
Zalcitabine	0.0258	0.9800	0.0662
Lamivudine	0.0337	2.1915	0.2298
API with Pheroid™			
5-Fluorouracil	0.0000	0.4819	0.0255
Acyclovir	0.1277	-1.1028	0.0815
Ketoconazole	0.0000	0.0035	0.0000
Scopolamine	0.1038	-0.7338	0.0299
Arginine vasopressin	0.0000	0.0990	0.0077
Lidocaine HCl	0.7288	1.9493	0.1625
Prilocaine HCl	1.0037	0.0312	0.0000
Isoniazid	0.0000	2.8373	0.4017
Rifampicin	0.0000	0.0790	0.0017

Zalcitabine	0.1271	0.9263	0.0598
Lamivudine	0.1535	-0.1263	0.0012

Table 4: Predicted concentrations (from Eq. 11), studentized deleted residuals (d_i/s_{di}) and Cook's distance (CD_i) of the APIs used to derive the model

Drug	Predicted concentration	d_i/s_{di}	CD_i
API in PBS donor			
5-Fluorouracil	0.0691	-0.4836	0.0168
Acyclovir	0.0000	0.0668	0.0003
Ketoconazole	0.0855	-0.8872	0.1462
Scopolamine	0.1116	-0.6043	0.0205
Arginine vasopressin	0.0000	0.0970	0.0049
Lidocaine HCl	1.2149	2.8322	0.4252
Prilocaine HCl	1.4463	-1.8834	0.2238
Isoniazid	0.0027	0.4330	0.0120
Rifampicin	0.0899	-0.9511	0.1644
Zalcitabine	0.1751	0.8879	0.0567
Lamivudine	0.4545	0.2947	0.0089
API with Pheroid™			
5-Fluorouracil	0.2337	-0.7094	0.0489
Acyclovir	0.0000	0.0375	0.0000
Ketoconazole	0.0808	-0.3339	0.0220
Scopolamine	0.1521	-0.4899	0.0091
Arginine vasopressin	0.0000	0.0384	0.0008
Lidocaine HCl	0.8260	0.5964	0.5717
Prilocaine HCl	0.9929	0.0593	0.1068
Isoniazid	0.1506	0.2810	0.0093
Rifampicin	0.0843	-0.3174	0.0244

Zalcitabine	0.2452	-0.0659	0.0006
Lamivudine	0.1472	-0.0350	0.1777

Table 5: Selected NSAIDs used to test the model, with their physicochemical properties and cumulative diffused concentrations (mean ± STD) after 12 h

Drug	Molecular weight (g/mol)	Molecular volume ($Å^3$)	Log P	Concentration after 12 h (µmol/mL) in PBS	Concentration after 12 h (µmol/mL) in Pheroid™
Ibuprofen	206.30	211.86	1.15	0.8264 ± 0.0877	0.6224 ± 0.0524
Acetylsalicylic acid	180.20	154.77	-1.10	0.0000	0.0000
Ketoprofen	254.30	233.61	0.97	0.2379 ± 0.0267	0.3724 ± 0.0413
Sodium diclofenac	318.10	234.37	4.00	0.1528 ± 0.0487	0.0830 ± 0.0148
Indomethacin	357.80	300.90	3.80	0.0000	0.0000
Mefenamic acid	241.30	225.94	5.12	0.0344 ± 0.0025	0.0099 ± 0.0025
Acetaminophen	151.20	138.06	0.64	0.0966 ± 0.0598	0.1052 ± 0.0662
Piroxicam	331.35	268.00	1.80	0.0002 ± 0.0001	$0.0001 \pm 3.95e^{-5}$

Table 6: Predicted concentrations, studentized deleted residuals (d_i/s_{di}) and Cook's distance (CD_i) of the NSAIDs tested in this study

Drug	Calculated from molecular volume			Calculated from log P		
	Predicted concentration	d_i/s_{di}	CD_i	Predicted concentration	d_i/s_{di}	CD_i
NSAID in PBS donor						
Ibuprofen	0.8264	0.0000	0.0000	0.8575	-0.0580	0.0007
Acetylsalicylic acid	0.0000	0.0000	0.0000	-	-	-
Ketoprofen	0.0073	3.2368	0.2594	0.5362	-0.5897	0.0725
Sodium diclofenac	0.0052	1.3861	0.1073	0.0001	0.2916	0.0185
Indomethacin	0.0000	0.0000	0.0000	0.0007	-0.0013	0.0000
Mefenamic acid	0.1138	-0.6579	0.0288	0.0000	0.0796	0.0041
Acetaminophen	0.0000	1.0897	0.4141	0.1141	-0.0345	0.0004
Piroxicam	0.0000	0.0017	0.0000	1.1840	*	0.5634
NSAID with Pheroid™						
Ibuprofen	0.6224	0.0000	0.0000	0.5407	0.4401	0.0362
Acetylsalicylic acid	0.0000	0.0000	0.0000	-	-	-
Ketoprofen	0.0055	17.8568	0.3773	0.3791	-0.0358	0.0003

Diclofenac sodium	0.0039	0.4898	0.0177	0.0000	0.4546	0.0436
Indomethacin	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Mefenamic acid	0.0857	-0.4666	0.0151	0.0000	0.0647	0.0027
Acetaminophen	0.0000	0.8673	0.2822	0.0946	0.0594	0.0011
Piroxicam	0.0000	0.0006	$6.3259e^{-8}$	0.4199	*	0.5652

* $d_{piroxicam}/s_{dpiroxicam}$ has no real solution.

Figures

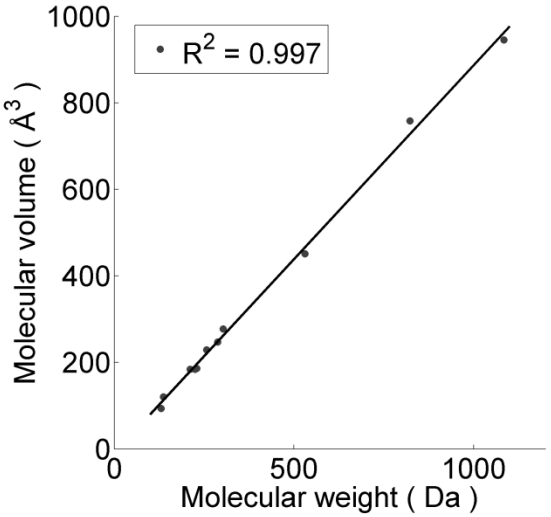

Figure 1: Representation of the strong linear correlation between molecular weight and molecular volume (single column fitting image).

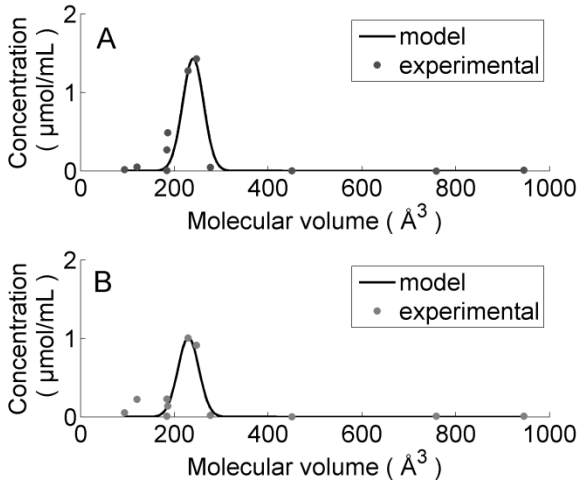

Figure 2: (A) Graphic representation of the model (Eq. 8) relating molecular volume to concentration (solid line) and the experimentally observed concentration values of drugs in PBS (dark grey circles). (B) Graphic representation of the model (solid line) and the experimentally observed concentration values of the drugs dispersed in Pheroid™ (light grey circles) (single column fitting image).

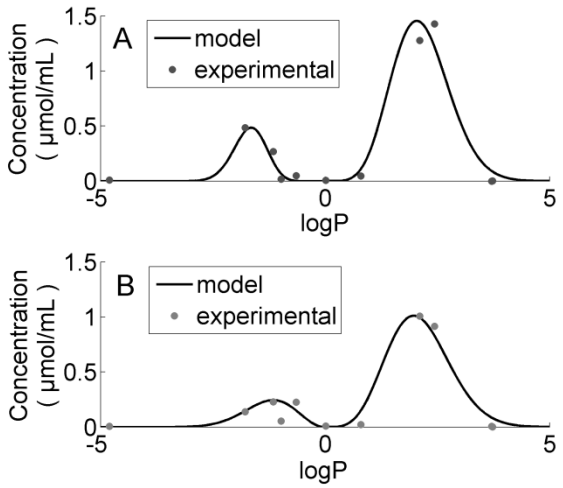

Figure 3: (A) Graphic representation of the model (Eq. 11) relating log P to concentration (solid line) and the experimentally observed concentration values of drugs in PBS (dark grey circles). (B) Graphic representation of the model (solid line) and the experimentally observed concentration values of the drugs dispersed in Pheroid™ (light grey circles) (single column fitting image).

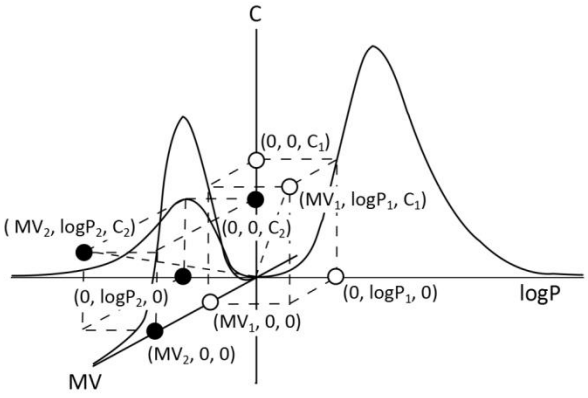

Figure 4: Visual representation of how the final model (Eq. 12) forms a coordinate system (single column fitting image).

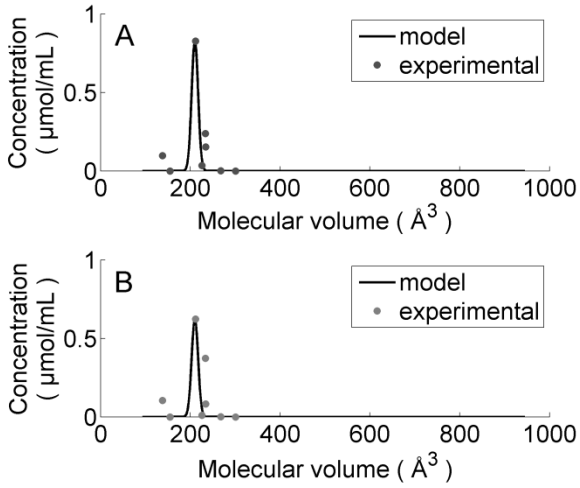

Figure 5: (A) Graphic representation of the model (Eq. 8) relating molecular volume to concentration (solid line) and the experimentally observed concentration values of the NSAIDs in PBS (dark grey circles). (B) Graphic representation of the model (solid line) and the experimentally observed concentration values of the NSAIDs dispersed in Pheroid™ (light grey circles) (single column fitting image).

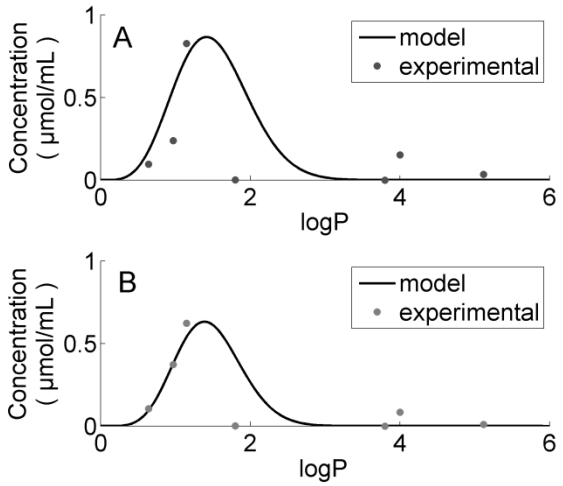

Figure 6: (A) Graphic representation of the model (Eq. 11) relating log P to concentration (solid line) and the experimentally observed concentration values of NSAIDs in PBS (dark grey circles). (B) Graphic representation of the model (solid line) and the experimentally observed concentration values of NSAIDs administered with Pheroid™ (light grey circles) (single column fitting image).

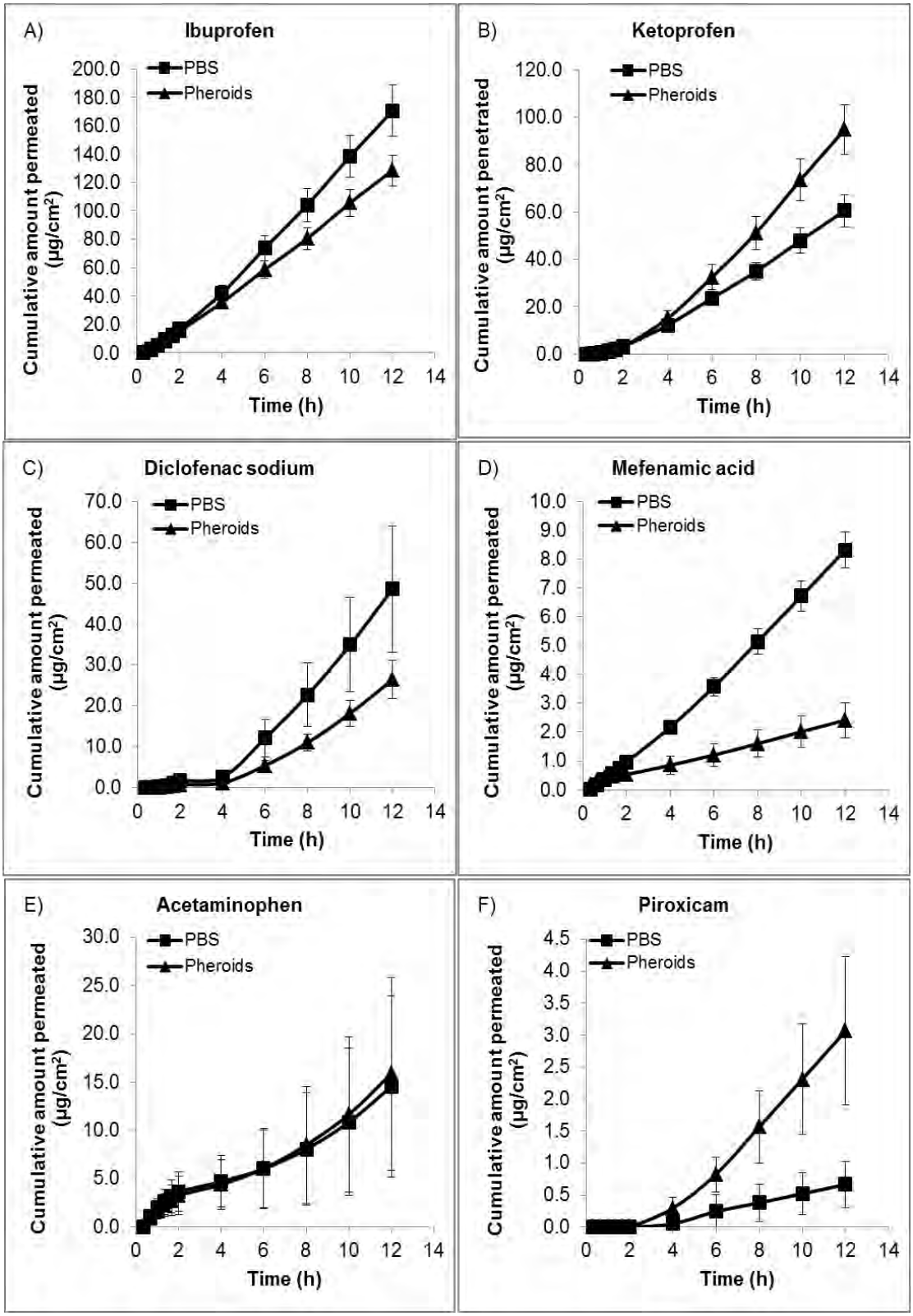

Figure 7: The average cumulative amount of API *versus* time that had permeated the skin in 12 h for the PBS control, and for the Pheroid™ delivery system (two column fitting image)

Chapter 4: Article for publication in Artificial Intelligence in Medicine

Chapter 4 is written in article format for the purpose of publication in Artificial Intelligence in Medicine. The complete author's guide is given in Annexure D. Please note that Chapter 4 is written in US English and not UK English and that all formatting is done according to the guide to authors. The text has also been justified to ease reading.

A cheminformatics and Bayesian network approach to investigating the probabilistic dependencies in transdermal drug delivery

Hendrik J.R. Lemmer, Dewald Kilian, Minja Gerber, Jan L. du Preez, Jeanetta du Plessis*

Centre of Excellence for Pharmaceutical Sciences (Pharmacen), North-West University, Private Bag X6001, Potchefstroom 2520, South Africa

* Corresponding author. Tel.: +2718 299-2274; Fax: +2718 299-2225. E-mail address: Jeanetta.duPlessis@nwu.ac.za (J du Plessis).

Abstract

The aim of the study was to investigate the potential of Bayesian networks to accurately model the probabilistic dependencies between physicochemical properties and skin permeability of selected drugs. Additionally, the ability of Bayesian networks to assist in early drug discovery and development decision making was investigated. Therefore, the physicochemical properties used in the training sets were collected from several bioinformatics and cheminformatics databases. Experimental skin permeability data were obtained from selected drugs dissolved in phosphate buffer solution (PBS) at pH 7.4, and dispersed in the lipid based delivery system, Pheroid™. Network structures were learned from training sets containing the physicochemical properties and experimental skin permeation data, and compared to known probabilistic dependencies and optimal physicochemical property ranges. The resulting network structures were also analyzed to identify differences in probabilistic dependencies between the physicochemical properties and skin permeabilities of the dissolved and dispersed drugs. The network structures learned from these datasets were consistent with our prior knowledge and even elucidated previously unknown causal relationships. For the dissolved drugs, skin permeability was found to be dependent on pKa. This dependency was not observed for the drugs dispersed in Pheroid™. In general, skin permeability was dependent on the ratio of topological polar surface area (TPSA) to molecular size, and a specific range of melting points. The best skin permeability was observed for drugs with low TPSA values, low

melting points and medium range molecular sizes. Our results also suggested that the skin permeability of drugs smaller than 400 Da decreases with an increase in TPSA and melting point, and a decrease in molecular size. The Bayesian networks were able to accurately model dependencies between physicochemical properties, correlate them with skin permeability and distinguish between dissolved and dispersed samples. This suggests that Bayesian networks can be valuable decision making tools, not only in early drug discovery, but also during the development phase.

Keywords: Bayesian networks, transdermal drug delivery, physicochemical properties, cheminformatics, drug delivery system.

1 Introduction

The skin is the largest organ in the body, with a surface area of approximately 1.8 to 2.0 m^2 [1]. One of its main functions is the protection of internal organs and tissue from exposure to harmful substances, radiation and microorganisms, by regulating permeation into the body. This barrier function is of particular interest to various scientific disciplines, including pharmaceutical sciences [2, 3], cosmetics [4, 5] and occupational health and safety [6, 7]. From a pharmaceutical point of view, the transdermal route has some advantages over other routes of drug administration. These include its non-invasiveness, ability to bypass first-pass metabolism, lack of gastrointestinal side-effects and ease of application. All of which can improve patient compliance.

When selecting a drug candidate for a specific route of administration, one usually starts with a thorough physicochemical evaluation of the drug, since the physicochemical properties impact the final product at several stages of the development processes [8]. Examples include the relationship between bioavailability, dissolution rate and aqueous solubility [9-11] as well as the influence of solid-state changes on the stability of the final formulation [12-14]. Based on its physicochemical properties, the drug can be classified as a good or poor candidate for a certain administration route. Well known classification systems for orally administered drugs include the biopharmaceutical classification system (BCS) [15] and Lipinski's rule of five [16]. Although originally developed to

identify suitable candidates for oral administration, the rule of five has been shown to also be relevant for other routes of administration [17-20]. Certain circumstances, like the age of the patient, the ability of the patient to swallow and the urgency of the treatment, may necessitate the formulation a drug for a route of administration for which it is unfavorable. Several formulation and pre-formulation strategies can then be employed to address the physicochemical shortcomings of the drug, including particle size reduction [21, 22], manipulation of the solid-state form or properties of the drug [23, 24] and incorporation into a specialized delivery system [25-28]. The Pheroid™ is one such delivery system, consisting of essential plant fatty acids emulsified at the submicron level in water and saturated with nitrous oxide [29].

Despite a large number of biopharmaceutics papers where artificial neural networks [30-33], discriminant and principle component analyses [18, 34-36] had been used, Bayesian networks have yet to get the same exposure in the field. In other fields of medicine and health care, Bayesian networks have seen such diverse applications as the detection of disease outbreaks [37], classification of human immunodeficiency virus (HIV) inhibitors [38], elucidation of the epidemiology of tuberculosis [39], the analysis of gene expression [40] and the modeling of treatments and prognoses [41, 42]. For this study, Bayesian networks were chosen because of their novelty in pharmaceutical sciences, their ability to identify causal relationships between variables, incorporate expert knowledge and be updated as new evidence becomes available.

Variations in experimental conditions can introduce unwanted uncertainty in data, e.g. the failure of previous studies to find consistent correlations between a drug's degree of ionization and its transdermal permeability [43-45], which could be traced back to inconsistencies in the pH values of the flux and solubility study solutions, respectively [46]. Even the frequently used Flynn dataset [47] contains skin permeability data from more than one type of donor solution, collected using different laboratory protocols. These differences in experimental conditions have been cited as major source of variability in the predictions made from models developed using this dataset [48, 49]. To minimize uncertainty, all transport data used in this study were collected from within our research group,

thereby ensuring that variations in working conditions, analytical equipment and protocols, pH, temperature and type of skin donor were kept at a minimum.

During the early drug discovery process, a potentially large number of drug development candidates are identified. Since it is impractical to synthesize and screen all of these compounds, certain physicochemical properties are calculated *in silico* and used for the candidate selection process. A more modern approach is the mining of available biomedical databases to identify targets for lead discovery [50]. Since some metrics used in early drug discovery decision making are purely abstract, and must be calculated, we also opted to collect the physicochemical properties of the drugs used in the skin permeation studies from online bioinformatics and cheminformatics databases. In this way, the physicochemical data used in this study was representative of the type of data used in early drug discovery. These databases sometimes use different software packages to calculate physicochemical properties and some also contain experimental results. Therefore, by collecting data from several of these databases we were able to cover a range of *in silico* predicted results.

In this study, we aimed to investigate the ability of structure learning algorithms to produce Bayesian networks that could accurately model the probabilistic dependencies between physicochemical properties collected from online bioinformatics and cheminformatics databases, and experimentally obtained transdermal permeation data. The transdermal transport data used in this study was the cumulative amount of drug that permeated the skin after 12 h, from samples dissolved in a buffer solution at pH 7.4 and dispersed in the Pheroid™ lipid based delivery system. Once the validity of the networks was established, the probabilistic dependencies, or network structures, of the dissolved and dispersed samples were compared to identify causal differences between them, and possible favorable classification ranges.

2 Background

As stated in the introduction, the aim of this study was to investigate the ability of Bayesian networks to model the statistical dependencies between variables important to transdermal delivery, and identify possible differences in these dependencies between drugs dissolved in a buffer and drugs suspended in a lipid based drug delivery system. A thorough understanding of the network structure and its meaning is therefore crucial. The following definitions and notation, as given by Nagarajan *et al.,* [51] will be used throughout the paper.

Bayesian networks are graphical models which give a representation of the probabilistic dependencies between a given set of random variables $\mathbf{X} = \{\mathrm{X}_1, \mathrm{X}_2, \cdots, \mathrm{X}_\mathrm{n}\}$. The graphical structure of these networks $\mathcal{G} = (\mathbf{V}, \mathrm{A})$ consists of a nonempty set **V** of nodes, representing the variables in **X**, and a finite set A of arcs connecting pairs of nodes. If an arc $a = (u, v)$ in the network has the property that its nodes form an ordered pair, i.e. u can be seen as the tail and v the head of the arc, then that arc is said to be directed from u to v, $(u \rightarrow v)$. If (u, v) is simply incident on the arc, the arc is said to be undirected, $(u - v)$. A graph that does not contain any cycles or loops is said to be acyclic. If a graph consists only of directed arcs and is acyclic, that graph is called a directed acyclic graph (DAG). The absence of arcs between certain nodes in a network causes a graphical separation between those nodes, which corresponds to conditional probabilistic independence between the corresponding variables. Moreover, if **A**, **B** and **C** are three disjoint subsets of nodes in a DAG, $\mathcal{G}$, then **C** is said to d-separate **A** from **B**, denoted $\mathbf{A} \perp_{\mathcal{G}} \mathbf{B}|\mathbf{C}$, if along every sequence of arcs between a node in **A** and a node in **B** there is a node v satisfying one of the following conditions:

1. v has two arcs pointing to v from adjacent nodes in the path and none of v or the nodes that can be reached from v are in **C**.
2. v is in **C** and does not have converging arcs [51].

Let $\prod_{X_i}$ denote the parent nodes of variable X_i. The Markov property of Bayesian networks can be used to factorize the joint probability distribution of **X** into a set of local probability distributions, giving the chain rule for Bayesian networks, presented here for the discrete case:

$$p_x(X_1, X_2, \cdots, X_n) = \prod_{i=1}^{n} p_x\left(X_i | \prod_{X_i}\right) \quad (1)$$

The chain rule therefore enables us to visualize and calculate the conditional dependencies of specific variables in the network, since the parent nodes, $\prod_{X_i}$, are all the nodes in the network with arcs directly directed to X_i.

Since Gaussian Bayesian networks cannot be used if there is a severe departure from normality in the data, an alternative is to discretize the data by transforming the real valued vector $\mathbf{v} = (v_1, v_2, \cdots v_n)$ into an integer-valued vector $\mathbf{d} = (d_1, d_2, \cdots d_n)$ with the properties that each element of **d** is in the set $\{0,1,\cdots, \mathrm{D}-1\}$ for some (usually small) positive integer D, called the degree of discretization and for all $1 < i, j < n$, we have $d_i < d_j$ if and only if $v_i < v_j$ [52]. However, median, quartile and quantile discretization techniques can cause information loss in datasets. The reason being that the mutual information between two random variables X_i and X_j can be expressed as,

$$I(\mathrm{X_i}; \mathrm{X_j}) = \sum_{x_i} \sum_{xj} p(x_i, x_j) \log \frac{p(x_i, x_j)}{p(x_i) p(x_j)} \ , \quad (2)$$

where $p(x_i, x_j)$ factorizes into $p(x_i)p(x_j)$ if X_i and X_j are independent. Therefore, mutual information can be lost if the discretization process accidentally splits pairs of variables that were dependent. Hartemink's algorithm [52] seeks to minimize the loss of pairwise mutual information between two real-valued vectors by collapsing the variables where mutual information exists, preventing the discretization process from splitting these pairs.

3 Materials and Methods

3.1 Materials

The drugs used in this study were donated by Adcock Ingram (South Africa). Analytical grade methanol, ethanol and phosphoric acid, PEG-400, as well as sodium chloride, disodium orthophosphate dehydrate, sodium dihydrogen orthophosphate dehydrate and dipotassium hydrogen orthophosphate anhydrous were purchased from Merck Laboratory Supplies (Midrand, South Africa). Double distilled deionized water was prepared with a Milli-Q water purification system (Millipore, Milford, USA) and used throughout the study. Vitamin F ethyl ester was obtained from Chemimpo (Johannesburg, South Africa), Cremophor® RH40 from BASF (Midrand, South Africa) and dl-α tocopherol from DSM (Basel, Switzerland). The Pheroid™ used was prepared in-house by The Centre of Excellence for Pharmaceutical Sciences (Pharmacen) at the North-West University (Potchefstroom Campus, South Africa).

3.2 High performance liquid chromatography

All high performance liquid chromatographic (HPLC) analyses were done using a Phenomenex™ Luna 5 µ C_{18} (250.00 x 4.60 mm) column at a flow rate of 1 mL/min. The HPLC system used for the analysis was an Agilent 1100 series, equipped with a variable wavelength ultraviolet (UV) detector, isocratic pump, auto sampler, and ChemStation (Rev. A.09.01 (1206)) data acquisition and analysis software. All analyses were performed using HPLC grade water and reactants. The temperature of the columns was kept at 25 °C throughout the analysis. All methods were validated by the Analytical Technology Laboratory at the North-West University (Potchefstroom Campus), and the data analysis was done by extrapolation from a least squares linear model fitted to peak area values obtained from a series of standard solutions. The standard solutions were prepared from a stock solution of 100 µg/mL (w/v) drug in phosphate buffer solution (PBS) of pH 7.4. This stock solution was diluted to give the series of standard solutions as 1.0, 2.5, 10.0, 25.0 and 100.0 µg/mL.

3.3 Solubility studies

Since the donor compartments of the Franz diffusion cells should contain a supersaturated solution of drug, the equilibrium solubility in PBS (pH 7.4) at skin temperature of each drug was determined. Over-saturated samples were stirred in a water bath at 32 °C for 24 h, after which each sample was filtered through a 0.22 μm Millipore filter, also at 32 °C, to remove any crystals or solids from the solution. The first 2 mL of solute was discarded, since the filter also had to be saturated with the active and with the solute. The filtrate was then diluted and the equilibrium solubility concentration determined according to the HPLC method described above. The experiment was performed in triplicate for each drug.

3.4 Preparation of Pheroid™ vesicles

The Pheroid™ vesicles were prepared and stored in-house at Pharmacen (North-West University, Potchefstroom Campus) according to the method described by Du Plessis *et al*. [53].

3.5 Diffusion studies

Prior approval for the project, "*In vitro* transdermal delivery of drugs through human skin", had been granted by the North-West University Ethics Committee (reference number NWU-00114-11-A5). Caucasian female skin from informed consenting patients was obtained after cosmetic surgery. To minimize variations among skin samples originating from different anatomical locations, only abdominal skin was used. The skin was collected immediately after surgical removal and was prepared within 24 h post-surgery. The preparation entailed rinsing with deionized water and blotting with clean tissue paper, after which residual fat was removed from the subcutaneous fat layer and surface sebaceous lipids by carefully wiping once with an ethanol moistened cotton swab. A final skin layer (thickness 400 μm and width of 2.5 cm) including the stratum corneum, viable epidermis and the upper dermis was cut using a dermatome (Zimmer Inc., Warsaw, IN, USA). The prepared skin was placed dermal side down on filter paper, wrapped in aluminum foil and frozen at -20 °C until used. All skin samples were used within one month after preparation. An hour prior to commencing

209 with the permeation experiments, the skin was removed from the freezer and thawed at room
210 temperature and cut into circular pieces, 15 mm in diameter.
211
212 **3.6 Permeation studies**
213 Vertically mounted Franz diffusion cells, each with a donor capacity of 1.0 mL and receptor capacity
214 of 2.0 mL, were used. The integrity of the skin was tested by measuring the electrical resistance
215 across it prior to use. This was done by placing the prepared skin between the donor and receptor
216 compartments, epidermal side facing the donor compartment, and filling both the donor and receptor
217 compartments with a 0.9% aqueous sodium chloride solution that had been degassed in an ultrasonic
218 water bath for 15 min. The Franz cells were then placed in a 37 ± 1 °C water bath and left to
219 equilibrate for 30 min. After this time, the electrical resistance of the skin was measured using a
220 Tinsley LCR Databridge Model 6401 (Tinsley Precision Instruments, Croydon, United Kingdom) at 1
221 kHz, with a maximum voltage of 300 mV root-mean-square (rms) in the parallel equivalent circuit
222 mode, employing an alternating current [54]. The aqueous sodium chloride solution was then
223 removed and those cells with a resistance of less than 10 kΩ were rejected. The effective diffusion
224 area was calculated as 1.13 cm^2.
225
226 The PBS (pH 7.4) was degassed in an ultrasonic bath, prior to filling the receptor compartment. Care
227 was taken to ensure that no air bubbles were trapped in the compartment, or under the skin, as this
228 would decrease the effective diffusion area. Each bottle of the prepared Pheroid™ and drug, was
229 removed from the fridge and placed in a water bath at 32 °C to warm under constant mechanical
230 shaking. This was done in order to ensure that any drug that might have crystallized during storage in
231 the fridge, would dissolve again to be encapsulated by the Pheroid™ vesicles. Consistent with the
232 results obtained by [55], it was found that addition of the drug to the Pheroid™ system during or after
233 the manufacturing process had no effect on the entrapment efficacy of the carrier vesicle. The freshly
234 prepared PBS (pH 7.4) solution containing only the drug was also placed in a water bath at 32 °C and
235 allowed to equilibrate. The contents of the receptor compartments were continuously stirred at 500

236 rpm, while maintaining the temperature at 37 °C using a water bath. This way the stratum corneum
237 surface temperature could simulate human skin temperature.
238
239 The donor compartments were filled with 1 mL of Pheroid™ (experimental cells, n = 8), or 1 mL of
240 the PBS (pH 7.4) solution (control cells, n = 7), each containing a different drug and covered with
241 Parafilm™ to prevent evaporation during the 12 h experimental run time. The Pheroid™ delivery
242 system had been prepared using PBS (pH 7.4) as dispersion medium, to facilitate comparison with the
243 control where each drug was dissolved in PBS (pH 7.4).
244
245 During sample collection, the entire receptor phase of each cell was removed at 20, 40, 60, 80 and 100
246 min and at 2, 4, 6, 8, 10 and 12 h. After removal, the receptor phase was immediately replaced with
247 an equal volume of fresh PBS (pH 7.4), also kept at 37 °C, to maintain sink conditions and the
248 concentration of the drug in the removed receptor phase was analyzed by HPLC.
249

250 ### 3.7 Selection of physicochemical properties

251 The choice of which physicochemical properties to investigate were partially based on the variables
252 used in the most popular QSPR models. These include the original (Eq. 3) and updated (Eq. 4)
253 models of Potts and Guy [56, 57], the model of Barratt (Eq. 5) [58], Lien and Gao (Eq. 6) [59] and
254 Abraham *et al*. (Eq. 7 and Eq. 8) [60, 61]:

$$\log K_p = 0.71 \log P - 0.0061MW - 6.3 \quad (3)$$

$$\log K_p = 0.0256MV - 1.72\sum\alpha_2^H - 3.93\sum\beta_2^H - 4.85 \quad (4)$$

$$\log K_p = 0.82 \log P - 0.0093MV - 0.039MP - 5.9163 \quad (5)$$

$$\log K_p = 0.84 \log P - 0.07 \log P^2 - 0.27H_b - 1.84 \log MW + 0.8337 \quad (6)$$

$$\log K_p = -0.59\pi_2^H - 0.63\sum\alpha_2^H - 3.48\sum\beta_2^H + 1.79V_x - 5.05 \quad (7)$$

$$\log K_p = 0.44R^2 - 0.49\pi_2^H - 1.48\sum\alpha_2^H - 3.44\sum\beta_2^H + 1.94V_x - 5.13 \quad (8)$$

255 Where log P is the octanol-water partition coefficient, MW is the molecular weight, MV the
256 molecular volume, MP the melting point, H_b is the number of hydrogen bonds, π_2^H the dipolarity,

$\sum\alpha_2^H$ the solute hydrogen bond acidity, $\sum\beta_2^H$ the solute hydrogen bond basicity and V_x is the McGowan characteristic molecular volume and R^2 is the excess molar refraction.

In this study, we used some of the variables mentioned above, e.g. log P, MW, MV, MP, but substituted H_b, $\sum\alpha_2^H$ and , $\sum\beta_2^H$ with TPSA (topological polar surface area) as a measure of the surface polarizability of the molecule. Molecular polar surface area (PSA) and TPSA are essentially the same, and differ mainly in the method of calculation, as described in Ertl *et al*, [62]. Since TPSA is the most efficient in terms of computing time, and has become a common subroutine in many cheminformatics platforms, notably ChemAxon (http://www.chemaxon.com) and Molinspiration Cheminformatics (http://www.molinspiration.com), we opted to use TPSA. Both TPSA and MV are abstract metrics that must be calculated *in silico*. In recent years these two metrics have shown good correlations with bioavailability, especially the polar surface area [62-65]. The pKa values were also included in this investigation, since the degree of ionization can influence the permeability of a drug [66]. Some of the drugs used in this study had more than one pKa value. In those cases the isoelectric point was used instead, but was still referred to in the text as pKa. Although pKa and the isoelectric point do not refer to the same thing, they were only used as an indicator of the shift in the distribution of charge in the sample at a certain pH.

The databases used to collect values of the physicochemical properties mentioned above were DrugBank version 4.2 [67], ZINC [68], ChEMBL [69], PubChem [70], IUPHAR/BPS [71], ChemSpider [72] and ChemBank [73]. The training set constructed from the results of the skin permeation studies and the physicochemical properties collected from the above-mentioned databases are presented here in Table 1.

3.8 Statistical software packages

All data analyses, discretization and network structure learning were performed using R version 3.1.3 (2015-03-09), the R Foundation for Statistical Computing, Vienna, Austria, (http://www.R-project.org) and the "bnlearn" package [51] in R.

3.9 Data discretization

Hartemink's Information-Preserving Discretization (IPD) [52] was used to discretize the data in this study.

3.10 Network cross-validation and structure learning

The network structure was cross-validated by fitting the discretized data to network structures learned from several constraint- and score-based algorithms, and evaluating the log-likelihood loss. The constraint-based algorithms used were the Grow-Shrink (GS) algorithm [74] and the Incremental Association Markov Blanket (IAMB) algorithm [75]. Score-based algorithms used were the hill-climbing algorithm with random restarts and the tabu search algorithm [76]. The resulting networks were scored using the Bayesian Dirichlet equivalent (BDe) [77] and the Bayesian Information Criterion (BIC).

The structure learning algorithm that resulted in the least log-likelihood loss was then repeated several times and the resulting networks were averaged [78] to produce a more robust, single averaged network containing only the arcs present in more than 85% of the networks .

4 Results and Discussion

Since the normality assumptions required by Gaussian Bayesian networks did not hold in the current training set, the data was discretized using Hartemink's IPD algorithm. The results of the data discretization are presented here in Table 2. Allocation of names to the different ranges in which the data were discretized was arbitrary, based on the entries in the dataset. The log-likelihood loss was

minimized by learning the network structure with the tabu search algorithm, scored with the BDe and including an imaginary sample size of 10. Bootstrap resampling was done using these structure learning parameters to learn a set of 1000 network structures. Arcs between the variables were considered significant if they appeared in at least 85% of the networks and most frequently in the same direction. The averaged networks for the datasets containing the drug dissolved in PBS and dispersed in Pheroid™ are presented here in Figures 1 and 2, respectively. The cumulative amount of drug, dissolved in PBS, which permeated the skin after 12 h, is denoted as CPBS. Similarly CPher refers to the cumulative amount of drug, dispersed in Pheroid™. The strongest arcs in both networks were: from MW to MV, MW to pKa, MP to pKa, pKa to log P, TPSA to log P, MP to cumulative amount permeated (CPBS and CPher) and TPSA to cumulative amount permeated (CPBS and CPher).

The Bayesian networks were successfully able to distinguish between skin permeation results obtained from drugs dissolved in a buffer and dispersed drugs in a lipid-based delivery system. This is seen most clearly in the probabilistic dependence between pKa and CPBS in Figure 1, where the drugs were fully dissolved in PBS at a pH of 7.4. This dependency does not exist for the network learned from the permeation data obtained from drug dispersed in the lipid micelles of the Pheroid™. Also, the probabilistic dependencies seen in Figure 1 bear a striking resemblance to the QSPR models presented in Section 3.7, all of which were constructed using the Flynn dataset [47], which consists mostly of data collected from aqueous solutions, and some ethanolic solutions. The absence of an arc between log P and permeation was initially surprising, especially considering the sheer volume of literature where log P had been used as a predictor of skin permeability. However, careful experimentation by Goodwin *et al*. [79] have indicated that log P itself is actually a measure of MV, or surface area, and hydrogen bond acceptor potential, and that these latter variables are the ones that contribute to permeability. This observation is consistent with the learned network structures presented here. Similar findings have been reported by Abraham *et al*. [61] and were used to construct the models in Eq. 7 and 8. This was not an easy correlation to make, and stands in testimony to Bayesian networks' ability to identify underlying causal relationships between variables.

The networks were also able to identify MV and TPSA as independent variables. As stated in Section 3.7, PSA and TPSA differ in the method of calculation. While PSA is calculated directly from the volume of the molecule, TPSA is calculated by summing tabulated surface contributions of polar fragments. In the network presented in Figure 2, we have an arc from MV to TPSA. The arc is included to counterbalance the shared effects and indicate that, should CPher and log P be omitted, there is an underlying dependency between MV and TPSA in this dataset. Similarly, the arcs between TPSA and MW and TPSA and pKa in Figure 1 were introduced by the conditional dependence brought about by the shared effect, cumulative permeated amount (CPBS and CPher).

The relationship between MW and pKa is well known. Using the principles of stoichiometry to find the MW of an unknown acid from its pKa value is common practice, and similar results hold for MW and the isoelectric point [80, 81]. The networks were also able to identify the probabilistic dependency between pKa and log P, which is consistent with our knowledge that the concentration of ionized drug will influence the extent to which the drug can partition between oil and water phases. In the dataset used in this study, there was also a strong linear correlation between MW and MV, ρ = 0.93. The relationship between MP and pKa is not as well documented as those described until now. From prior knowledge on pKa values and the relationship between MP and the functional groups in a compound, we would expect crystals of compounds with highly polarizable functional groups, e.g. carboxylic acid or amine groups, to have higher melting points because of stronger intermolecular interactions [82]. The same functional groups that increase the melting points of crystals are also responsible for very high or low pKa values. Performing a conditional probability query on the pKa node of Figure 1, we find the probability of a drug in our dataset having a pKa = (5.96, 8.80], given that MW = (254, 358], TPSA = (32.00, 54.37] and MP = (58, 170] is 0.875, while the probability of a drug having a low pKa value, pKa = (3.50, 5.96], given that same ranges of MW, TPSA and MP is 0. If we maintain the ranges of MW and TPSA, but increase the range of MP to (170, 266], we find P(pKa = (3.50, 5.96] | MW,TPSA,MP) = 1. Similar results hold if we increase the range of MP to (266, 286]. Performing a conditional probability query on the pKa node in Figure 2, where the arc

365 from TPSA to pKa is missing, we find a similar trend in pKa with an increase in MP. The
366 probabilistic dependencies between MP and pKa in these networks are therefore consistent with our
367 expectations, based on prior knowledge.
368
369 For the network learned from the permeation data of drugs dissolved in PBS, we find the probability
370 that CPBS will be in the range (1140, 1610], given TPSA = (32.00, 54.37], MW = (254, 358], MP =
371 (58, 170] and pKa = (5.96, 8.80], to be 0.929. This probability drops to 0.5 if the pKa range increases
372 to (8.80, 9.86] and to 0 if the pKa range decreases to (3.5, 5.96]. This suggests that the best
373 transdermal permeability is associated with a low TPSA, high MW (relative to the current dataset),
374 low MP and a pKa close to the pH of the buffer solution. This is consistent with previous results of
375 Magnusson *et al*. [18] that showed a correlation between lower MP values and higher permeabilities.
376 The negative coefficient in Eq. 5 also suggests that permeability should increase as MP decreases.
377 Based on previous studies, we would also expect passive diffusion to increase with a decrease in
378 TPSA [63] and if the compound exists in a balance between ionized and unionized species at
379 experimental pH [83]. A conditional probability query on the CPher node in Figure 2 indicates that
380 the best transdermal delivery for drugs dispersed in Pheroid™ was obtained from the same TPSA and
381 MP ranges as the dissolved drug, and an MV range of (233.6, 277]. In both networks, the lowest
382 transdermal permeabilities (CPBS = (0.0, 181.0], CPher = (0.0, 53.7]) were observed for drugs with
383 medium range TPSA values (TPSA = (54.37, 87.96]), high MP (MP = (266, 286]) and small
384 molecular size descriptors (MW = (130, 137], MV = (93.6, 120.4]), and a pKa range of (5.96, 8.80]
385 for the network in Figure 1. This observation is consistent with our discussion thus far, since an
386 increase in MP is associated with an increase in polarizability, smaller molecules may be more
387 affected by polarization than larger molecules, simply because of the total size of the molecule
388 relative to the number of its polarizable functional group(s) contributing to its surface charge. The
389 extent of which is limited by the TPSA of the molecule. Therefore, the larger the ratio of TPSA to
390 molecular size the larger the total area of the drug's surface that can be polarized. These small yet
391 highly polarized molecules can undergo stronger interactions with the constituents of the stratum
392 corneum, slowing their permeation rates. The observation that skin permeability is dependent on the

393 ratio of TPSA to molecular size, in combination with specific MP ranges is novel, and might serve as
394 a more general classification tool, since it is independent of whether the drug was dissolved in a
395 buffer or dispersed in a lipid based delivery system.
396
397 To assist in visualizing these relationships, 3D scatterplots of the TPSA to molecular size ratio, MP
398 and cumulative amount of drug that permeated the skin after 12h are presented here in Figure 3. The
399 plots show the favorable regions identified by the Bayesian networks in the ranges of TPSA to
400 molecular size ratio < 0.3 and MP < 200°C. Above a MP of 200°C there is a marked decrease in
401 permeability regardless of the TPSA to molecular size ratio, at least in this dataset. We also see that,
402 in this dataset, there were no drugs with melting points below 150°C and TPSA to molecular size
403 ratios of above 0.3. This might be a general observation, although the ranges are likely to shift as the
404 dataset is expanded. Still, since a large TPSA to molecular size ratio is indicative of a molecule with
405 a large polar surface contribution relative to its total size, we would not expect such a molecule to
406 exhibit a low MP. There is also the possibility that such molecules may not exist in solid form at
407 standard temperature and pressure, e.g. water, chloroform, methanol etc. In these cases, MP may not
408 be an appropriate variable to use for classification. Thus far, no mention has been made regarding the
409 upper boundary for molecular size. This boundary has been well documented, and is considered to be
410 MW ~ 400 Da [20]. None of the drugs tested in this study had a MW > 400 Da, allowing us to focus
411 on the factors influencing skin permeability of drugs below this upper molecular size boundary.
412
413 The results suggest that Bayesian networks can serve as valuable decision making tools in the early
414 drug discovery and development processes. Let us consider, for example, that the molecular size,
415 TPSA and MP of a drug candidate falls within the favorable ranges for transdermal delivery identified
416 here, but it's predicted pKa value is either too high or low, we now know that the permeability of a
417 drug dispersed in Pheroid™ is independent of its pKa value, and we may continue with the
418 development process, knowing that the final formulation should be in a lipid based delivery system
419 such as the Pheroid™.
420

5 Conclusion

In this study, we investigated the ability of Bayesian networks to learn the correct probabilistic dependencies between skin permeability and the physicochemical properties of selected drugs dissolved in PBS and dispersed in a lipid based drug delivery system. The skin permeability data were collected from within our research group, and the corresponding physicochemical properties were collected from several online bioinformatics and cheminformatics databases.

The Bayesian network structures learned from the training sets of dissolved and dispersed drugs were consistent with known relationships between physicochemical properties and also elucidated new causal relationships between these properties and skin permeability. The networks identified a probabilistic dependence between pKa and skin permeability for drugs dissolved in PBS at pH 7.4, which was not observed for the same drugs dispersed in the lipid micelles of the Pheroid™ drug delivery system. For both dissolved and dispersed drugs the same probabilistic dependencies existed between TPSA, MP and cumulative amount of drug that permeated the skin after 12 h (CPBS and CPher). Although both networks shared a causal relationship between permeability and molecular size descriptors, the network learned from the dissolved drugs displayed a probabilistic dependence between MW and permeability, while the dispersed drugs displayed a dependence between MV and permeability. Both networks identified similar physicochemical regions for optimal transdermal delivery, i.e. low MP, small TPSA to molecular size ratio and, in the case of the dissolved drugs, a pKa value close to the pH of the buffer solution. Regions for poor transdermal delivery were also identified, i.e. high MP and a TPSA to molecular size ratio close to 0.5. The results suggest that Bayesian networks learned from online bioinformatics and cheminformatics databases can be viable classification tools in early drug discovery and development, and can aid in the identification of suitable drug candidates and formulation strategies.

Future studies will include an expansion of the training set, to include more drugs, more formulations and different routes of administration, thereby constructing a set of Bayesian networks representing the probabilistic dependencies of different drug delivery strategies.

Acknowledgements

This work was carried out with the financial support of the National Research Foundation of South Africa (NRF) (Grants no. IFRR81178 and CPRR13091742482) and The Centre of Excellence for Pharmaceutical Sciences (Pharmacen) of the North-West University, Potchefstroom Campus, South Africa.

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668

Tables:

Table 1: The training set of drugs used in this study, their physicochemical properties and cumulative amounts diffused after 12 h presented as mean ± STD where available (n = 8)

Drug	MW (g/mol)	MV ($Å^3$)	TPSA ($Å^2$)	MP (°C)	log P	pKa	Permeation PBS (nmol/mL)	Permeation Pheroid™ (nmol/mL)
5-Fluorouracil	130.08	93.60	60.53 ± 4.438	283.00 ± 1.685	-0.59 ± 0.612	7.62 ± 0.509	17.0 ± 12.0	529.0 ± 257.0
Acyclovir	225.00	183.96	115.21 ± 1.632	254.00 ± 0.916	-1.50 ± 0.209	5.74 ± 0.233	7.4 ± 4.5	10.1 ± 5.2
Scopolamine	303.35	277.00	62.19 ± 0.155	59.00 ± 0.760	1.05 ± 0.612	7.49 ± 0.412	46.2 ± 9.9	21.4 ± 6.8
Lidocaine HCl	288.00	247.26	32.89 ± 1.348	159.50 ± 0.535	2.15 ± 0.580	7.83 ± 0.147	1428.4 ± 164.2	914.5 ± 250.5
Prilocaine HCl	256.80	229.37	42.15 ± 1.985	169.00 ± 0.926	2.30 ± 0.729	8.37 ± 0.467	1277.2 ± 136.5	1007.3 ± 328.5
Isoniazid	137.14	120.44	68.24 ± 0.656	160.00 ± 0.926	-0.74 ± 0.095	8.06 ± 0.434	49.8 ± 34.5	225.2 ± 149.8
Zalcitabine	211.22	183.91	88.45 ± 0.738	217.38 ± 0.518	-1.03 ± 0.399	4.96 ± 1.454	267.4 ± 296.3	227.8 ± 215.5
Lamivudine	229.25	186.04	97.27 ± 16.861	164.50 ± 4.660	-1.03 ± 0.252	8.36 ± 0.922	485.8 ± 454.7	139.1 ± 69.8
Ibuprofen	206.30	211.86	37.60 ± 0.976	76.00 ± 0.926	3.61 ± 0.190	4.54 ± 0.214	826.4 ± 87.7	622.4 ± 52.4
Ketoprofen	254.30	233.61	54.66 ± 0.956	93.38 ± 1.060	3.54 ± 0.645	4.22 ± 0.230	237.9 ± 26.7	372.4 ± 41.3
Na diclofenac	318.10	234.37	49.62 ± 0.969	281.00 ± 7.746	4.38 ± 0.160	4.16 ± 0.017	152.8 ± 48.7	83.0 ± 14.8

Indomethacin	357.80	300.90	69.43 ± 2.785	152.00 ± 1.600	3.66 ± 0.882	3.85 ± 0.156	0.000	0.000
Mefenamic acid	241.30	225.94	49.62 ± 0.969	230.88 ± 0.835	4.85 ± 0.431	4.08 ± 0.183	34.4 ± 2.5	9.9 ± 2.5
Acetaminophen	151.20	138.06	49.68 ± 1.277	169.50 ± 0.926	0.91 ± 0.511	9.45 ± 0.207	96.6 ± 59.8	105.2 ± 66.2

Table 2: Results of the data discretization and allocation of classifications

Variables	**Low**	**Medium**	**High**
MW	(130, 137]	(137, 254]	(254, 358]
MV	(93.6, 120.4]	(120.4, 233.6]	(233.6, 277]
TPSA	(32, 54.37]	(54.37, 87.96]	(87.96, 133]
MP	(58, 170]	(170, 266]	(266, 286]
log P	(-1.78, 0.987]	(0.987, 2.51]	(2.51, 5.40]
pKa	(3.50, 5.96]	(5.96, 8.80]	(8.80, 9.86]
CPBS	(0.0, 181]	(181, 1140]	(1140, 1610]
CPher	(0.0, 53.7]	(53.7, 234]	(234, 1640]

Figures:

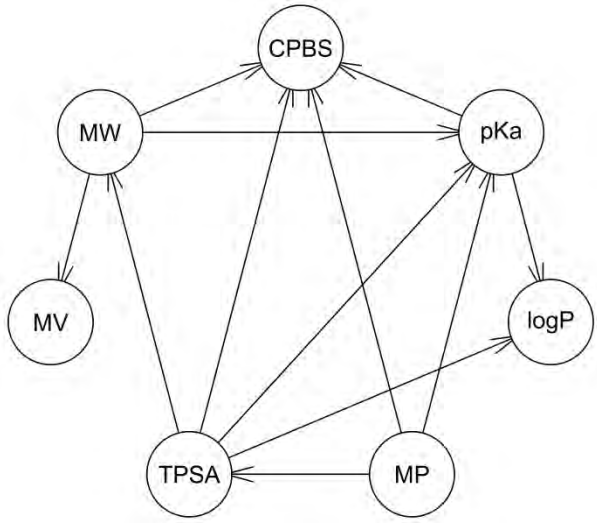

Figure 1: Averaged network of physicochemical properties and permeation data of drugs dissolved in PBS.

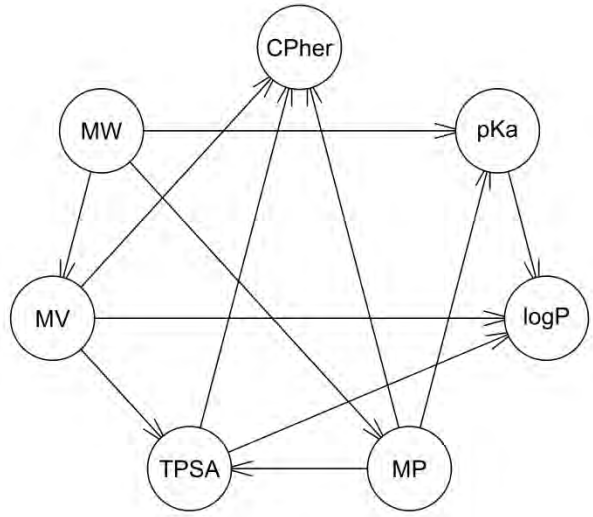

Figure 2: Averaged network of physicochemical properties and permeation data of drugs dispersed in Pheroid™.

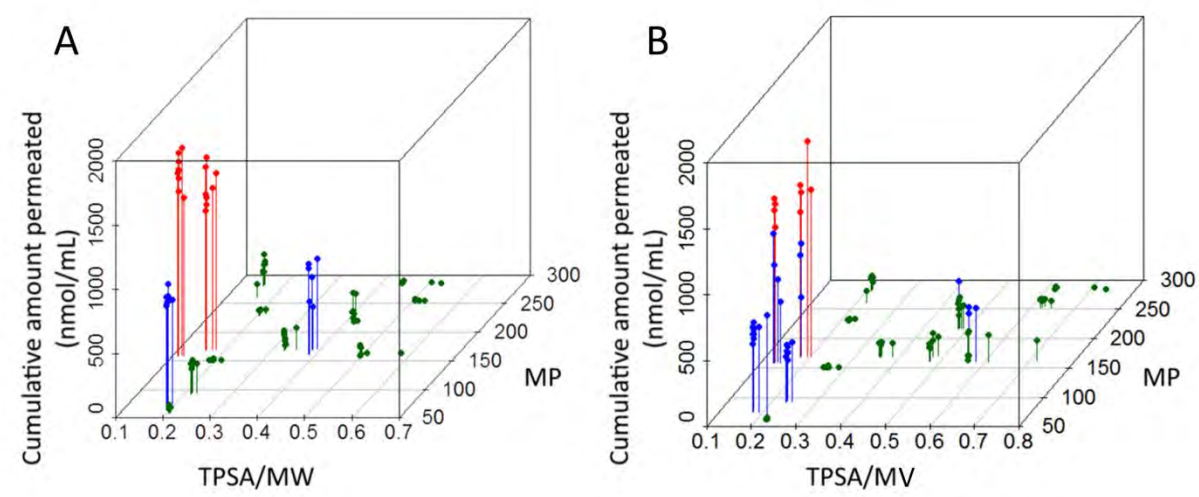

Figure 3: A) Scatterplot of the TPSA to MW ratio, MP and cumulative amount of permeated drug after 12h for the drugs dissolved in PBS. B) Scatterplot of the TPSA to MV ratio, MP and cumulative amount of permeated drug after 12h for the drugs dispersed in Pheroid™. Coloring: red indicates cumulative amount permeated ≥ 1000 nmol/mL, blue indicates 300 nmol/mL ≤ cumulative amount permeated < 1000 nmol/mL and dark green indicates cumulative amount permeated < 300 nmol/mL.

Chapter 5: Article for publication in Pharmaceutical Statistics

Chapter 5 is written in article format for the purpose of publication in Pharmaceutical Statistics. The complete author's guide is given in Annexure E. Please note that Chapter 5 is written in US English and not UK English and that all formatting is done according to the guide to authors. The text has also been justified to ease reading.

Exploratory data analysis of the dependencies between skin permeability, molecular weight and log P

D. Kilian, H.J.R. Lemmer, M. Gerber, J.L. du Preez, J. du Plessis*

Centre of Excellence for Pharmaceutical Sciences (Pharmacen), North-West University, Private Bag X6001, Potchefstroom 2520, South Africa

* Corresponding author. Tel.: +2718 299-2274; Fax: +2718 299-2225. E-mail address: Jeanetta.duPlessis@nwu.ac.za (J du Plessis).

Abstract

Molecular weight and log P remain the most frequently used physicochemical properties in models that predict skin permeability. However, several reports over the past two decades have suggested that predictions made by these models may not be sufficiently accurate. In this study, exploratory data analysis of the probabilistic dependencies between molecular weight, log P and log K_p was performed on a dataset constructed from the combination of several popular datasets. The results suggest that, in general, molecular weight and log P are poorly correlated to log K_p. However, after employing several exploratory data analysis techniques, regions within the dataset of statistically significant dependencies were identified. As an example of the possible applicability of the information extracted from the exploratory data analyses, a multiple linear regression model was constructed, bounded by the ranges of dependence. This model gave reasonable approximations to log K_p values obtained from skin permeability studies of selected non-steroidal ant-inflammatory drugs (NSAIDs) administered from a buffer solution and a lipid-based drug delivery system. Knowing the ranges within which molecular weight and log P are statistically related to log K_p can

supplement existing methods of screening, risk analysis or early drug development decision making to add confidence to predictions made regarding skin permeability.

Keywords: Transdermal drug delivery, Exploratory data analysis, Multivariate analysis, Physicochemical properties, Pheroid™

1. Introduction

Over the past 50 years, *in vitro* skin permeability studies had become a major research topic in various fields, ranging from pharmaceutical sciences to cosmetics and risk assessment (Fitzpatrick et al 2004; Hadgraft and Lane 2005; Shen et al. 2014). From a pharmaceutics perspective, transdermal delivery offers an attractive alternative to other routes of administration. Advantages include the ease of application and elimination of gastric side-effects, both of which can improve patient compliance, as well as bypassing of the first-pass metabolic effect (Hadgraft and Lane 2005). The latter can potentially lower the amount of drug needed to elicit the same therapeutic response obtained from other routes of administration, which may in turn decrease the cost of the formulation and potentially increase the availability of drugs to aid organizations and poorer countries.

One of the most important factors that determine a drug's ability to permeate the skin is its physiochemical properties. These, along with the drug's biopharmaceutical properties, are used extensively in drug discovery and development decision making (Bharate and Vishwakarma 2013). Popular classification systems for selecting favorable drug candidates for oral delivery include the biopharmaceutical classification system (BCS) and Lipinski's rule of five (Amidon et al. 1995, Lipinski et al. 2001). However, when it comes to transdermal delivery, reaching an agreement on which physicochemical properties, and in

what ranges, to use for classification becomes more complicated. Molecular weight (MW), aqueous solubility, melting point and oil/water partition coefficient (log P) have been suggested as important indicators of skin permeability (Roy 1997), with nondescriptive "cut-off" points, like a MW of less than 600 Da (Daltons), good solubility in both oil and water, and a low melting point (Barry 2001). Referring back to Lipinski's rule of five, we find that this classification system selects favorable drug candidates based on the following criteria: (1) no more than 5 H-bond donors, (2) at most 10 H-bond acceptors, (3) a maximum MW of 500 Da and (4) a log P value not higher than 5. Because the stratum corneum forms a more formidable barrier than the intestinal epithelium, some studies caution against the direct use of the rule of five to identify potential drug candidates for transdermal delivery (Choy 2011). Most authors, however, agree that the rule of five is still applicable to transdermal delivery, as long as some restrictions are placed on the physicochemical properties' selection criteria. These restrictions include the addition of a lower boundary for the log P values, i.e. log $P \geq 0$, with optimal values at around 2 to 3, as well as a lowering of the upper boundary of MW to between 400 and 500 Da, with ideal values below 400 Da and optimal values at around 150 Da (Choy 2011; Bos and Meinhardi 2000; Magnusson et al. 2004; Wiedersberg and Guy 2014).

In the absence of definitive physicochemical property ranges for classifying potential drug candidates, most researchers turn to the skin permeability constant, K_p, as an indicator of expected skin permeability. Mathematical methods that predict K_p include quantitative structure-permeability relationship (QSPR) models, artificial neural networks, random walks and mechanistic models (Chen et al. 1993; Frasch 2002; Mitragotri 2002). QSPR models specifically have seen extensive use in transdermal permeability studies. From the early to mid-1990's, several of these multiple linear regression models were developed in an attempt to estimate the K_p value of a chemical entity directly from certain physicochemical properties,

most commonly molecular size and log P (Abraham et al. 1995; Barratt 1995; Lien and Gao 1995; Potts and Guy 1992; Potts and Guy 1995; Wilscut et al. 1995;). Numerous QSPR models have been developed since then, and are still being developed, mainly because of the poor predictions associated with applying these models to datasets other than the ones used to derive them. In a study by Lian et al. (2008) the predictive accuracy of several popular QSPR models, and a more recent mechanistic model, was investigated using a dataset containing experimental skin permeability results of 124 compounds. Some of the models returned R^2 values as low as 0.364 and 0.463, while R^2 values as low as 0.159 were reported when restricting the dataset to hydrophobic solutes. It should also be noted that even in the original QSPR model by Potts and Guy (1992) only about two thirds of the variance in skin permeability data could be explained by the model (R^2 = 0.67). However, a detailed discussion on the mathematical and statistical methods used to derive and validate these models is not the purpose of this study. Rather, we will take the two structural descriptors most frequently used in transdermal permeability studies, i.e. molecular size and log P, and investigate the probabilistic dependencies between these variables and the skin permeability constant.

In this study, MW was used as the molecular size descriptor, since it is easily obtained and frequently used. By combining datasets from several previous studies, a dataset larger than any previously reported was constructed. Several exploratory data analysis techniques were used to extract information from this dataset. In the process, new insights regarding the probabilistic dependencies between the skin permeability constant, MW and log P were obtained. From the exploratory data analysis, subsets of probabilistically dependent data points were identified. As an example of how the information gathered from the exploratory data analyses can be used, a multiple linear regression model was proposed that could estimate log K_p from MW and log P without assumptions about Fickian laws. Finally, a skin

97 permeability study of selected non-steroidal anti-inflammatory drugs (NSAIDs), dissolved in
98 phosphate buffer solution (PBS), and dispersed in a lipid-based drug delivery system, was
99 conducted. The log K_p values were calculated and tested against those predicted from the
100 multiple linear regression model. The Pheroid™ drug delivery system was used for this
101 study. The Pheroid™ is a colloidal delivery system, consisting of essential and plant fatty
102 acids that, for the current study, had been emulsified in PBS saturated with nitrous oxide.
103 Although the ability of the Pheroid™ to entrap and deliver drugs over different biological
104 membranes has been reported (Du Plessis et al 2010), previous investigations by our group
105 into the influence of the Pheroid™ on the importance of NSAIDs' physicochemical
106 properties regarding transdermal permeability were inconclusive.

107

108 **2. Investigations and results**

109 The correlation matrices of the individual and combined datasets are presented here in Table
110 1. The results suggest that MW and log P are not strongly correlated to log K_p, and may not
111 be the best choice of independent variables in quantitative models. Yet, these two variables
112 are used far more frequently than any other. The reason for this can be traced back to
113 assumptions made while deriving the first QSPR models (Potts and Guy 1992). Similar
114 assumptions can also be found in mechanistic models, where the so called "brick and mortar"
115 model of the skin is employed (Micheals et al. 1975; Mitragotri 2002). These models are all
116 based on the steady state flux formula of Fick's first law of diffusion:

$$J_{ss} = \frac{D\nabla C_s}{h} \quad (1)$$

Where J_{ss} is the steady state flux, D the diffusion coefficient, ∇C_s is the molar concentration gradient across the skin and h is the path length. The skin permeability constant, K_p, can then be expressed as:

$$K_p = \frac{J_{ss}}{\nabla C_v} = \frac{\nabla C_s D}{\nabla C_v h} = \frac{K_{mv} D}{h} \tag{2}$$

Where K_{mv} is the membrane/vehicle partition coefficient. Based on the assumption that the solute concentration is essentially zero on one side of the skin, K_{mv} is calculated simply as the ratio of solute concentration in the skin to the solute concentration in the vehicle, $K_{mv} = C_s/C_v$. The next simplifying assumption is that K_{mv} can be substituted with K_{ow}, the octanol/water partition coefficient. K_{ow} may be much easier to calculate, but whether the partitioning of a drug between octanol and water is similar to its partitioning between the skin and water remains debatable. Since D can also be difficult to calculate, a further assumption is made that D can be expressed as a negative exponential function of MW alone. This assumption cannot account for chemical and physical interactions between the diffusing molecules and the constituents of the stratum corneum, which results in viscous drag. Incorporating these assumptions into Eq. 2, and performing a logarithmic transformation, gives the general form of the QSPR models as

$$\log K_p = \alpha \log K_{ow} - \beta MW + \log \frac{D_0}{h} \tag{3}$$

Where D_0 is the diffusivity of a hypothetical infinitesimally small (zero volume) molecule, α and β are constants and log K_{ow} is the same as log P. Similarly, the general form of mechanistic models can be expressed as

$$K_p = \frac{D_{lip} K_{mv}}{\tau h} = K_{ow}^n \cdot \exp(-\beta r^2) \cdot \frac{D_0}{\tau h} \tag{4}$$

Where τ is the effective tortuosity, r is the molecular radius and K_{ow} is related to K_{mv} through $K_{mv} = K_{ow}^{n}$, where n can vary between 0.7 and 0.86. Despite the shortcomings of the simplifying assumptions described above, and the poor correlations seen from the correlation matrices, log P and MW remain the mainstay structural descriptors used by pharmaceutical scientists for transdermal permeability predictions. We will therefore investigate whether we can still extract valuable skin permeability information from these two variables by applying the appropriate statistical methods.

To assist in visualizing the data contained in the correlation matrices, scatterplot matrices of the datasets are presented here in Figure 1. The cells on the main diagonals contain the smoothed histograms of the corresponding random variables. From this visual representation of the datasets, several interesting observations emerged. First, the distributions of the molecular weights of substances tested for transdermal delivery were consistently positively skewed. Also, despite several previous papers claiming an upper cut-off boundary for MW to be 500 Da, we see that very little data is actually available above this region. Whether this is the result of insufficient data, or the choice of the original authors not to include such compounds in their datasets, is uncertain. Probably the most interesting, and troubling, observation is that the increase in dataset size elucidated a bimodal distribution in log K_p, suggesting that the same ranges of MW and log P that correspond to "good" log K_p values also correspond to "poor" ones.

Because of the severe departure from normality in the data, it was decided to change the data from continuous to ordinal categorical variables. Initial attempts to discretize the data using Hartemink's Information-Preserving Discretization (IPD) algorithm were unsuccessful (Hartemink 2001). The spread of the data lead to disproportionately large size allocations to areas where the data was sparse, while allocating small regions to areas where the data was clustered. It was therefore decided to cut the continuous data into intervals, chosen *a priori*,

160 based on literature described in the introduction and prior knowledge of the field. The
161 regions into which the data was split, along with each region's ordinal value, are presented in
162 Table 2. Since a lower boundary for log P of 0 had been suggested, and optimal values to be
163 between 2 and 3, this was taken into account when deciding on the cut-off regions. Similarly,
164 an upper MW boundary of 400 Da and optimal values at around 150 Da had been reported,
165 and were incorporated in this study. Log K_p values above 1 correspond to exceptionally high
166 K_p values, and are not expected to have a high frequency of occurrence in nature, therefore
167 this cut-off value was chosen as a region. Also, log K_p values below 7 correspond to
168 extremely poor permeability and was used as a cut-off. For the middle regions of log K_p, the
169 cut-off values were chosen in such a way as to not introduce median splits in the data, which
170 can lead to unnecessary information loss (see Figure 1). A 3-way contingency table of the
171 combined dataset is presented in Table 3. To assist in visualizing this table, a mosaic plot of
172 the data was constructed (Figure 2). The mosaic plot was also used to test for independence.
173 Since the Pearson residuals are ~N(0,1) distributed, $\alpha = 0.05$ and $\alpha = 0.0001$ levels of
174 significance correspond to absolute residual values of 2 and 4, respectively. More than half
175 of the cells did not differ from the null model (expected values mosaic plot) by a statistically
176 significant margin, indicating that no statistically significant dependence exists among the
177 variables in these regions. Pearson's Chi-square test was subsequently used to test for
178 independence. The test statistic was significantly larger than the critical value, $\chi^2 > \chi^2_\alpha = 248$
179 > 97.351, allowing us to reject the null hypothesis at the 5% level of significance and
180 conclude that the variables are not independent. From the mosaic plot, we see that the
181 probability of observing good permeability constants, log $K_p = (3, 1]$, are significantly higher
182 than expected for MW = (100, 200] Da, log P = (2, 4] and MW $\leq$ 100 Da, log P = (0, 2].
183 Also, the probability of observing log K_p values > 1 is highly unlikely, with a marginal
184 probability of only 0.0114. Therefore, when looking for MW and log P classification regions

for good skin permeability, a realistic region would be log K_p = (3, 1]. As mentioned above, the mosaic plot already showed that the regions, MW = (100, 200] Da, log P = (2, 4] and MW ≤ 100 Da, log P = (0, 2], are suitable for this classification.

If we let B denote the event where a drug displayed a log K_p value within one of the regions given in Table 2, such that the sample space Ω can be given by $\bigcup_{i=1}^{5} B_i = \Omega$, and let A denote the event where a drug had a MW and log P within the regions specified, then the law of total probability gives

$$P(A) = \sum_{i=1}^{5} P(A|B_i)P(B_i) \tag{5}$$

The conditional probability of observing a drug within the region log K_p = 4, given that it had MW and log P values within the regions, MW = 1, log P = 2, was calculated using Bayes' Rule:

$$P(B_4|A) = \frac{P(A|B_4)P(B_4)}{\sum_{i=1}^{5} P(A|B_i)P(B_i)} \tag{6}$$

The conditional probability obtained was 0.425. Similarly, the conditional probability of observing a drug within the region log K_p = 4, given that it had MW and log P values within the regions, MW = 2, log P = 3, was calculated to be 0.185, which is much lower than expected. The reason for this is that 2 of the five drugs that displayed log K_p values > 1 were within this MW and log P region, giving $P(A|B_5)$ = 0.4. If not for this, the MW and log P regions, MW = 2, log P = 3, might be considered equal or better than MW = 1, log P = 2, since P(log K_p = 4 | MW = 2, log P = 3) = 0.299 whereas P(log K_p = 4 | MW = 1, log P = 2) = 0.233. To address this issue, a multiple correspondence analysis was performed on the dataset.

For brevity, the results from the correspondence analysis were included in the supplementary information and only summarized results and the correspondence map (Figure 3) will be discussed here. The total inertia was larger than the χ^2 critical value at the 5% level of significance, allowing us to conclude that the variables are not independent. This observation is consistent with the results from the χ^2 test for independence from the contingency table (Table 2). From the correspondence map we can see that no variable differs significantly, as a group, from the other variables. MW made the largest contribution to the total inertia, and the first axis opposes medium to large MWs to MWs smaller than 100 Da. The second axis opposes medium regions of log P to large and negative regions, and also opposes good log K_p regions from poor regions. The correspondence map suggests that the frequency of observing good skin permeability constants, log K_p = 4 and 5, are higher than expected in the regions MW = 2 and log P = 3, respectively. Also, poor skin permeability constant values, log K_p = 2, are observed more frequently in high MW regions, MW = 5, while the poorest values, log K_p = 1, are observed more frequently than expected with negative log P values. These isolated occurrences of higher than average observation frequency tendencies are consistent with those observed from the mosaic plot. As we might expect from the discussion thus far, the identification of clusters among groups of individual variables from the correspondence map was not straight forward.

To investigate clustering, we returned to the original dataset. Because of the severe departure from normality in the distributions of MW and log K_p, it was decided not to use model-based clustering. Instead, we investigated clustering via nonparametric density estimation (Azzalini 2007, Menardi 2014). Density estimation was done using the kernel method and calculated by a product estimator:

$$\hat{f}(y) = \sum_{i=1}^{n} \frac{1}{n h_{i,1} \cdots h_{i,d}} \prod_{j=1}^{d} K\left(\frac{y_j - x_{i,j}}{h_{i,j}}\right) \tag{7}$$

Where K is the kernel function and h_i is the bandwidth, which can either be fixed, $h_i = h$, or adaptive, $h_i = (h_{i,1} \cdots h_{i,d})^{\mathrm{T}}$. The data was then clustered using the block-sequential criterion of allocation proposed by Azzalini and Torelli (2007), where for a fixed number of stages, K, an estimated density, $f_m(x_u)$, of the unallocated data x_u was computed, based only on the data already allocated to group m, using the log-ratios:

$$r_m(x_u) = \log \frac{\hat{f}_m(x_u)}{\max\limits_{l \neq m} \hat{f}_l(x_u)}, \qquad m = 1,2,\cdots,M \tag{8}$$

The density-based silhouette (*bds*) was used as a diagnostic tool to assess the quality of the clusters obtained from Eq. 8, and is defined as

$$dbs_i = \frac{\log\left(\frac{\hat{\tau}_{m0}(x_i)}{\hat{\tau}_{m1}(x_i)}\right)}{\max\limits_{x_i} \left|\log\left(\frac{\hat{\tau}_{m0}(x_i)}{\hat{\tau}_{m1}(x_i)}\right)\right|} \tag{9}$$

Where m_0 is the group to which x_i has been allocated and m_1 is the group for which $\tau_m(x_i)$ takes the second largest value. Therefore, large values of *dbs* are evidence of a well clustered data point, while small values indicate low confidence in the classification. The results of the cluster analysis are presented in Figure 4. The three clustering techniques employed were spatial tessellation with fixed and adaptive bandwidths and pairwise connections. The least amount of misclassified points was obtained from the adaptive bandwidth spatial tessellation technique, and therefore that technique was chosen for this study. A detailed list of which drugs in the dataset were classified into what groups can be found in the supplementary information. The diagnostic plot (Figure 4.D) showed *dbs* values appreciably higher than zero, suggesting that the partitions identified were sound. Furthermore, from the six groups

identified, the smallest dissimilarity was between groups 1 and 2. These groups consisted of molecules with MW averages of 128.18 ± 65.693 and 135.06 ± 116.327 Da, log P averages of 1.57 ± 1.486 and 1.01 ± 1.54, and log K_p averages of -2.36 ± 1.113 and -2.76 ± 0.942 for groups 1 and 2, respectively.

The results from the exploratory data analyses presented in this paper suggest that, as a whole, molecular weight and log P are poorly correlated with the skin permeability constant, and might not be the best choice for predictive models. However, statistically significant probabilistic dependencies between molecular weight, log P and log K_p do exist within specific regions, and mutual information can be extracted from these variables using the appropriate exploratory statistical analyses. Specific regions of dependence was identified and, although some of these ranges may seem similar to those mentioned in the introduction, the addition of log K_p ranges in which the probabilistic dependencies hold are novel. Since the applicability of the knowledge gained from the exploratory data analyses is important, we returned once more to the topic of multiple linear regression.

Quantitative forecasting models remain an attractive predictive technique, due to its usability by a wide range of scientists regardless of their mathematical proficiency. The clade of groups 1 and 2 was used to construct a smaller dataset, this time consisting only of variable ranges within which the variables are probabilistically dependent at a statistically significant level. The ranges of these variables were MW = [18, 336.47], log P = [-2.26, 4.57] and log K_p = [-5.22 -0.85]. From the discussion thus far, we see that these ranges coincide with regions where dependencies between the variables have been shown to exist. Employing least squares techniques on this new dataset gave the following multiple linear regression model:

$$\log K_p = 0.739 \log P - 0.0089MW - 2.36 \quad (10)$$

The coefficients of determination for the model were $R^2 = 0.856$ and adjusted $R^2 = 0.854$. Details on the model fitting diagnostics can be found in the supplementary information. The only model assumption not entirely satisfied was the normality of the residues, which were slight positively skewed. It should be noted that this model is not complete. As stated earlier, at least one other variable related to the extent of each compound's interaction with the skin should be added, e.g. viscous drag. However, the objective of this paper was only the investigation of the relationships between MW, log P and log K_p. The model presented in Eq. 10 is not a QSPR model in the general sense, since it was not based on Fickian laws or assumptions, but was rather a statistical predictive model obtained from multiple linear regression using two readily accessible physicochemical properties and based on the notion that MW and log P may contain valuable information regarding the skin permeability capacity of a chemical entity.

The NSAIDs used in this study, their physicochemical properties and experimentally determined log K_p values are presented in Table 4. The model presented in Eq. 10 was used to predict log K_p values for these NSAIDs. As a reference, the QSPR model of Potts and Guy (1992), abbreviated "P&G" in Table 4, was also used to predict log K_p values, since it contained the same variables as the model in Eq. 10 and had been shown to be the most accurate QSPR model (Lian et al.2008). The predicted log K_p values were included in Table 4. The values predicted by Eq. 10 appear to be better approximations of the experimental values. The mean squared error of the estimates for experimental and predicted, using Eq. 10, were 0.79 and 1.564, for the NSAIDs dissolved in PBS and dispersed in Pheroid™, respectively. For the experimental log K_p values and those predicted using the Potts and Guy equation, the mean squared error of the estimates were 9.595 and 8.227, for the NSAIDs dissolved in PBS and dispersed in Pheroid™, respectively. To supplement the forecasting error statistics, a Wilcoxon rank-sum test was performed as a nonparametric test of group

differences. The results suggest that we may conclude at the 5% level of significance that the experimental log K_p values and those predicted from Eq. 10 were from identical populations, p-values = 0.4848 and 0.3095 for NSAIDs dissolved in PBS and dispersed in Pheroid™, respectively. We may also conclude at the 5% level of significance that the experimental log K_p values and those predicted from the Potts and Guy equation were from nonidentical populations, p-values = 0.0021 and 0.0043, respectively. From the skin permeation profiles of the different NSAIDs, Figure 5, we see that in each case the NSAID dispersed in Pheroid™ displayed poorer permeation than its dissolved counterpart, possibly due to differences in the solubilized state of the drug and/or the additional drug release step from the delivery system. Regardless of these differences, Eq. 10 was still able to predict log K_p with reasonable certainty. Since the regression model in Eq. 10 is linear in its coefficients, it is still possible that negative log P values can cause a loss in predictive accuracy.

The results suggest that valuable information regarding the dependencies between MW, log P and log K_p can indeed be extracted from large datasets and applied to predict skin permeability with reasonable certainty, as long as the investigation is restricted to regions of dependency. As seen from Table 4, the log P value of mefenamic acid was well outside the ranges of probabilistic dependencies used to construct Eq. 10, resulting in a poor prediction of log K_p.

3. Discussion

In this study, we investigated the probabilistic dependencies between log K_p and the two physicochemical properties most frequently used in skin permeability predictions, i.e. MW and log P. Initial observations based on a dataset constructed from several popular datasets used to construct and/or test predictive models, suggested that MW and log P are poorly

316 correlated with log K_p. However, exploratory data analyses indicated the existence of regions
317 of statistically significant probabilistic dependence between these variables. Based on these
318 observations, a cluster analysis was performed on the dataset and two similar clusters were
319 identified. The ranges of these clusters coincided with the regions of probabilistic
320 dependencies identified from other exploratory data analyses and the clusters were used to
321 construct a new, refined dataset. As an example of a possible application of the knowledge
322 gained from the exploratory data analyses, a multiple linear regression model was constructed
323 from this refined dataset and tested on experimental skin permeation data of selected
324 NSAIDs. The regression model presented in this work (Eq. 10) gave reasonable
325 approximations to experimental log K_p values.

326 Although MW and log P are not strongly correlated with log K_p, they remain the most
327 frequently used predictors of skin permeability, are easily accessible and calculable, and have
328 intuitive meaning in the transport process. By identifying and restricting investigations to
329 regions of statistically significant dependencies, valuable skin permeability information can
330 be extracted from these physicochemical properties, and the confidence in predictions made
331 regarding the skin permeability of compounds can be enhanced.

332

333 **4. Experimental**

334 **4.1. Materials**

335 The NSAIDs used in this study were donated by Adcock Ingram (South Africa). Analytical
336 grade methanol, ethanol and phosphoric acid, PEG-400, as well as sodium chloride, disodium
337 orthophosphate dehydrate, sodium dihydrogen orthophosphate dehydrate and dipotassium
338 hydrogen orthophosphate anhydrous were purchased from Merck Laboratory Supplies

339 (Midrand, South Africa). Double distilled deionized water was prepared with a Milli-Q water
340 purification system (Millipore, Milford, USA). HPLC grade water was used throughout the
341 study. Vitamin F ethyl ester was obtained from Chemimpo (Johannesburg, South Africa),
342 Cremophor® RH40 from BASF (Midrand, South Africa) and dl-α tocopherol from DSM
343 (Basel, Switzerland). The Pheroid™ used was prepared in-house by The Centre of
344 Excellence for Pharmaceutical Sciences (Pharmacen) at the North-West University
345 (Potchefstroom Campus, South Africa).

346 **4.2. HPLC analysis**

347 The high performance liquid chromatographic (HPLC) system used for the analysis was an
348 Agilent 1100 series, equipped with a variable wavelength ultraviolet (UV) detector, isocratic
349 pump, autosampler, and ChemStation (Rev. A.09.01 (1206)) data acquisition and analysis
350 software. All analyses were performed using HPLC grade water and reactants. The
351 temperature of the columns was kept at 25°C throughout the analysis. All HPLC analyses
352 were done, using a Phenomenex™ Luna 5 µ C_{18} (250 x 4.60 mm) column at a flow rate of 1
353 ml/min. All methods had previously been validated by the Analytical Technology
354 Laboratory at the North-West University (Potchefstroom Campus). The NSAIDs (10 mg
355 each) were accurately weighed and transferred into a 100 ml volumetric flask and made up to
356 volume with PBS (pH of 7.4) to produce a 100.0 µg/ml stock solution. Dilutions with
357 concentrations of 1.0; 2.5; 10.0; 25.0 and 100.0 µg/ml were prepared from the 100.0 µg/ml
358 stock solution and used to construct a standard curve for solubility studies.

359 The solubility of each API included in this study, was determined in PBS (pH 7.4). All of the
360 solubility determinations were done in triplicate. The Pheroid™ was prepared with PBS (pH
361 7.4) as dispersion medium. The samples were left to stir in a water bath at 32°C for 24 h
362 under supersaturated conditions. Each sample was then filtered through a 0.22 µm Millipore

363 filter at 32°C. The filtrate was then diluted and HPLC analysis was performed to determine
364 the solubility.

365 **4.3. Preparation of Pheroid™ vesicles**

366 The Pheroid™ vesicles were prepared in-house at Pharmacen (North-West University,
367 Potchefstroom Campus), according to a previously described method (Du Plessis et al. 2010).

368 **4.4. Skin permeation experiments**

369 Prior approval for the project, *In vitro* transdermal delivery of drugs through human skin, had
370 been granted by the North-West University Ethics Committee (reference number NWU-
371 00114-11-A5). Caucasian, female skin from informed consenting patients was obtained after
372 cosmetic surgery. To minimize permeability variations among different skin, only abdominal
373 skin was used. The full thickness skin was collected immediately after surgical removal and
374 was prepared within 24 h post-surgery. The skin was rinsed with deionized water and blotted
375 dry with clean tissue paper. To remove any residual fat from the subcutaneous fat layer and
376 surface sebaceous lipids, the skin was carefully wiped once with an ethanol moistened cotton
377 swab.

378 A skin layer 400 µm thick, including the stratum corneum, the viable epidermis and the upper
379 dermis, with a width of 2.5 cm, was cut using a dermatome (Zimmer Inc., Warsaw, IN,
380 USA). The prepared skin was placed dermal side down on filter paper. It was then wrapped
381 in aluminum foil and frozen at -20°C, until used. All skin samples were used within one
382 month after preparation. An hour prior to commencing with the permeation experiments, the
383 skin was removed from the freezer and thawed at room temperature and cut into circular
384 pieces, 15 mm in diameter.

385 Vertically mounted Franz diffusion cells, each with a donor capacity of 1.0 ml and receptor
386 capacity of 2.0 ml, were used. Preceding the permeation experiments, the integrity of the
387 skin to be used was tested by measuring the electrical resistance across it, using a Tinsley
388 LCR Databridge Model 6401 (Tinsley Precision Instruments, Croydon, United Kingdom) at 1
389 kHz, with a maximum voltage of 300 mV root-mean-square (rms) in the parallel equivalent
390 circuit mode, by employing an alternating current (Fasano et al. 2002). After completion of
391 the resistance measurements, the contents of the donor and receptor compartments were
392 removed and cells with resistances of less than 10 kΩ were rejected. The effective diffusion
393 area was calculated as 1.13 cm^2.

394 The PBS (pH 7.4) was degassed on an ultrasonic bath, prior to filling the receptor
395 compartment. Care was taken to ensure that no air bubbles were trapped in the compartment
396 or under the skin, as this would decrease the effective diffusion area. Each bottle of
397 Pheroid™ with entrapped NSAID, was removed from the fridge and placed in a water bath at
398 32°C under constant mechanical shaking prior to use.

399 The donor compartments were filled with 1 ml of Pheroid™ (experimental cells, n = 8), or 1
400 ml of the PBS (pH 7.4) solution (control cells, n = 7), each containing a different NSAID, and
401 covered with Parafilm™ to prevent evaporation. The Pheroid™ delivery system was
402 prepared by using the same dissolution medium as the control (PBS).

403 Sampling was done by removing the entire receptor phase at 20, 40, 60, 80 and 100 min, and
404 at 2, 4, 6, 8, 10 and 12 h. After extraction, the receptor phase was immediately replaced with
405 an equal volume of fresh PBS (pH 7.4), preheated to 37°C, to maintain sink conditions. The
406 concentration of the API that had penetrated the stratum corneum was recovered in the
407 receptor compartment and after sampling analyzed by HPLC.

408

4.5. The dataset

The dataset used in this study was constructed by combining the datasets of Lian et al. (2008), Moss and Cronin (2002), and Wilscut et al. (1995), giving a total dataset size of n = 437. Many researchers have expressed concerns regarding the validity of the steroid data contained in the Flynn dataset (1990). For this reason the dataset of Moss and Cronin (2002), which incorporates updated steroid data, was chosen for this study.

4.6. Statistical software and analyses

All data analyses were performed using R version 3.1.3 (2015-03-09), the R Foundation for Statistical Computing, Vienna, Austria, (http://www.R-project.org). Mosaic plots were constructed using the 'vcd' package in R and multivariate correspondence analysis was performed using the 'ca' package in R (Meyer et al. 2006; Zeileis et al. 2007; Nenadic and Greenacre 2007). Finally, cluster analysis was performed using the 'pdfCluster' package in R (Azzalini and Menardi 2014).

Acknowledgements

This work was carried out with the financial support of the National Research Foundation of South Africa (NRF) (Grants no. IFRR81178 and CPRR13091742482) and The Centre of Excellence for Pharmaceutical Sciences (Pharmacen) of the North-West University, Potchefstroom Campus, South Africa.

Disclaimer

Any opinion, findings and conclusions, or recommendations expressed in this material are those of the authors and therefore the NRF does not accept any liability in regard thereto.

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506

Tables

Table 1: Correlation matrices of the datasets used in this study, Lian *et al*. (**A**), Moss and Cronin (**B**), Wilscut *et al*. (**C**) and the combined set (**D**)

A

	MW	log P	log K_p
MW	1.0000	0.4085	-0.2852
log P	0.4085	1.0000	0.5983
log K_p	-0.2852	0.5983	1.0000

B

	MW	log P	log K_p
MW	1.0000	0.4548	-0.2515
log P	0.4548	1.0000	0.6214
log K_p	-0.2515	0.6214	1.0000

C

	MW	log P	log K_p
MW	1.0000	0.2617	-0.4248
log P	0.2617	1.0000	0.4913
log K_p	-0.4248	0.4913	1.0000

D

	MW	log P	log K_p
MW	1.0000	0.3716	-0.2105
log P	0.3716	1.0000	0.2783
log K_p	-0.2105	0.2783	1.0000

Table 2: The cut-off points for the data discretization and ordinal values attributed to each region

MW				
≤ 100	(100, 200]	(200, 300]	(300, 400]	> 400
1	2	3	4	5
log P				
≤ 0	(0, 2]	(2, 4]	> 4	
1	2	3	4	
log *Kp*				
≤ -7	(-7, -5]	(-5, -3]	(-3, -1]	> -1
1	2	3	4	5

515 **Table 3:** 3-way contingency table of the random variables used in this study

MW, log P, log K_p		log K_p					MW, log P
MW	log P	≤ -7	(-7, -5]	(-5, -3]	(-3, -1]	> -1	
	≤ 0	9	12	21	6	0	48
≤ 100	(0, 2]	0	16	5	36	0	57
	(2, 4]	0	0	3	1	1	5
	> 4	0	0	0	0	0	0
	≤ 0	9	2	2	0	0	13
(100, 200]	(0, 2]	4	29	7	30	1	71
	(2, 4]	0	19	17	46	2	84
	> 4	0	0	2	2	0	4
	≤ 0	0	1	0	1	0	2
(200, 300]	(0, 2]	7	4	9	2	0	22
	(2, 4]	0	13	8	9	0	30
	> 4	0	12	0	2	1	15
	≤ 0	1	2	1	0	0	4
(300, 400]	(0, 2]	9	3	9	0	0	21
	(2, 4]	1	5	6	7	0	19
	> 4	0	4	2	4	0	10
	≤ 0	1	1	1	0	0	3
> 400	(0, 2]	2	1	1	2	0	6
	(2, 4]	2	7	5	4	0	18
	> 4	0	1	2	2	0	5
log K_p		45	132	101	154	5	437

516

Table 4: Selected NSAIDs tested, their MWs, log P values, experimental and predicted skin permeability constants (in cm/s)

Drug	**MW (g/mol)**	**Log P**	**Experimental log K_p**		**Predicted log K_p**	
			PBS	**Pheroid™**	**P&G**	**Eq.10**
Ibuprofen	206.30	3.97	-1.262	-1.587	-4.739	-1.265
Ketoprofen	254.30	3.1	-1.424	-1.607	-5.650	-2.335
Sodium diclofenac	318.10	4	-2.373	-2.549	-5.400	-2.238
Mefenamic acid	241.30	5.12	-2.474	-3.162	-4.137	-0.727
Acetaminophen	151.20	0.64	-3.486	-3.318	-6.768	-3.234
Piroxicam	331.35	3.06	-3.931	-4.699	-6.149	-3.051

Figure captions

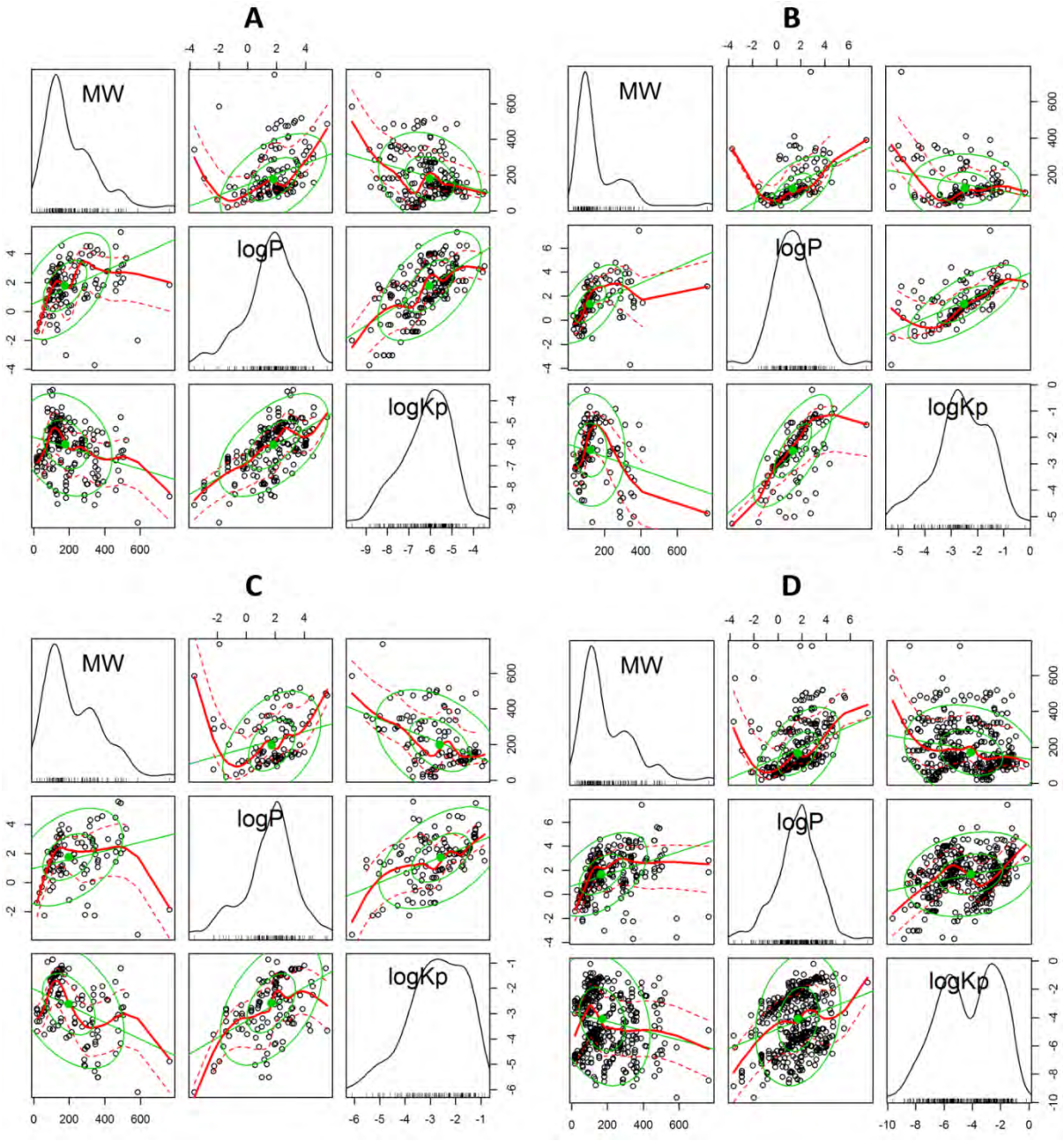

Figure 1: Scatterplot matrices of the datasets of Lian et al. (**A**), Moss and Cronin (**B**), Wilscut et al. (**C**) and the combined set (**D**).

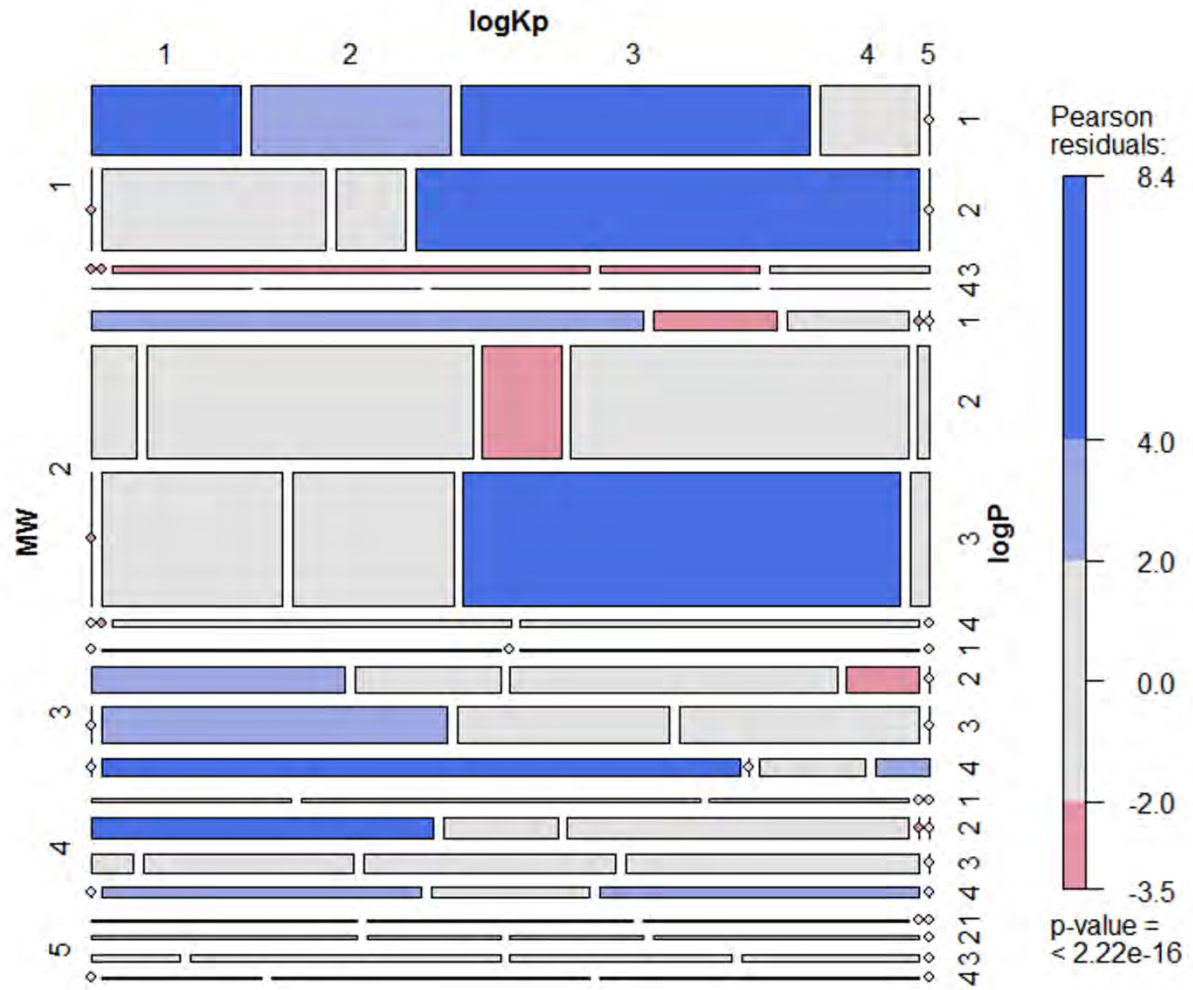

Figure 2: Mosaic plot of the combined dataset, shaded based on Pearson residual values.

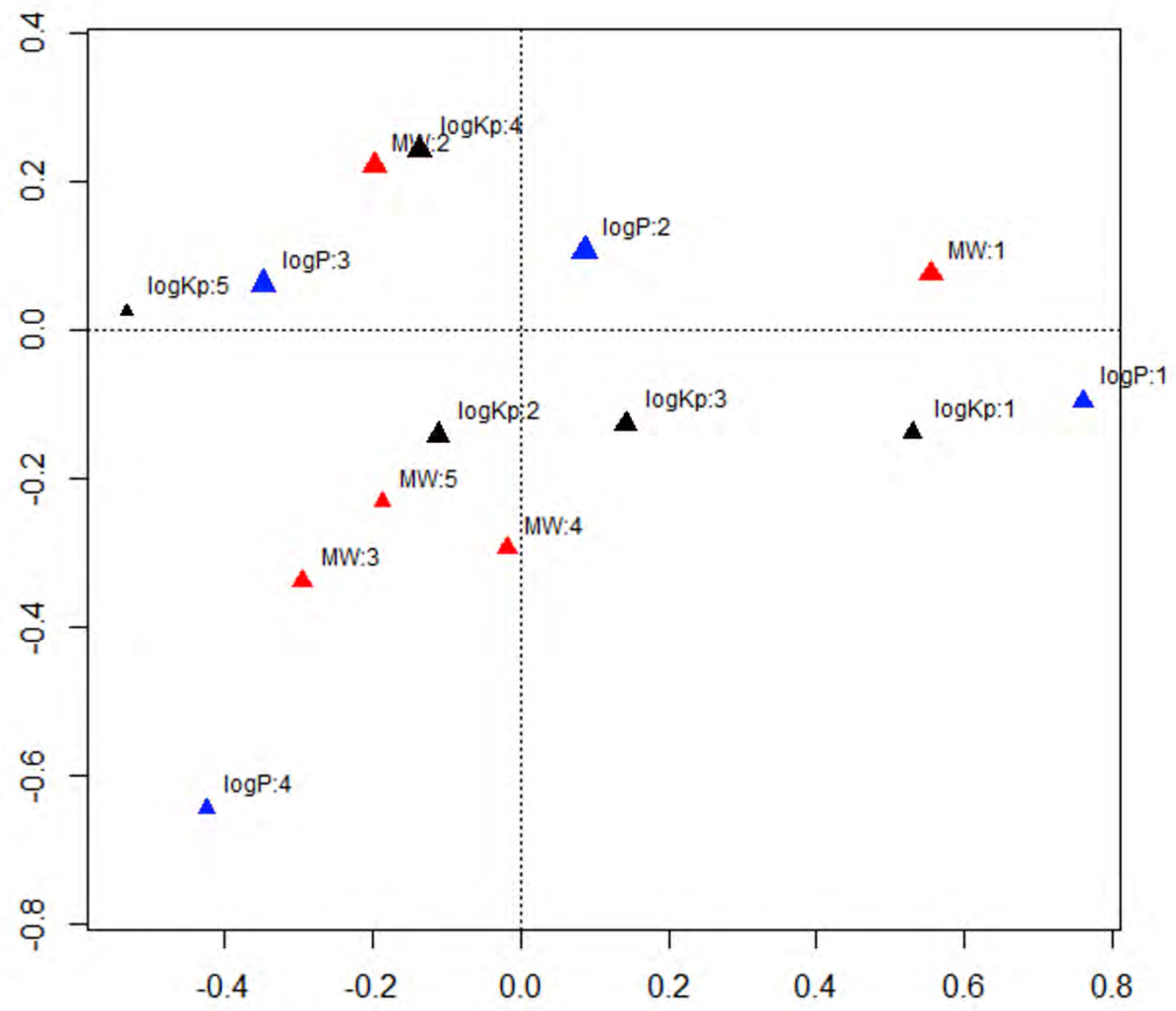

Figure 3: Correspondence map of the categorical variables. The size of the points indicates the mass of the variable. Dimension 1 is plotted on the horizontal axis and dimension 2 on the vertical axis.

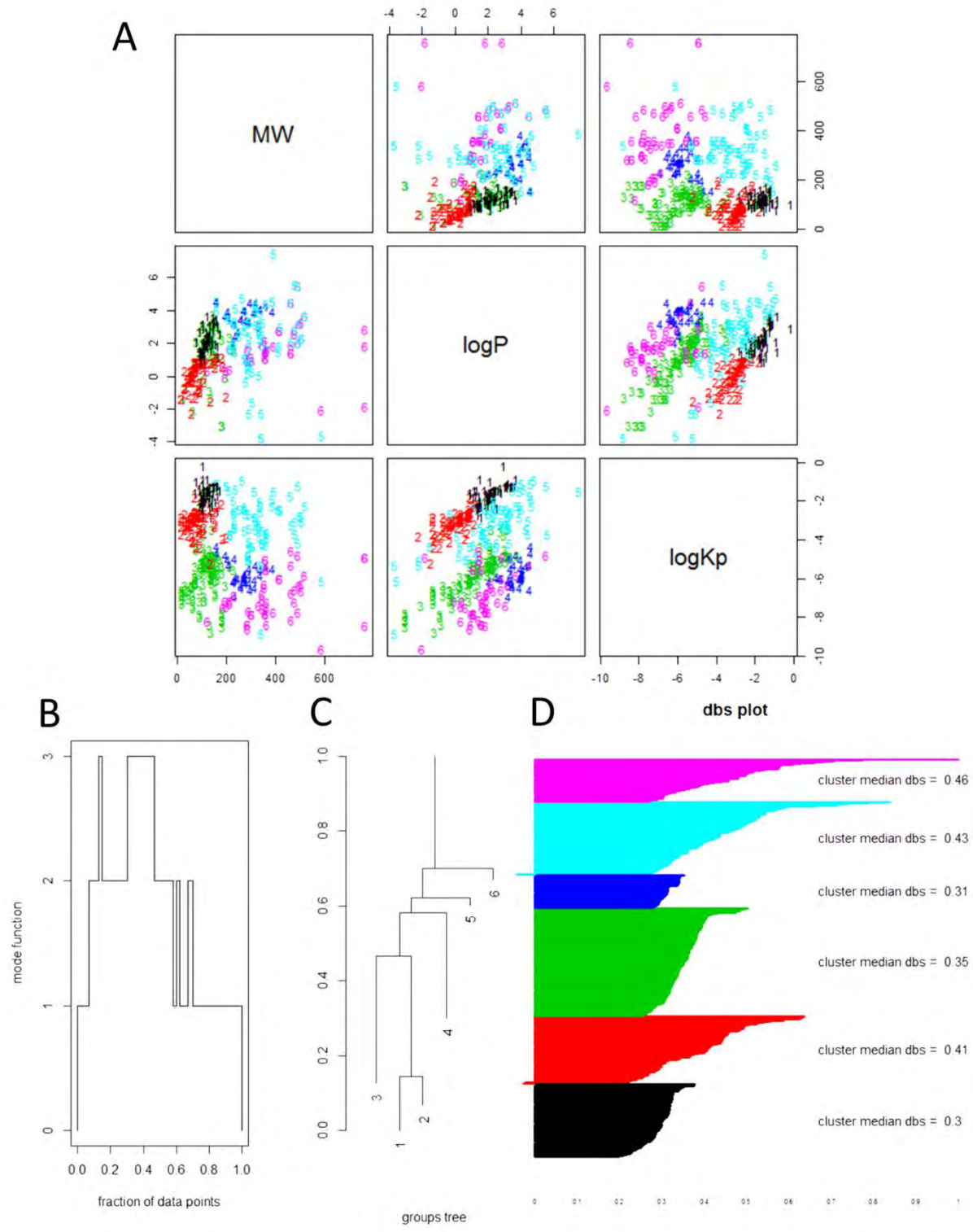

Figure 4: Results of the estimated density cluster analysis. **A)** Clustering of the groups into which the data were divided, group 1 – black, 2 – red, 3 – green, 4 – blue, 5 – turquoise, 6 – purple. **B)** Mode function. **C)** The cluster tree. **D)** The *dbs* plot.

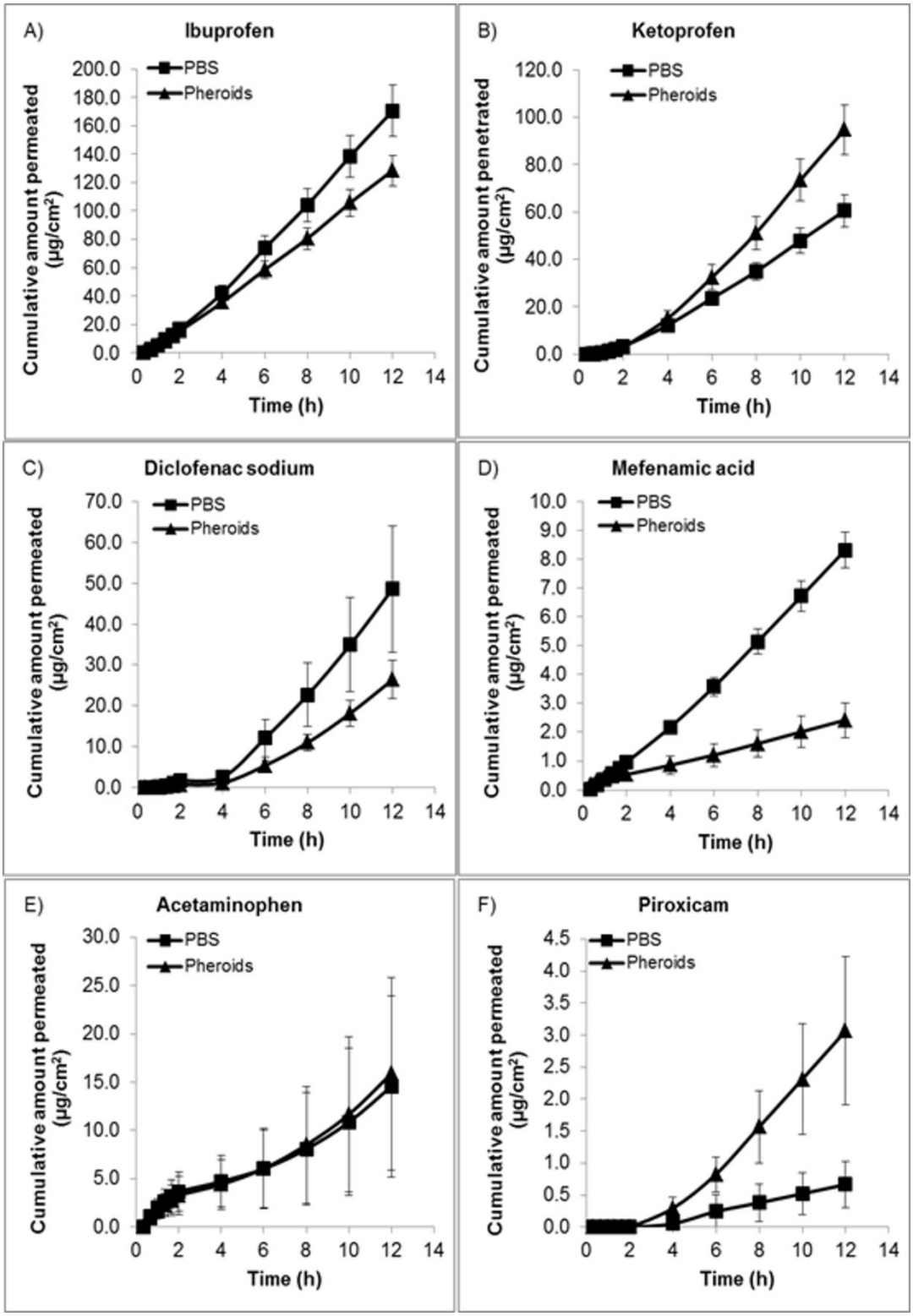

Figure 5: The average cumulative amount of NSAID that permeated the skin over a 12 h period.

SUPPLEMENTARY INFORMATION

Exploratory data analysis of the dependencies between skin permeability, molecular weight and log P

Dewald Kilian, Hendrik J.R. Lemmer, Minja Gerber, Jan L. du Preez, Jeanetta du Plessis*

Centre of Excellence for Pharmaceutical Sciences (Pharmacen), North-West University, Private Bag X6001, Potchefstroom 2520, South Africa

* Corresponding author. Tel.: +2718 299-2274; Fax: +2718 299-2225. E-mail address: Jeanetta.duPlessis@nwu.ac.za (J du Plessis).

Results of the correspondence analysis

Eigenvalues:

	1	2	3	4	5	6
Value	0.102646	0.041923	0.007296	0.002791	8e-05	4.5e-05
Percentage	49.55%	20.24%	3.52%	1.35%	0.04%	0.02%

Columns:

	MW:1	MW:2	MW:3	MW:4	MW:5	logP:1	logP:2
Mass	0.083905	0.131198	0.052632	0.041190	0.024409	0.053394	0.135011
ChiDist	1.133028	0.759867	1.396438	1.582381	2.087905	1.510746	0.717258
Inertia	0.107714	0.075753	0.102634	0.103137	0.106407	0.121865	0.069458
Dim. 1	1.729786	-0.612988	-0.915460	-0.056047	-0.582792	2.368524	0.271261
Dim. 2	0.374072	1.084533	-1.651541	-1.434305	-1.133712	-0.466370	0.522053

	logP:3	logP:4	logKp:1	logKp:2	logKp:3	logKp:4	logKp:5

Mass	0.118993	0.025934	0.034325	0.100686	0.077040	0.117468	0.003814
ChiDist	0.850731	2.113422	1.800294	0.892374	1.075877	0.824033	5.395931
Inertia	0.086121	0.115837	0.111249	0.080180	0.089175	0.079764	0.111045
Dim. 1	-1.082517	-1.321684	1.656040	-0.347368	0.442805	-0.422636	-1.661321
Dim. 2	0.303141	-3.148455	-0.676914	-0.687720	-0.614749	1.186344	0.126568

Principal inertias (eigenvalues):

dim	value	%	cum%	scree plot
1	0.299125	22.0	22.0	*****
2	0.220744	16.2	38.2	****
3	0.152317	11.2	49.4	***
4	0.135832	10.0	59.4	**
5	0.115120	8.5	67.9	**
6	0.114116	8.4	76.2	**
7	0.099109	7.3	83.5	**
8	0.084443	6.2	89.7	**
9	0.076950	5.7	95.4	*
10	0.051901	3.8	99.2	*
11	0.010681	0.8	100.0	
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Total:	1.360338	100.0		

Columns:

	name	mass	qlt	inr	k=1	cor	ctr	k=2	cor	ctr
1	MW:1	84	721	79	946	697	251	-176	24	12
2	MW:2	131	644	56	-335	195	49	-510	450	154
3	MW:3	53	437	75	-501	129	44	776	309	144
4	MW:4	41	182	76	-31	0	0	674	181	85
5	MW:5	24	88	78	-319	23	8	533	65	31
6	logP:1	53	756	90	1295	735	300	219	21	12
7	logP:2	135	160	51	148	43	10	-245	117	37
8	logP:3	119	512	63	-592	484	139	-142	28	11
9	logP:4	26	607	85	-723	117	45	1479	490	257
10	logKp:1	34	284	82	906	253	94	318	31	16
11	logKp:2	101	176	59	-190	45	12	323	131	48
12	logKp:3	77	123	66	242	51	15	289	72	29
13	logKp:4	117	536	59	-231	79	21	-557	458	165
14	logKp:5	4	28	82	-909	28	11	-59	0	0

Results of the cluster analysis

Solute	logP	MW	logKp	Group
2,4,6-Trichlorophenol	3.69	197.44	-1.23	1
2-Butoxyethanol	0.83	118.17	-2.85	1
2-Chlorophenol	2.15	128.55	-1.48	1
2-Cresol	1.95	108.14	-2	1
2-Heptanone	1.98	114.18	-2	1
2-Pentanone	0.91	86.13	-2.6	1
2-Toluidine	1.32	107.15	-1.44	1
3-Cresol	1.96	108.14	-2	1
3-Xylene	3.2	106.16	-1.1	1
4-Bromophenol	2.59	173	-1.44	1
4-Chlorophenol	2.39	128.55	-1.44	1
4-Cresol	1.94	108.14	-2	1
4-Ethylphenol	2.47	122.16	-1.46	1
Atropine	1.83	289.37	-4.12	1
Butobarbital	1.73	212.24	-3.71	1
Catechol	0.88	110.11	-2.77	1
Cortisone	1.47	360.44	-5	1
Ethanolamine	-1.31	61.08	-4.02	1
Ethyl-acrylate	1.32	100.11	-2.39	1
Ethylamine	-0.13	45.08	-3.09	1
Formaldehyde	0.35	30.02	-2.65	1
Hexachloroethane	4.14	236.74	-1.4	1
Ibuprofen	3.5	206.28	-1.44	1
Isopropylamine	0.26	59	-2.9	1
Morpholine	-0.86	88.12	-3.86	1
Naproxen	3.34	230.26	-2.54	1
n-Decanol	4.57	158.28	-1.1	1
n-Heptanol	2.72	116.2	-1.5	1
n-Hexanol	2.03	102.17	-1.89	1
N-Nitrosodiethanolamine	-1.51	134.13	-5.22	1
n-Octanol	3	130.23	-1.28	1
n-Propanol	0.25	60.09	-2.91	1
Octanoic-acid	2.11	144.21	-1.6	1
Phenobarbital	1.47	232.23	-3.34	1
Phenol	1.47	94.11	-2	1
Resorcinol	0.8	110.11	-2.82	1
Scopolamine	0.26	303.35	-4.3	1
Testosterone	3.32	288.42	-2.66	1
Thymol	3.3	150.22	-1.28	1
Toluene	2.73	92.14	-1.3	1
urea	-2.26	60.6	-3.830	1
water	-1.38	18	-3.301	1

methanol	-0.7	32	-3.301	1
n-butanol	0.88	74.1	-2.602	1
octanol	3.15	130.2	-1.284	1
hexanol	2.03	102.2	-1.886	1
pentanol	1.4	88.2	-2.222	1
methylhydroxybenzoate	1.96	152.1	-2.040	1
b-naphtol	2.8	144.2	-1.554	1
2,4-dichlorophenol	3.15	163	-1.221	1
p-bromophenol	2.58	173	-1.442	1
m-nitrophenol	2	139.1	-2.249	1
p-bromophenol	1.92	108.1	-1.757	1
p-nitrophenol	1.96	139.1	-2.253	1
o-cresol	2	108.1	-1.804	1
2,4,6-trichlorophenol	3.69	197.5	-1.226	1
chloroxylenol	3.39	156.6	-1.229	1
phenol	1.46	94.1	-2.085	1
resorcinol	0.78	110.1	-3.620	1
p-chlorophenol	2.39	128.6	-1.440	1
thymol	3.34	150.2	-1.277	1
o-chlorophenol	2.19	128.6	-1.481	1
chlorocresol	3.1	142.6	-1.260	1
salicyclic-acid	2.21	138.1	-3.480	1
barbital	0.65	184.2	-3.951	1
nicotine	1.2	162.3	-1.721	1
butobarbital	1.65	212.4	-3.721	1
2-ethoxyethanol	-0.54	90.1	-3.602	1
n-butanol	0.88	74.1	-2.523	1
water	-1.38	18	-2.854	1
water	-1.38	18	-3.000	1
methanol	-0.7	32	-3.000	1
octanol	3.15	130.2	-1.284	1
heptanol	2.41	116.2	-1.495	1
2-amino-4-nitrophenol	1.13	154.1	-3.180	1
banzyl-alcohol	1.1	108.1	-1.770	1
benzaldehyde	1.5	106.1	-0.854	1
aniline	0.9	93.1	-1.658	1
anisole	2.1	108.1	-1.602	1
17-Hydroxyprogesterone	3.17	330.46	-3.22	2
2,4-Dichlorophenol	3.06	163	-1.22	2
2-Butanone	0.29	72.1	-2.95	2
2-Hexanone	1.38	100.16	-2.35	2
4-Methyl-2-pentanol	1.53	102	-2.33	2
Acetaldehyde	-0.22	44.05	-3.15	2
Acetic-acid	-0.17	60.05	-3.21	2
Acetone	-0.24	58.08	-3.29	2
Acetonitrile	-0.34	41.05	-3.21	2

Acrolein	-0.01	56.06	-3.07	2
Acrylic-acid	0.35	72.06	-3.05	2
Aldosterone	1.08	360.44	-4.24	2
Amobarbital	2.07	226.27	-2.64	2
Aniline	0.9	93.12	-2.65	2
Benzoic-acid	1.87	122.12	-1.6	2
Butyl-acrylate	2.36	128.17	-2	2
Butyric-acid	0.79	88.1	-3	2
Cumene	3.66	120.19	-0.85	2
Diclofenac	4.4	318.13	-3	2
Diethanolamine	-1.31	77.08	-4.38	2
Digitoxin	2.83	764.94	-4.89	2
Dimethyl-acetamide	-0.77	87.12	-2.8	2
Dioxane	-0.42	88.1	-3.45	2
Estrone	3.13	270.37	-2.44	2
Ethanol	-0.31	46.06	-3.1	2
Ethyl-benzene	3.15	106.16	-1.15	2
Ethyl-ether	0.89	74.12	-1.8	2
Ethyl-formate	0.26	74.07	-3.01	2
Ethylene-dichloride	1.47	98.96	-2	2
Fentanyl	3.89	336.47	-2.25	2
Isoamyl-alcohol	1.16	88.14	-1.44	2
Isobutyl-alcohol	0.76	74	-2.65	2
Isopropyl-alcohol	0.05	60	-3.05	2
Meperidine	2.45	247.33	-2.43	2
Methanol	-0.77	32.04	-3.46	2
Methyl-acrylate	0.8	86.09	-2.68	2
Methyl-acrylic-acid	-0.01	70.09	-2.58	2
Methyl-cellosolve	-0.77	76.09	-3.73	2
Methyl-nicotinate	0.83	137.14	-2.41	2
Morphine	0.76	287.35	-5.03	2
Nicotine	1.87	162.23	-2.48	2
n-Pentanol	1.56	88.14	-2.22	2
Progesterone	3.87	314.46	-1.52	2
Propionic-acid	0.33	74.07	-2.94	2
Propylene-oxide	0.03	58.08	-3.05	2
Pyridine	0.65	79.1	-2.74	2
Triethylamine	1.45	101.19	-2.31	2
Vinyl-acetate	0.73	86.09	-2.73	2
water	-1.38	18	-2.807	2
benzene	2.13	78.1	-0.955	2
scopolamine	-1.58	303.4	-4.301	2
atropine	-2.22	289.4	-5.066	2
water	-1.38	18	-3.699	2
heptanol	2.41	116.2	-1.495	2
propanol	0.34	60.1	-2.921	2

ethanol	-0.3	46.1	-3.097	2
decanol	4	158.3	-1.097	2
p-ethylphenol	2.39	122.2	-1.457	2
diclofenac	4.31	318	-3.447	2
salicyclic-acid	2.21	138.1	-2.201	2
1,1,1-trichloroetlume	2.49	133.4	-2.347	2
heptanol	2.41	116.2	-1.425	2
ethylether	0.8	74.1	-2.796	2
propanol	0.34	60.1	-2.770	2
sucrose	-2.26	342.3	-5.284	2
propanol	0.34	60.1	-2.854	2
ethanol	-0.3	46.1	-3.000	2
hexanol	2.03	102.2	-1.886	2
pentanol	1.4	88.2	-2.222	2
n-butanol	0.88	74.1	-2.602	2
o-phenylenediamine	0.15	108.1	-3.347	2
2-nitro-p-phenylenediamine	0.53	153.1	-3.301	2
p-phenylenediamine	-0.3	108.1	-3.620	2
4-amino-2-nitrophenol	0.96	154.1	-2.553	2
4-chloro-m-phenylenediamine	0.85	142.6	-2.678	2
1,1,1-Trichloroethane	2.49	133	-5.9	3
2,3-Butanediol	-0.92	90	-7.95	3
2,4,6-Trichlorophenol	3.69	197	-4.78	3
2,4-Dichlorophenol	3.06	163	-4.78	3
2-Amino-4-nitrophenol	1.26	154	-6.74	3
2-Butanone	0.29	72	-5.9	3
2-Chlorophenol	2.15	129	-5.04	3
2-Ethoxyethanol	-0.32	90	-7.16	3
2-Naphthol	2.7	144	-5.15	3
2-Naphthol	2.7	144	-5.11	3
2-nitro-p-phenylenediamine	0.53	153	-6.86	3
2-Phenylethanol	1.36	122	-5.44	3
3,4-Xylenol	2.3	122	-5	3
3-Nitrophenol	2	139	-5.81	3
4-Amino-2-nitrophenol	0.96	154	-6.11	3
4-Bromophenol	2.59	173	-5	3
4-Chloro-4-phenylenediamine	0.85	142	-6.23	3
4-Chlorocresol	3.1	142	-4.82	3
4-Chlorophenol	2.39	128	-5	3
4-Ethylphenol	2.58	122	-5.02	3
4-Nitrophenol	1.91	139	-5.81	3
5-Fluorouracil	-0.89	130	-8.34	3
Amylobarbital	2.07	226	-6.2	3
Aniline	0.9	93	-5.21	3
Barbital	0.65	184	-7.51	3
Benzaldehyde	1.48	106	-4.77	3

Benzaldehyde	1.48	106	-4.41	3
Benzene	2.13	78	-4.51	3
Benzene	2.13	78	-4.35	3
Benzoic-acid	1.87	122	-5.16	3
Benzyl-alcohol	1.1	108	-5.78	3
Benzyl-alcohol	1.1	108	-5.33	3
Benzyl-nicotinate	2.4	213	-5.35	3
Boric-acid	-0.22	62	-7.48	3
Boric-acid	-0.22	62	-7.09	3
Boric-acid	-0.22	62	-6.86	3
Butanol	0.88	74	-6.21	3
Butanol	0.88	74	-6.16	3
Butobarbital	1.73	212	-7.27	3
Butyl-nicotinate	2.27	179	-5.34	3
Caffeine	-0.07	194	-7.14	3
Caffeine	-0.07	194	-6.35	3
Corticosterone	1.94	346	-6.81	3
Coumarin	1.39	146	-5.6	3
Dihydromorphine	0.93	287	-8.38	3
Dimethylethylamine	0.7	73	-6.26	3
Dimethylethylamine	0.7	73	-5.95	3
Estradiol	4.01	272	-5.77	3
Ethanol	-0.31	46	-7.06	3
Ethanol	-0.31	46	-6.65	3
Ethanol	-0.31	46	-6.56	3
Ethyl-benzene	3.15	106	-3.48	3
Ethyl-nicotinate	1.32	151	-5.77	3
Ethyl-nicotinate	1.32	151	-5.74	3
Griseofulvin	2.18	353	-6.27	3
Heptanoic-acid	2.42	130	-5.26	3
Heptanol	2.31	116	-5.05	3
Heptanol	2.31	116	-4.98	3
Hexanoic-acid	1.92	116	-5.41	3
Hexanol	2.03	102	-5.44	3
Hexanol	2.03	102	-5.26	3
Indomethacin	4.27	358	-5.39	3
Lidocaine	2.44	234	-5.96	3
Mannitol	-3.01	182	-8.51	3
Mannitol	-3.01	182	-8.18	3
Mannitol	-3.01	182	-8.16	3
Mannitol	-3.01	182	-7.92	3
Mannitol	-3.01	182	-7.77	3
Meperidine	2.72	247	-5.99	3
Methanol	-0.77	32	-6.86	3
Methanol	-0.77	32	-6.56	3
Methanol	-0.77	32	-6.35	3

Methyl-nicotinate	0.83	137	-6.07	3
Methyl-nicotinate	0.83	137	-6.02	3
Methyl-nicotinate	0.83	137	-5.97	3
Nicorandil	0.43	211	-7.13	3
Nicotine	1.17	162	-6.04	3
Nicotine	1.17	162	-5.54	3
Nicotinic-acid	0.36	123	-8.18	3
Nitroglycerine	1.62	227	-5.51	3
n-Nitrosodiethanolamine	-1.28	134	-8.78	3
Nonanol	3.77	144	-4.78	3
o-Cresol	1.95	108	-5.36	3
Octanoic-acid	3.05	144	-5.16	3
Octanol	3	130	-4.84	3
Octanol	3	130	-4.77	3
Octanol	3	130	-4.51	3
Ouabain	-2	585	-9.66	3
p-Cresol	1.94	108	-5.31	3
Pentanoic-acid	1.39	102	-6.26	3
Phenobarbital	1.47	232	-6.89	3
Phenol	1.46	94	-5.64	3
Phenol	1.46	94	-5.44	3
Piroxicam	3.06	331	-7.37	3
Progesterone	3.87	314	-6.08	3
Propanol	0.25	60	-6.48	3
Propanol	0.25	60	-6.41	3
Propranolol	3.48	257	-6.33	3
Resorcinol	0.8	110	-7.18	3
Salicyclic-acid	2.26	138	-5.76	3
Salicyclic-acid	2.26	138	-5.48	3
Salicyclic-acid	2.26	138	-5.44	3
Salicyclic-acid	2.26	138	-5.42	3
Scopolamine	0.98	303	-7.86	3
Testosterone	3.32	288	-4.95	3
Thymol	3.3	150	-4.84	3
Toluene	2.73	92	-3.56	3
Trichloromethane	1.97	119	-5.35	3
Urea	-2.11	61	-7.39	3
Water	-1.38	18	-7.26	3
Water	-1.38	18	-7.08	3
Water	-1.38	18	-6.86	3
Water	-1.38	18	-6.8	3
Water	-1.38	18	-6.72	3
Water	-1.38	18	-6.71	3
Water	-1.38	18	-6.68	3
naproxen	3.18	230.3	-3.151	3
2,3-Butanediol	-0.92	90	-7.86	4

Aldosterone	1.08	360	-7.79	4
Chloroxylenol	3.27	156	-4.83	4
Coumarin	1.39	146	-5.46	4
Decanol	4.57	158	-4.65	4
Dexamethasone	1.83	392	-7.75	4
Diclofenac	4.51	296	-6.56	4
Ephedrine	1.13	165	-5.78	4
Estradiol	4.01	272	-6.08	4
Estradiol	4.01	272	-6.05	4
Estradiol	4.01	272	-6.02	4
Estradiol	4.01	272	-6.01	4
Estradiol	4.01	272	-5.97	4
Estradiol	4.01	272	-5.95	4
Estradiol	4.01	272	-5.94	4
Estradiol	4.01	272	-5.94	4
Estradiol	4.01	272	-5.84	4
Estradiol	4.01	272	-5.82	4
Etorphine0	2.79	411	-6	4
Fentanyl	4.05	336	-5.81	4
Hydromorphone	1.6	285	-8.38	4
Ibuprofen	3.97	206	-5	4
Isoquinoline	2.08	129	-5.33	4
m-Cresol	1.96	108	-5.38	4
Morphine	0.89	285	-8.595	4
Naproxen	3.18	230	-6.95	4
Naproxen	3.18	230	-6.1	4
Naproxen	3.18	230	-4.97	4
p-Phenylenediamine	-0.3	108	-7.18	4
Progesterone	3.87	314	-6.44	4
Propanol	0.25	60	-6.33	4
Propranolol	3.48	257	-6.48	4
Sucrose	-3.7	342	-8.84	4
Sufentanil	3.95	387	-5.48	4
Testosterone	3.32	288	-6.21	4
Testosterone	3.32	288	-5.83	4
Testosterone	3.32	288	-5.48	4
Styrene	2.95	104	-3.74	5
1,2-Dichloropropene	1.76	110.97	-2	5
2,3-Butanediol	-0.92	90.12	-4.39	5
Acrylonitrile	0.25	53.06	-2.87	5
Allyl-alcohol	0.17	58.08	-2.95	5
Aspirin	1.19	180.16	-2.14	5
Benzyl-alcohol	1.1	108.14	-2.22	5
Chloroxylenol	3.48	156.61	-1.28	5
Cortexolone	3.25	346.46	-4.13	5
Cyclohexanone	0.81	98.14	-2.74	5

Epichlorohydrin	0.45	92.52	-3.43	5
Estradiol	4.01	272.39	-2.4	5
Estriol	2.45	288.38	-4.4	5
Ethylene-glycol	-0.31	62.06	-4.07	5
Ethylhexyl-phthalate	7.45	390.56	-1.52	5
Etorphine	1.47	409.52	-2.44	5
Heptanoic-acid	2.41	130.18	-1.7	5
Hexachlorobutadiene	4.78	260.76	-0.92	5
Hexanoic-acid	1.92	116.16	-1.85	5
Hydrocortisone	1.61	362.46	-3.64	5
Hydromorphone	0.21	285.34	-4.82	5
Isoquinoline	2.08	129.16	-1.78	5
Monomethylhydrazine	-1.05	46.07	-3.75	5
NN-dimethyl-aniline	2.31	121.18	-1.7	5
n-Butyl-alcohol	0.88	74.12	-1.55	5
Pentanoic-acid	1.39	102.13	-2.7	5
Phenylglycicyl-ether	1.6	134.17	-2.84	5
Salicylic-acid	2.26	138.12	-1.86	5
Styrene	2.95	104.15	-0.19	5
Sucrose	-3.7	342.29	-5.28	5
ephedrine	0.93	165.2	-2.222	5
diethylcarbamazine	-1.19	199.3	-3.886	5
digitoxin	-1.85	764.9	-4.886	5
chlorpheniramine	-0.33	274.8	-2.658	5
nitroglycerine	2	227.1	-1.959	5
fentanyl	2.3	336.5	-2.000	5
estradiol	1.08	272.4	-2.284	5
nonanol	3.62	144.3	-1.222	5
3,4-xylenol	2.34	122	-1.444	5
m-cresol	2.01	108.1	-1.818	5
naproxen	3.18	230.3	-3.398	5
piroxicam	0.05	331.4	-3.812	5
indomethacin	4.42	357.8	-3.668	5
phenol	1.46	94.1	-1.710	5
isoquinoline	2.08	129.2	-1.777	5
amylobarbital	1.95	226.3	-2.638	5
phenobarbital	1.47	232.2	-3.347	5
hydrocortisone	1.55	362.5	-3.921	5
hexanol	2.03	102.2	-1.558	5
testosterone	3.29	288.4	-3.398	5
estriol	2.47	288.4	-4.398	5
cortisone	1.42	360.5	-5.000	5
pregnenolone	3.13	316.5	-2.824	5
cortexone	2.88	330.5	-3.347	5
progesterone	3.87	314.5	-2.824	5
hydrocortisone	1.55	362.5	-5.523	5

corticosterone	1.94	346.5	-4.222	5
hydroxyprogesterone	2.74	330.5	-3.222	5
aldosterone	1.08	360.4	-5.523	5
estradiol	1.08	272.4	-3.523	5
hyydrocortisone-methylsuccinate	5.58	476.6	-3.678	5
hydrocortisone-hexanoate	4.48	460.6	-1.745	5
hydrocortisone-methylpimelate	3.7	518.6	-2.268	5
hydrocortisone-N,N,-dimethyisuccinate	2.03	489.6	-4.174	5
hydrocortisone-hemisuccinate	2.11	462.5	-3.201	5
fluocinonide	3.19	494.6	-2.770	5
hydrocortisone-octanoate	5.49	488.7	-1.208	5
hydrocortisone-succinamate	1.43	461.6	-4.585	5
hydrocortisone-hydroxyhexanoate	2.79	476.6	-3.041	5
hydrocortisone-hemipimelate	2.31	503.6	-2.745	5
hydrocortisone-pimelamate	2.31	503.6	-3.051	5
2-phenylethanol	1.76	122.2	-1.420	5
etorphine	1.86	411.5	-2.444	5
sufentanil	4.59	386.6	-2.260	5
fentanyl	2.3	336.5	-2.523	5
meperidine	2.72	247.4	-2.432	5
sufentanil	4.59	386.6	-1.921	5
morphine	0.62	285.3	-5.032	5
codeine	0.89	299.4	-4.310	5
fentanyl	2.3	336.5	-2.252	5
Acetylsalicylic-acid	1.19	180	-5.7	6
Aldosterone	1.08	360	-7.86	6
Anisole	2.11	108	-4.69	6
Atropine	1.83	289	-7.68	6
Butanol	0.88	74	-6.08	6
Butyric-acid	0.79	88	-6.56	6
Chlorpheniramine	3.38	275	-6.22	6
Codeine	1.19	299	-7.87	6
Corticosterone	1.94	346	-7.56	6
Corticosterone	1.94	346	-7.08	6
Decanol	4.57	158	-4.3	6
Diclofenac	4.51	318	-5.3	6
Diethylcarbamazine	0.37	199	-7.45	6
Digitoxin	1.85	765	-8.44	6
Ethylether	0.89	74	-5.36	6
Fentanyl	4.05	336	-5.56	6
Fluocinonide	3.19	494	-6.33	6
Griseofulvin	2.18	353	-6.44	6
Hexanol	2.03	102	-5.111	6
Hydrocortisone	1.61	362	-8.35	6
Hydrocortisone	1.61	362	-7.48	6
Hydrocortisone	1.61	362	-7.19	6

Hydrocortisone-hemipimelate	3.26	504	-6.3	6
Hydrocortisonehemisuccinate	1.89	462	-6.76	6
Hydrocortisone-hexanoate	4.48	461	-5.3	6
Hydrocortisone-hydroxyhexanoate	2.79	477	-6.6	6
Hydrocortisone-methylpimelate	3.7	519	-5.82	6
Hydrocortisone-methylsuccinate	2.6	476	-7.23	6
Hydrocortisone-N,N-dimethylsuccinamate	2.03	489	-7.72	6
Hydrocortisone-octanoate	5.49	489	-4.76	6
Hydrocortisone-pimelamate	2.3	504	-6.61	6
Hydrocortisone-propinate	2.8	418	-6.02	6
Hydrocortisone-succinamate	1.43	462	-8.14	6
Methylhydroxybenzoate	1.96	152	-5.6	6
N-Hexyl-nicotinate	3.51	207	-5.3	6
Nicotine	1.17	162	-5.28	6
o-Phenylenediamine	0.15	108	-6.9	6
Pentanol	1.51	88	-5.78	6
Phenol	1.46	94	-5.27	6
Salicyclic-acid	2.26	138	-5.07	6
Corticosterone	1.94	346.46	-3.52	6
Diethylamine	0.58	73.13	-2.75	6
ouabain	-3.58	584.6	-6.108	6
hydroxypregnenolone	3	330.5	-3.222	6
cortexolone	2.52	346.5	-4.125	6
estrone	2.76	270.4	-2.444	6
hydrocortisone-propinate	3	418.5	-2.469	6

R-output of multiple linear regression diagnostics

Call:

lm(formula = logKp ~ MW + logP, data = groups)

Residuals:

Min	1Q	Median	3Q	Max
-0.8555	-0.1923	-0.1033	0.1704	0.9046

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	-2.360219	0.058375	-40.43	<2e-16 ***
MW	-0.008904	0.000513	-17.36	<2e-16 ***
logP	0.738540	0.025499	28.96	<2e-16 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.3532 on 141 degrees of freedom

Multiple R-squared: 0.8561, Adjusted R-squared: 0.8541

F-statistic: 419.5 on 2 and 141 DF, p-value: < 2.2e-16

ASSESSMENT OF THE LINEAR MODEL ASSUMPTIONS

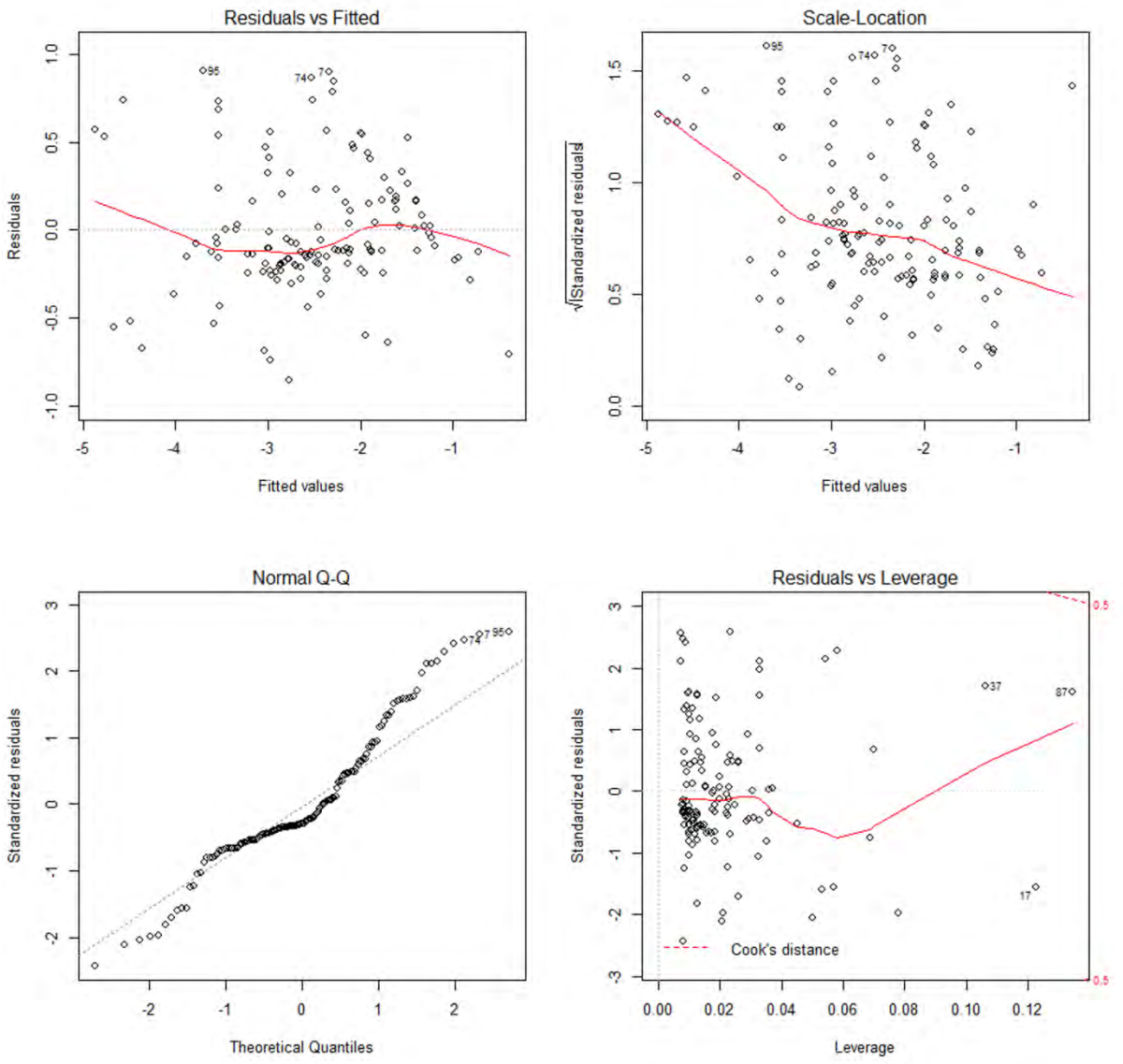
USING THE GLOBAL TEST ON 4 DEGREES-OF-FREEDOM:

Level of Significance = 0.05

Call:

gvlma(x = fit1)

	Value	p-value	Decision
Global Stat	7.5301	0.110388	Assumptions acceptable.
Skewness	6.6991	0.009646	Assumptions NOT satisfied!
Kurtosis	0.7049	0.401154	Assumptions acceptable.
Link Function	0.0283	0.866399	Assumptions acceptable.
Heteroscedasticity	0.0979	0.754361	Assumptions acceptable.



Diagnostics of linear model assumptions based on the fit of Eq. 10 to the dataset consisting of groups 1 and 2 from the cluster analysis

Chapter 6: Final conclusions and future prospects

In the second chapter which is a review article on all previous work done with Pheroid™ an attempt was made to establish a pattern or some commonality between the different APIs that were used and their respective physiochemical characteristics for both groups, those in which the flux was increased by the Pheroid™ and those where the flux was less or the same with the Pheroid™ when compared to the control for each API during transdermal diffusion studies. No clear pattern or commonality could be established so it is still unclear based on the review article in Chapter 2 if an API requires certain specific physicochemical characteristics in order for the Pheroid™ to enhance its permeation. It was however observed that the different types of formulations had a significant impact on the API's transdermal delivery behaviour.

To aid in the investigation of the optimal physiochemical properties an NSAID must have when administered transdermally with the Pheroid™ delivery system, a novel flux-independent mathematical model, based on restrictions made to Lipinski's rule of five, was derived and tested. The results suggest that the same molecular size and log P ranges that determine the skin permeability of an API dissolved in PBS (pH of 7.4) also determine its permeability when dissolved in Pheroid™, and that these ranges are consistent with the previously described restrictions that should be placed on Lipinski's rule of five to account for the formidability of the stratum corneum. The model gave reasonable approximations of the experimentally observed concentrations. The results suggest that the restrictions placed on Lipinski's rule of five, discussed in the introduction of Chapter 3, can be used to accurately model transdermal delivery.

The ability of Bayesian networks to learn the correct probabilistic dependencies between skin permeability and the physicochemical properties of selected drugs dissolved in PBS (pH of 7.4) and dispersed in a lipid based drug delivery system was investigated in Chapter 4. The Bayesian network structures learned from the training sets of dissolved and dispersed drugs were consistent with known relationships between physicochemical properties and also elucidated new causal relationships between these properties and skin permeability. The networks identified a probabilistic dependence between pKa and skin permeability for drugs dissolved in PBS at pH 7.4, which was not observed for the same drugs dispersed in the lipid micelles of the Pheroid™ drug delivery system. For both dissolved and dispersed drugs the same probabilistic dependencies existed between TPSA, MP and cumulative amount of drug that permeated the skin after 12 h (CPBS and CPher). Although both networks shared a causal relationship between permeability and molecular size descriptors, the network learned from the

dissolved drugs displayed a probabilistic dependence between MW and permeability, while the dispersed drugs displayed a dependence between MV and permeability. Both networks identified similar physicochemical regions for optimal transdermal delivery, i.e. low MP, small TPSA to molecular size ratio and, in the case of the dissolved drugs, a pKa value close to the pH of the buffer solution. Regions for poor transdermal delivery were also identified, i.e. high MP and a TPSA to molecular size ratio close to 0.5. The results suggest that Bayesian networks learned from online bioinformatics and cheminformatics databases can be viable classification tools in early drug discovery and development, and can aid in the identification of suitable drug candidates and formulation strategies.

In Chapter 5 an investigation into the probabilistic dependencies between log Kp and the two physicochemical properties most frequently used in skin permeability predictions, i.e. MW and log P was done. Initial observations based on a dataset constructed from several popular datasets used to construct and/or test predictive models, suggested that MW and log P are poorly correlated with log Kp. However, exploratory data analyses indicated the existence of regions of statistically significant probabilistic dependence between these variables. Based on these observations, a cluster analysis was performed on the dataset and two similar clusters were identified. The ranges of these clusters coincided with the regions of probabilistic dependencies identified from the previous exploratory data analyses and these two groups were used to construct a new, refined dataset. As an application of the knowledge gained from the exploratory data analyses, a predictive model was constructed from this new dataset and tested on experimental skin permeation data of selected NSAIDs. The quantitative model presented in this work gave reasonable approximations to experimental log Kp values. Although MW and log P are not strongly correlated with log Kp, they remain the most frequently used predictors of skin permeability, are easily accessible and calculable, and have intuitive meaning in the transport process. By identifying and restricting investigations into regions of statistically significant dependencies, valuable skin permeability information can still be extracted from these physicochemical properties.

Future prospects and studies could include optimisation of the Pheroid™ preparation technique and the effects this may have on the short and long term stability of Pheroid™ formulations. Formulation stability and future investments is crucial factors for successfully transforming laboratory scale studies into viable commercial products.

Future studies based on the permeation model presented in Chapter 3 could focus on how it scales with time, possibly as a function of the permeability constant. This can give us a way to predict changes in skin permeation of a chemical entity as it changes structure over time, e.g. as a result of decomposition.

Annexure A: Transdermal diffusion studies

A.1 Introduction

Transdermal diffusion studies were done over a 12 h time period for each of the following NSAID APIs: ibuprofen, ketoprofen, diclofenac sodium, mefenamic acid, acetaminophen and piroxicam. The APIs were entrapped in the Pheroid™ delivery system and this served as the experimental cells or a saturated solution of the respective API was made in PBS (pH of 7.4) and this served as the control cells for each respective API diffusion experiment. For each of the APIs there were seven control cells and eight experimental cells. The concentration of the API present in the receptor phase after HPLC analysis at each time interval is given in the tables in µg/ml. The electrical resistance across the human skin in the Franz diffusion cells were measured using a Tinsley LCR Databridge Model 6401 (Tinsley Precision Instruments, Croydon, UK). The reading was determined at 1 kHz with a maximum voltage of 300 mV root-mean-square in the parallel equivalent circuit mode using an alternating current. Cells with a resistance lower than 10 kΩ were rejected and for comparison studies the cells with similar resistance values within one skin donor were chosen for diffusion studies.

The transdermal diffusion experiments were done in order to determine whether an increase in the flux for the different APIs across the skin could be seen when the API was incorporated with the Pheroid™ delivery system as compared to just using a saturated solution of the API in PBS (pH of 7.4) buffer as the donor delivery medium.

Table A.1: Cumulative amount (μg/cm^2) of ibuprofen that permeated through the skin from the PBS (pH of 7.4) and Pheroid™ donor solutions over the 12 h Franz cell diffusion experiments

		Time (h)												
Cell no	**ER (kΩ)**	**0.33**	**0.67**	**1.00**	**1.33**	**1.67**	**2.00**	**4.00**	**6.00**	**8.00**	**10.00**	**12.00**	**J (μg/cm^2.h)**	**r^2**
PBS														
1	21.8	0.00	1.84	4.28	7.68	10.83	14.54	36.52	63.51	90.75	119.96	147.83	12.8	0.998
2	20.5	0.00	2.06	4.70	8.68	12.08	16.40	42.08	74.41	106.91	142.21	176.03	15.2	0.998
3	21.8	0.00	2.65	5.60	8.94	12.20	16.10	40.14	71.03	101.95	137.12	170.24	14.6	0.997
4	22.0	0.00	2.39	5.58	9.57	13.45	17.54	44.68	78.29	109.96	146.13	179.81	15.5	0.998
5	38.7	0.00	2.20	4.21	7.90	10.21	13.26	34.75	63.99	90.06	120.58	148.78	12.8	0.997
6	26.3	0.00	2.29	5.34	9.72	13.12	17.30	43.92	75.10	105.87	139.58	170.97	14.8	0.999
7	21.1	000	3.00	6.43	12.39	16.78	21.56	51.72	89.09	123.82	163.03	199.91	17.2	0.999
	Average	**0.0**	**2.3**	**5.2**	**9.3**	**12.7**	**16.7**	**42.0**	**73.6**	**104.2**	**138.4**	**170.5**	**14.7**	
	SD	**0.0**	**0.4**	**0.8**	**1.6**	**2.2**	**2.6**	**5.6**	**8.8**	**11.6**	**15.0**	**18.1**	**1.6**	
	RSD	**0.0**	**16.4**	**15.6**	**17.0**	**17.0**	**15.8**	**13.5**	**12.0**	**11.2**	**10.8**	**10.6**	**10.6**	
Pheroid™														
1	23.9	0.00	2.03	4.99	8.98	12.41	16.14	37.84	62.45	85.47	111.69	135.47	11.7	1.000
2	20.9	0.00	1.99	4.35	7.30	10.90	14.37	33.73	55.36	77.48	101.95	125.16	10.8	0.999
3	18.7	0.00	2.55	5.84	9.86	13.47	17.68	39.95	65.19	88.74	115.21	140.05	12.1	1.000
4	28.0	0.00	1.94	4.37	7.49	10.57	13.99	32.45	53.47	74.18	96.56	117.72	10.2	0.999
5	31.3	0.00	1.77	4.14	7.56	10.40	13.69	30.71	51.08	69.80	91.71	112.00	9.6	0.999
6	20.6	0.00	2.88	6.64	10.66	14.54	18.91	42.67	67.94	92.26	119.77	143.32	12.4	1.000
7	24.5	0.00	1.35	3.45	7.35	10.31	13.12	32.12	54.15	75.76	101.34	124.97	10.7	0.998
8	27.4	0.00	1.96	4.59	7.30	10.19	14.02	34.77	58.76	80.58	106.11	128.54	11.2	0.999
	Average	**0.0**	**2.1**	**4.8**	**8.3**	**11.6**	**15.2**	**35.5**	**58.6**	**80.5**	**105.5**	**128.4**	**11.1**	
	SD	**0.0**	**0.5**	**1.0**	**1.3**	**1.7**	**2.1**	**4.2**	**6.1**	**7.7**	**9.5**	**10.8**	**0.9**	
	RSD	**0.0**	**22.8**	**21.1**	**16.1**	**14.4**	**13.8**	**11.8**	**10.4**	**9.6**	**9.0**	**8.4**	**8.5**	

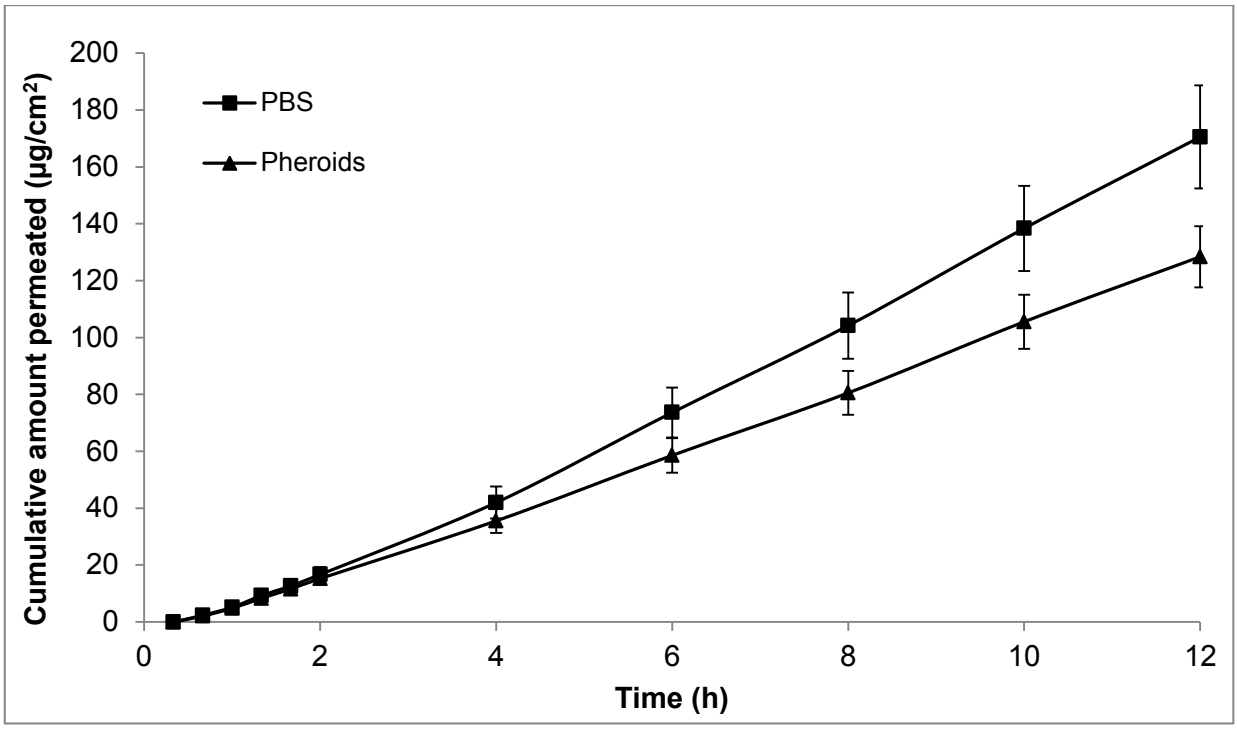


Figure A.1: The cumulative amount of ibuprofen (µg/cm^2) that permeated the skin from both the PBS (pH of 7.4) and Pheroid™ donors during the 12 h diffusion experiments are plotted versus time

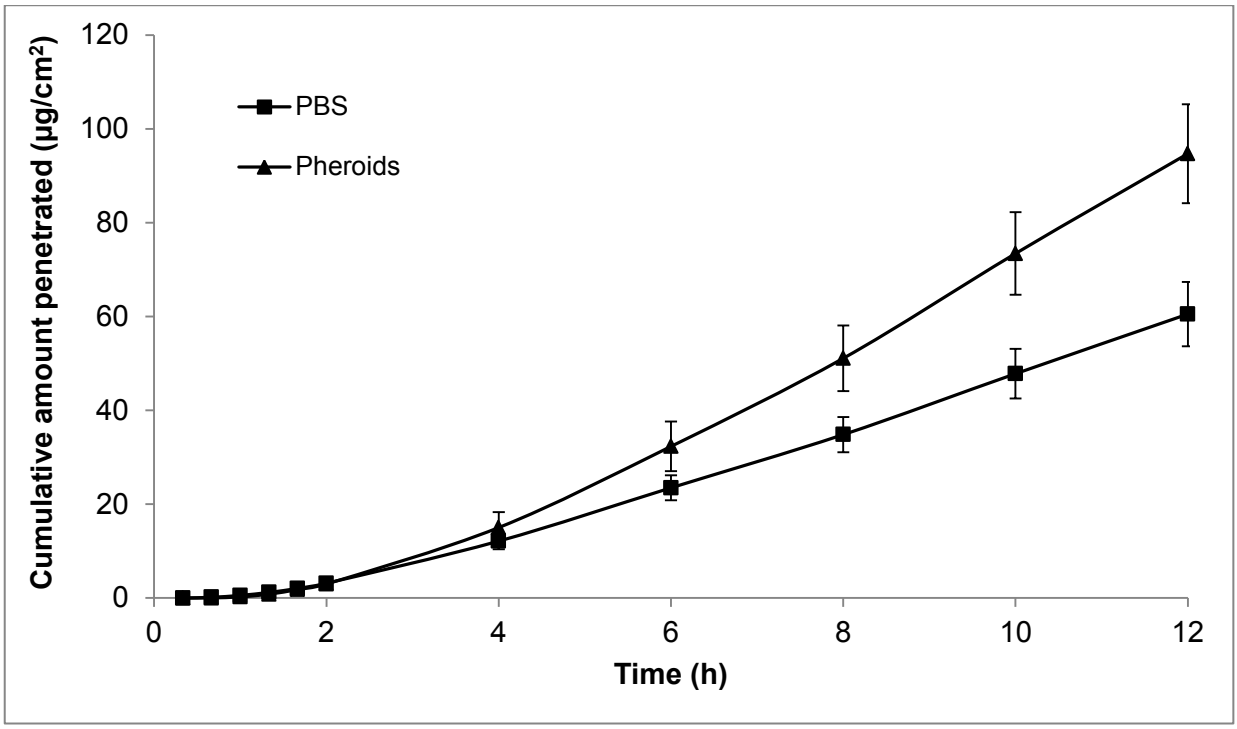


Figure A.2: The cumulative amount of ketoprofen (µg/cm^2) that permeated the skin from both the PBS (pH of 7.4) and Pheroid™ donors during the 12 h diffusion experiments are plotted versus time

Table A.2: Cumulative amount (μg/cm^2) of ketoprofen that permeated through the skin from the PBS (pH of 7.4) and Pheroid™ donor solutions over the 12 h Franz cell diffusion experiments

		Time (h)												
Cell no	**ER (kΩ)**	**0.33**	**0.66**	**1.00**	**1.33**	**1.66**	**2.00**	**4.00**	**6.00**	**8.00**	**10.00**	**12.00**	**J (μg/cm^2.h)**	**r^2**
PBS														
1	17.0	0.00	0.12	0.37	0.98	1.86	3.03	13.86	27.07	40.43	56.42	71.99	7.3	0.999
2	21.7	0.00	0.21	0.74	1.53	2.69	4.06	13.61	25.49	37.52	50.75	63.80	6.3	1.000
3	23.5	0.00	0.23	0.67	1.25	2.15	3.30	12.05	23.44	34.79	48.08	60.73	6.1	0.999
4	19.5	0.00	0.14	0.40	0.79	1.50	2.44	11.12	22.79	34.19	46.92	59.92	6.1	1.000
5	26.3	0.00	0.12	0.35	0.62	1.09	2.03	9.34	19.45	29.68	41.20	52.44	5.4	1.000
6	26.4	0.00	0.19	0.80	1.91	2.92	4.14	13.91	25.28	36.56	49.63	62.51	6.1	0.999
7	31.8	0.00	0.17	0.62	1.34	2.16	3.07	11.07	20.95	30.71	41.75	52.26	5.2	1.000
	Average	**0.0**	**0.2**	**0.6**	**1.2**	**2.1**	**3.2**	**12.1**	**23.5**	**34.8**	**47.8**	**60.5**	**6.1**	
	SD	**0.0**	**0.0**	**0.2**	**0.4**	**0.6**	**0.8**	**1.7**	**2.7**	**3.8**	**5.3**	**6.8**	**0.7**	
	RSD	**0.0**	**26.2**	**33.4**	**36.9**	**31.2**	**24.6**	**14.4**	**11.4**	**10.8**	**11.0**	**11.3**	**11.3**	
Pheroid™														
1	27.0	0.00	0.08	0.37	1.13	2.42	3.67	17.42	35.00	53.75	76.78	100.11	10.4	0.998
2	26.5	0.00	0.15	0.93	2.02	3.55	5.28	20.46	39.94	60.04	82.99	104.97	10.6	0.999
3	19.9	0.00	0.00	0.18	0.76	1.76	2.92	15.63	33.27	51.06	72.40	93.34	9.7	0.999
4	33.7	0.00	0.00	0.00	0.32	1.02	1.90	11.32	25.27	40.80	59.40	76.18	8.2	0.999
5	23.3	0.00	0.00	0.19	0.68	1.61	2.76	14.70	32.99	53.86	77.45	101.03	10.9	0.999
6	18.9	0.00	0.00	0.33	1.00	2.22	3.68	16.91	36.47	57.14	81.86	103.01	10.9	0.999
7	22.8	0.00	0.00	0.00	0.38	1.18	2.26	13.36	31.11	51.33	75.53	97.73	10.7	0.998
8	30.4	0.00	0.00	0.00	0.24	0.74	1.46	10.41	24.54	40.82	61.10	81.34	8.9	0.997
	Average	**0.0**	**0.0**	**0.2**	**0.8**	**1.8**	**3.0**	**15.0**	**32.3**	**51.1**	**73.4**	**94.7**	**10.0**	
	SD	**0.0**	**0.1**	**0.3**	**0.6**	**0.9**	**1.2**	**3.3**	**5.3**	**7.0**	**8.8**	**10.5**	**1.0**	
	RSD	**0.0**	**196.6**	**124.4**	**71.5**	**50.1**	**40.5**	**22.1**	**16.4**	**13.7**	**12.0**	**11.1**	**9.9**	

Table A.3: Cumulative amount (μg/cm^2) of diclofenac sodium that permeated through the skin from the PBS (pH of 7.4) and Pheroid™ donor solutions over the 12 h Franz cell diffusion experiments

		Time (h)												
Cell no	**ER (kΩ)**	**0.33**	**0.66**	**1.00**	**1.33**	**1.66**	**2.00**	**4.00**	**6.00**	**8.00**	**10.00**	**12.00**	**J (μg/cm^2.h)**	**r^2**
PBS														
1	13.8	0.00	0.00	0.29	0.82	1.54	2.37	3.65	12.63	23.10	34.79	47.55	5.5	0.998
2	17.4	0.00	0.00	0.00	0.22	0.54	0.95	1.56	6.41	12.06	18.61	25.91	3.0	0.997
3	14.2	0.00	0.00	0.00	0.16	0.43	0.83	1.32	8.15	16.78	27.26	39.46	4.8	0.994
4	13.2	0.00	0.00	0.24	0.69	1.29	2.16	3.28	15.20	29.73	45.67	62.44	7.4	0.998
5	17.3	0.00	0.00	0.14	0.40	0.82	1.35	2.12	15.75	25.27	36.50	48.94	5.7	0.999
6	12.8	0.00	0.00	0.49	1.30	2.38	3.53	4.81	18.42	34.43	52.79	73.39	8.6	0.997
7	14.5	0.00	0.00	0.00	0.18	0.49	0.92	1.48	8.50	17.73	28.92	42.37	5.1	0.993
	Average	**0.0**	**0.0**	**0.2**	**0.5**	**1.1**	**1.7**	**2.6**	**12.1**	**22.7**	**34.9**	**48.6**	**5.7**	
	SD	**0.0**	**0.0**	**0.2**	**0.4**	**0.7**	**1.0**	**1.3**	**4.5**	**7.8**	**11.5**	**15.5**	**1.8**	
	RSD	**0.0**	**0.0**	**113.0**	**78.6**	**66.8**	**58.1**	**51.1**	**37.4**	**34.4**	**33.0**	**31.9**	**31.5**	
Pheroid™														
1	14.1	0.00	0.00	0.00	0.15	0.37	0.64	1.34	4.91	9.41	14.72	21.00	2.5	0.994
2	16.2	0.00	0.00	0.12	0.32	0.62	1.03	1.63	7.45	14.72	23.50	33.83	4.0	0.994
3	14.3	0.00	0.00	0.00	0.00	0.15	0.37	0.75	5.12	11.07	19.11	28.95	3.5	0.988
4	12.5	0.00	0.00	0.00	0.00	0.16	0.38	0.77	5.00	10.77	18.30	26.23	3.2	0.993
5	12.9	0.00	0.00	0.00	0.00	0.00	0.17	0.48	4.35	9.91	17.10	25.60	3.1	0.990
6	12.6	0.00	0.00	0.00	0.15	0.33	0.59	1.03	6.12	12.70	21.29	30.83	3.7	0.992
7	14.5	0.00	0.38	0.79	1.13	1.52	2.11	2.59	6.70	11.77	17.91	25.01	2.8	0.994
8	17.3	0.00	0.00	0.00	0.00	0.14	0.65	0.96	4.05	7.89	13.52	19.85	2.4	0.989
	Average	**0.0**	**0.0**	**0.1**	**0.2**	**0.4**	**0.7**	**1.2**	**5.5**	**11.0**	**18.2**	**26.4**	**3.2**	
	SD	**0.0**	**0.1**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**1.2**	**2.1**	**3.2**	**4.7**	**0.6**	
	RSD	**0.0**	**282.8**	**241.8**	**177.0**	**118.1**	**82.0**	**56.1**	**21.6**	**19.0**	**17.9**	**17.9**	**18.8**	

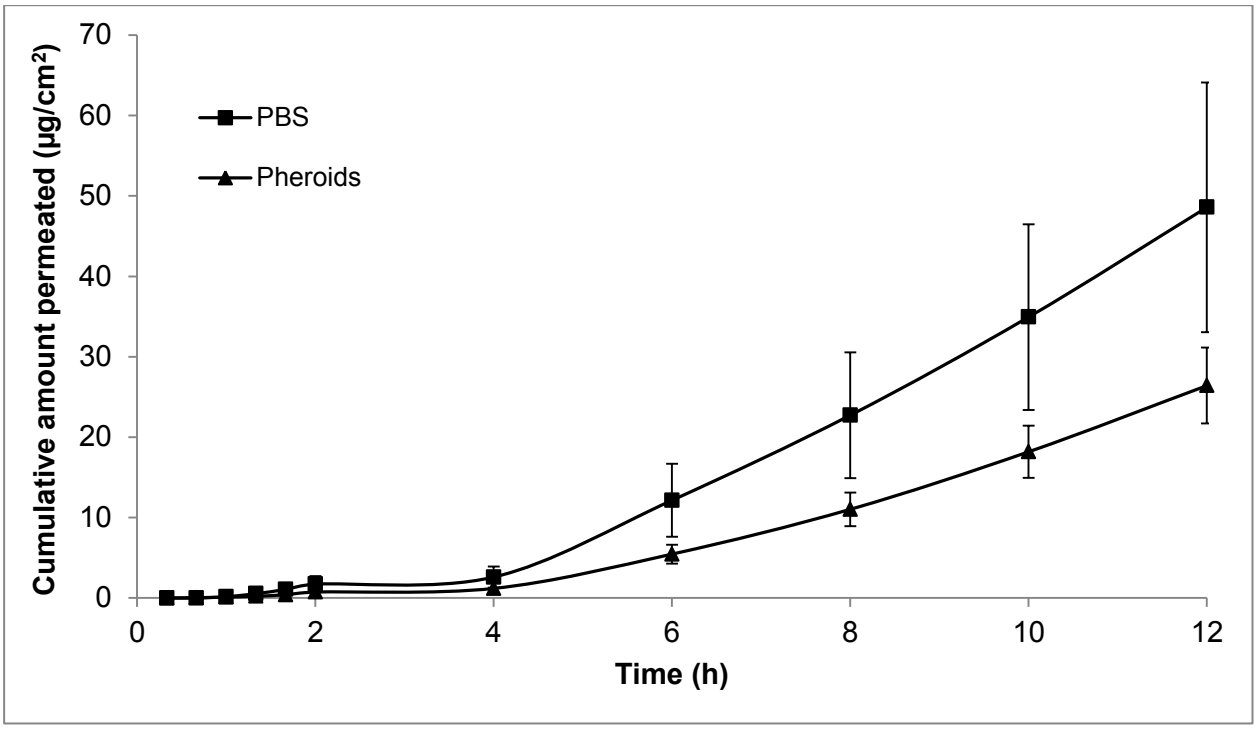


Figure A.3: The cumulative amount of diclofenac sodium (µg/cm^2) that permeated the skin from both the PBS (pH of 7.4) and Pheroid™ donors during the 12 h diffusion experiments are plotted versus time

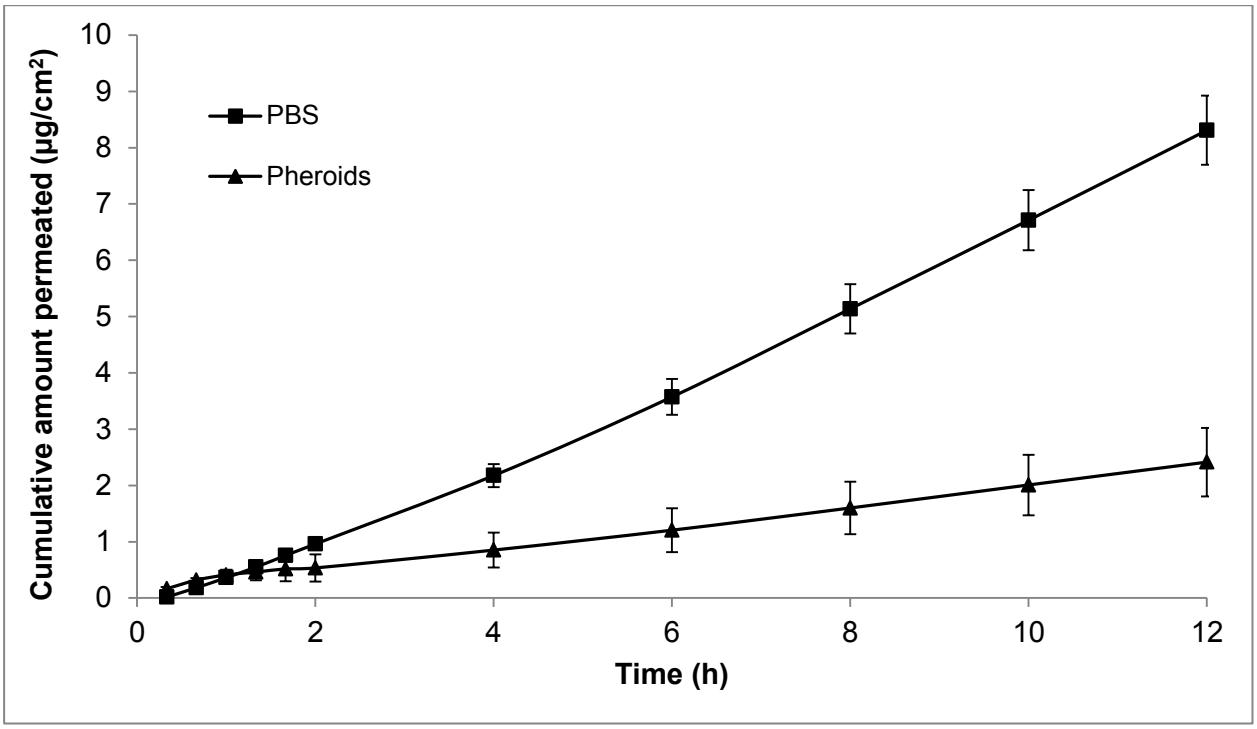


Figure A.4: The cumulative amount of mefenamic acid (µg/cm^2) that permeated the skin from both the PBS (pH of 7.4) and Pheroid™ donors during the 12 h diffusion experiments are plotted versus time

Table A.4: Cumulative amount (μg/cm^2) of mefenamic acid that permeated through the skin from the PBS (pH of 7.4) and Pheroid™ donor solutions over the 12 h Franz cell diffusion experiments

		Time (h)												
Cell no	**ER (kΩ)**	**0.33**	**0.66**	**1.00**	**1.33**	**1.66**	**2.00**	**4.00**	**6.00**	**8.00**	**10.00**	**12.00**	**J (μg/cm^2.h)**	**r^2**
PBS														
1	14.6	0.00	0.14	0.31	0.50	0.70	0.90	2.05	3.43	4.96	6.51	8.07	0.71	0.999
2	20.7	0.00	0.19	0.34	0.49	0.68	0.85	1.85	3.06	4.45	5.80	7.34	0.63	0.998
3	12.9	0.00	0.17	0.34	0.53	0.71	0.91	2.15	3.56	5.15	6.81	8.53	0.74	0.998
4	14.6	0.00	0.17	0.38	0.60	0.86	1.09	2.51	4.13	5.87	7.54	9.31	0.81	0.999
5	15.4	0.00	0.18	0.36	0.55	0.75	0.95	2.17	3.53	5.06	6.65	8.17	0.71	0.999
6	15.8	0.00	0.16	0.33	0.53	0.73	0.94	2.25	3.64	5.41	7.10	8.68	0.77	0.999
7	19.4	0.13	0.28	0.47	0.65	0.87	1.07	2.27	3.65	5.06	6.58	8.07	0.69	0.999
	Average	**0.0**	**0.2**	**0.4**	**0.5**	**0.8**	**1.0**	**2.2**	**3.6**	**5.1**	**6.7**	**8.3**	**0.72**	
	SD	**0.0**	**0.0**	**0.1**	**0.1**	**0.1**	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.06**	
	RSD	**264.6**	**26.0**	**14.2**	**10.2**	**10.1**	**9.3**	**9.4**	**8.9**	**8.5**	**8.0**	**7.4**	**7.96**	
Pheroid™														
1	18.9	0.15	0.29	0.43	0.56	0.73	0.86	1.26	1.67	2.14	2.58	3.04	0.22	0.999
2	21.3	0.19	0.34	0.47	0.63	0.75	0.75	1.13	1.55	2.00	2.43	2.85	0.21	0.999
3	15.7	0.17	0.34	0.55	0.66	0.83	0.83	1.28	1.76	2.25	2.79	3.31	0.24	0.999
4	32.2	0.21	0.35	0.48	0.48	0.48	0.48	0.67	0.87	1.12	1.42	1.70	0.11	0.993
5	32.7	0.14	0.28	0.39	0.39	0.39	0.39	0.59	0.82	1.09	1.41	1.73	0.13	0.993
6	27.7	0.13	0.27	0.27	0.27	0.27	0.27	0.65	1.06	1.53	2.00	2.45	0.21	0.997
7	23.8	0.18	0.37	0.37	0.37	0.37	0.37	0.60	0.89	1.22	1.59	1.96	0.15	0.994
8	22.3	0.15	0.32	0.32	0.32	0.32	0.32	0.64	1.01	1.44	1.85	2.26	0.19	0.996
	Average	**0.2**	**0.3**	**0.4**	**0.5**	**0.5**	**0.5**	**0.9**	**1.2**	**1.6**	**2.0**	**2.4**	**0.18**	
	SD	**0.0**	**0.0**	**0.1**	**0.1**	**0.2**	**0.2**	**0.3**	**0.4**	**0.5**	**0.5**	**0.6**	**0.05**	
	RSD	**16.7**	**10.6**	**22.1**	**31.5**	**42.1**	**45.0**	**36.6**	**32.3**	**29.3**	**26.7**	**25.1**	**25.82**	

Table A.5: Cumulative amount (μg/cm^2) of acetaminophen that permeated through the skin from the PBS (pH of 7.4) and Pheroid™ donor solutions over the 12 h Franz cell diffusion experiments

		Time (h)												
Cell no	**ER (kΩ)**	**0.33**	**0.66**	**1.00**	**1.33**	**1.66**	**2.00**	**4.00**	**6.00**	**8.00**	**10.00**	**12.00**	**J (μg/cm^2.h)**	**r^2**
PBS														
1	15.8	0.00	0.61	1.20	1.70	2.08	2.41	2.94	3.22	3.93	5.19	7.04	0.78	0.994
2	12.2	0.00	2.11	3.91	5.47	6.93	8.20	8.75	9.89	11.99	15.19	19.75	1.94	0.995
3	14.3	0.00	1.34	2.13	2.62	3.15	3.52	8.47	13.42	19.66	26.23	33.03	3.34	1.000
4	22	0.00	1.12	1.78	2.23	2.66	3.05	3.61	4.66	6.57	9.63	14.10	1.88	0.994
5	18.1	0.00	0.95	1.57	2.12	2.52	2.91	3.08	3.45	4.17	5.34	7.07	0.72	0.994
6	12.5	0.00	0.91	1.54	2.07	2.46	2.82	3.11	3.67	4.65	6.10	8.10	0.86	0.996
7	12.5	0.00	0.98	1.64	2.04	2.41	2.78	3.11	3.98	5.59	8.41	12.86	1.82	0.992
	Average	**0.0**	**1.1**	**2.0**	**2.6**	**3.2**	**3.7**	**4.7**	**6.0**	**8.1**	**10.9**	**14.6**	**1.62**	
	SD	**0.0**	**0.5**	**0.9**	**1.3**	**1.7**	**2.0**	**2.7**	**4.0**	**5.8**	**7.6**	**9.4**	**0.94**	
	RSD	**0.0**	**41.9**	**45.9**	**49.6**	**53.2**	**55.2**	**56.4**	**66.1**	**71.8**	**70.1**	**64.3**	**57.75**	
Pheroid™														
1	11.3	0.00	1.82	3.52	5.18	6.60	8.22	8.64	9.60	11.30	13.94	17.69	1.60	0.995
2	12.6	0.00	0.81	1.35	1.81	2.13	2.51	8.48	15.12	22.63	30.73	39.54	4.23	1.000
3	14.9	0.00	0.73	1.23	1.72	2.06	2.42	2.96	4.17	6.37	9.64	14.29	1.98	0.995
4	15.3	0.00	0.88	1.40	1.82	2.24	2.67	3.07	3.98	5.56	7.67	10.49	1.23	0.997
5	16.3	0.00	0.83	1.50	2.01	2.38	2.77	3.40	4.42	6.17	7.86	11.34	1.29	0.980
6	15.1	0.00	1.01	1.53	2.07	2.38	2.82	3.09	3.80	4.96	6.67	8.83	0.97	0.998
7	18.6	0.00	0.74	1.21	1.66	2.04	2.35	2.76	3.85	6.14	9.56	14.46	2.08	0.995
8	16.3	0.00	0.72	1.39	1.85	2.25	2.64	2.96	3.62	5.08	7.25	10.54	1.37	0.993
	Average	**0.0**	**0.9**	**1.6**	**2.3**	**2.8**	**3.3**	**4.4**	**6.1**	**8.5**	**11.7**	**15.9**	**1.84**	
	SD	**0.0**	**0.4**	**0.8**	**1.2**	**1.6**	**2.0**	**2.6**	**4.2**	**6.0**	**8.0**	**10.0**	**1.03**	
	RSD	**0.0**	**38.9**	**46.8**	**52.4**	**56.5**	**60.5**	**57.9**	**68.5**	**70.9**	**68.9**	**62.7**	**56.14**	

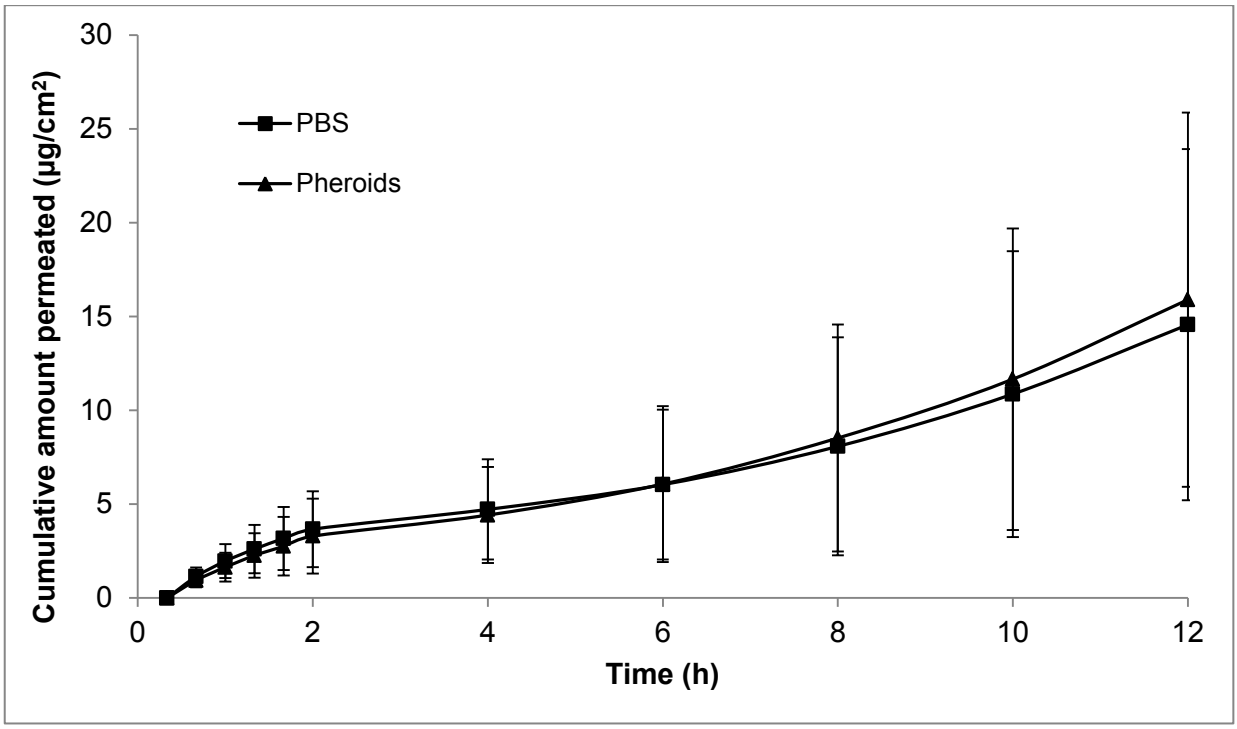


Figure A.5: The cumulative amount of acetaminophen (µg/cm^2) that permeated the skin from both the PBS (pH of 7.4) and Pheroid™ donors during the 12 h diffusion experiments are plotted versus time

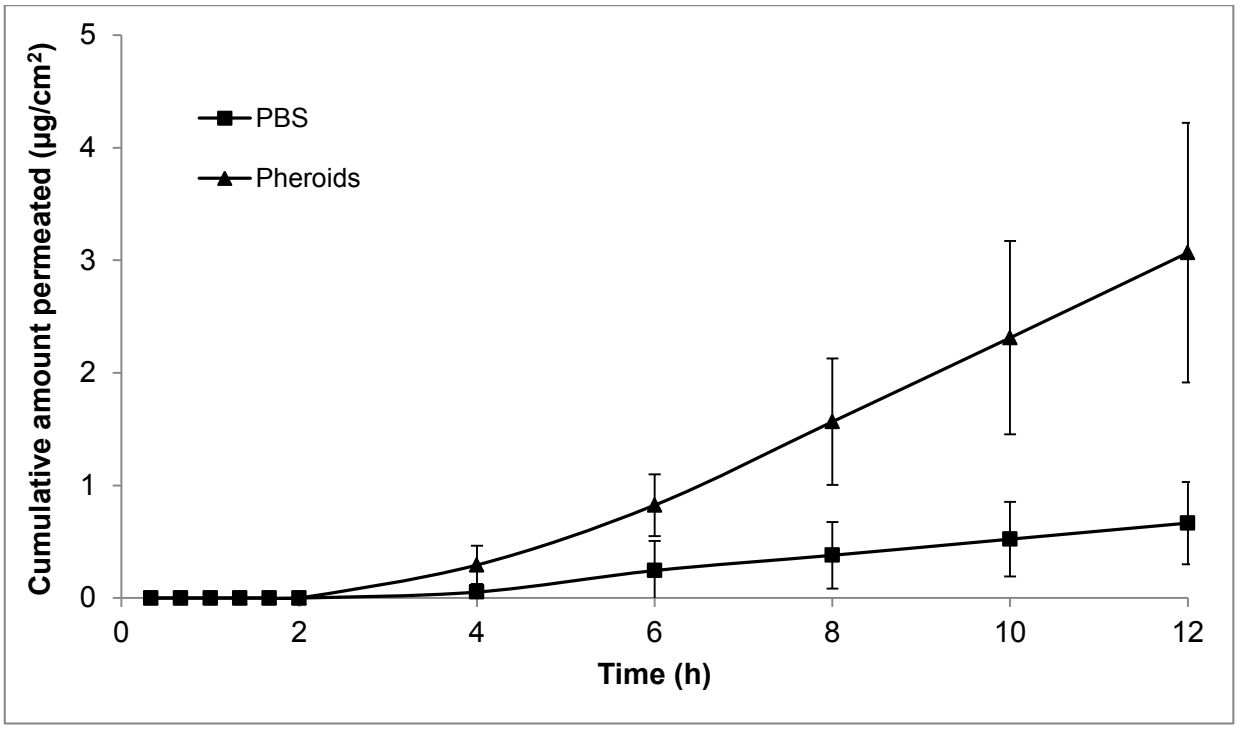


Figure A.6: The cumulative amount of piroxicam (µg/cm^2) that permeated the skin from both the PBS (pH of 7.4) and Pheroid™ donors during the 12 h diffusion experiments are plotted versus time

Table A.6: Cumulative amount (μg/cm^2) of piroxicam that permeated through the skin from the PBS (pH of 7.4) and Pheroid™ donor solutions over the 12 h Franz cell diffusion experiments

		Time (h)												
Cell no	**ER (kΩ)**	**0.33**	**0.66**	**1.00**	**1.33**	**1.66**	**2.00**	**4.00**	**6.00**	**8.00**	**10.00**	**12.00**	**J (μg/cm^2.h)**	**r^2**
PBS														
1	14.3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.20	0.36	0.51	0.066	0.989
2	22.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.14	0.22	0.31	0.039	0.997
3	17.6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.14	0.24	0.34	0.042	0.997
4	21.2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.24	0.35	0.47	0.058	0.999
5	11.3	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.31	0.47	0.63	0.78	0.082	0.999
6	12.8	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.29	0.50	0.73	0.94	0.099	0.994
7	12.6	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.79	0.96	1.14	1.31	0.140	0.934
	Average	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.1**	**0.2**	**0.4**	**0.5**	**0.7**	**0.075**	
	SD	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.1**	**0.3**	**0.3**	**0.3**	**0.4**	**0.036**	
	RSD	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**135.3**	**107.9**	**78.0**	**63.4**	**54.8**	**47.5**	
Pheroid™														
1	18.5	0.00	0.00	0.00	0.00	0.00	0.00	0.50	0.89	1.85	2.81	3.83	0.429	0.990
2	16.4	0.00	0.00	0.00	0.00	0.00	0.00	0.23	0.42	0.90	1.39	1.89	0.214	0.990
3	22.8	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.50	0.77	1.05	1.36	0.152	0.998
4	23	0.00	0.00	0.00	0.00	0.00	0.00	0.25	1.12	1.62	2.16	2.72	0.299	0.994
5	21.1	0.00	0.00	0.00	0.00	0.00	0.00	0.56	1.17	2.31	3.35	4.43	0.495	0.996
6	14.2	0.00	0.00	0.00	0.00	0.00	0.00	0.38	0.81	1.58	2.37	3.16	0.355	0.995
7	19.2	0.00	0.00	0.00	0.00	0.00	0.00	0.21	0.69	1.29	1.94	2.60	0.301	0.998
8	13.4	0.00	0.00	0.00	0.00	0.00	0.00	0.10	1.00	2.21	3.43	4.56	0.568	0.999
	Average	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.3**	**0.8**	**1.6**	**2.3**	**3.1**	**0.352**	
	SD	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.2**	**0.3**	**0.6**	**0.9**	**1.2**	**0.140**	
	RSD	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**58.2**	**33.2**	**35.9**	**37.2**	**37.6**	**39.8**	

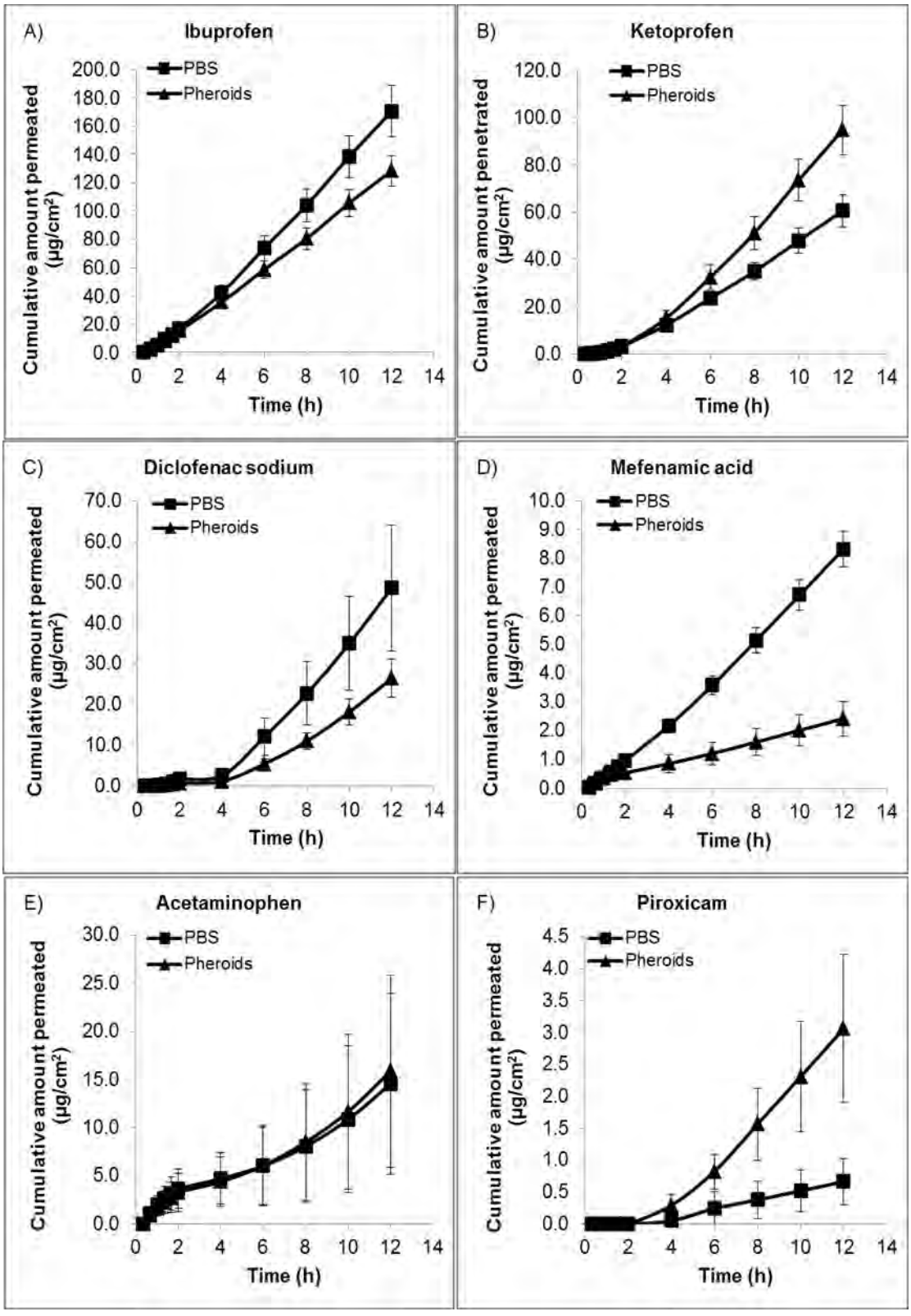


Figure A.7: A summary of the average cumulative amount against time for the different NSAIDs that permeated the skin over a 12 h period from both the PBS (pH of 7.4) and Pheroid™ donor solutions

A.2 Discussion

In Figure 7 the cumulative amount of each API that permeated the skin during the 12 h diffusion experiments from both the Pheroid™ and the control PBS (pH of 7.4) donor solutions are plotted versus time. It is clear from the graphical representation of the results that there were differences in the flux for the different APIs from the different delivery vehicles and also for the same API from the two respective donor solutions. The reasons for these differences in the data are explored in the different preceding articles of this study.

Annexure B: Current Pharmaceutical Design: Guide for Authors

B.1 Online manuscript submission

An online submission and tracking service *via* Internet facilitates a speedy and cost-effective submission of manuscripts. The full manuscript has to be submitted online *via* Bentham's Content Management System (CMS) at bsp-cms.eurekaselect.com

Manuscripts must be submitted by one of the authors of the manuscript, and should not be submitted by anyone on their behalf. The principal/corresponding author will be required to submit a Copyright Letter along with the manuscript, on behalf of all the co-authors (if any). The author(s) will confirm that the manuscript (or any part of it) has not been published previously or is not under consideration for publication elsewhere. Furthermore, any illustration, structure or table that has been published elsewhere must be reported, and copyright permission for reproduction must be obtained.

For all online submissions, provide soft copies of all the materials (main text in MS Word or Tex/LaTeX), figures/illustrations in TIFF, PDF or JPEG, and chemical structures drawn in ChemDraw (CDX)/ISISDraw (TGF) as separate files, while a PDF version of the entire manuscript must also be included, embedded with all the figures/illustrations/tables/chemical structures *etc*. It is advisable that the document files related to a manuscript submission should always have the name of the corresponding author as part of the file name, i.e., “Cilli MS text.doc”, “Cilli MS Figure 1”, etc.

It is imperative that before submission, authors should carefully proofread the files for special characters, mathematical symbols, Greek letters, equations, tables, references and images, to ensure that they appear in proper format.

References, figures, tables, chemical structures etc. should be referred to in the text at the appropriate place where they have been first discussed. Figure legends/captions should also be provided.

A successful electronic submission of a manuscript will be followed by a system-generated acknowledgement to the principal/corresponding author. Any queries therein should be addressed to manuscript@benthamscience.org

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The Journal publishes peer-reviewed mini- and full-length review articles and research papers written in English. Single topic/thematic issues may also be considered for publication.

B.3.1 Single topic issues

These special issues are peer-reviewed and may contain invited or uninvited review/mini-review articles. A Single Topic Issue Editor will offer a short perspective and co-ordinate the solicitation of manuscripts between 3-5 (for a mini-hot topic) to 6-10 (for full-length hot topic) from leading scientists. Authors interested in editing a single topic issue in an emerging topic of drug design may submit their proposal to the Editor-in-Chief at cpd@benthamscience.org for consideration.

B.3.2 Conference proceedings

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B.4 Manuscript length

B.4.1 Mini-reviews

Mini-reviews should be 3000-6000 words excluding figures, structures, photographs, schemes, tables, etc.

B.4.2 Full-length reviews

Full-length reviews should be 8000-40000 words excluding figures, structures, photographs, schemes, tables, etc.

B.4.3 Research articles

Research articles should be 4000-8000 words excluding figures, structures, photographs, schemes, tables, etc. There is a quota of 20% of published Research articles per issue in this journal.

There is no restriction on the number of figures, tables or additional files e.g. video clips, animation and datasets, that can be included with each article online. Authors should include all relevant supporting data with each article (Refer to Supplementary Material section).

B.5 Manuscript preparation

The manuscript should be written in English in a clear, direct and active style. All pages must be numbered sequentially, facilitating in the reviewing and editing of the manuscript. Download the Template

For further convenience, our contracted service provider Eureka Science can provide assistance to authors for the preparation of manuscripts.

B.6 Manuscript sections for papers

Manuscripts may be divided into the following sections:

- Copyright letter
- Title
- Title page
- Structured abstract
- Graphical abstract
- Keywords
- Text organization
- Conclusion
- List of abbreviations (if any)
- Conflict of interest
- Acknowledgements
- References
- Appendices
- Figures/illustrations (if any)
- Chemical structures (if any)
- Tables (if any)
- Supportive/supplementary material (if any)

B.6.1 Copyright letter

It is mandatory that a signed copyright letter should also be submitted along with the manuscript by the author to whom correspondence is to be addressed, delineating the scope of the submitted article declaring the potential competing interests, acknowledging contributions from authors and funding agencies, and certifying that the paper is prepared according to the *'Instructions for Authors'*. All inconsistencies in the text and in the reference section and any typographical errors must be carefully checked and corrected before the submission of the manuscript. The article should not contain any such material or information that may be unlawful, defamatory, fabricated, plagiarized, or which would, if published, in any way whatsoever, violate the terms and conditions as laid down in the copyright agreement. The authors acknowledge that the publishers have the legal right to take appropriate action against the authors for any such violation of the terms and conditions as laid down in the copyright agreement. Download the Copyright letter

B.6.2 Title

The title of the article should be precise and brief and must not be more than 120 characters. Authors should avoid the use of non-standard abbreviations. The title must be written in title case except for articles, conjunctions and prepositions.

Authors should also provide a short 'running title'. Title, running title, byline, correspondent footnote and keywords should be written as presented in original manuscript.

B.6.3 Title page

Title page should include paper title, author(s) full name and affiliation, corresponding author(s) names complete affiliation/address, phone, fax, email and keyword should be written as presented in original manuscript.

B.6.4 Structured abstract

The abstract of an article should be its clear, concise and accurate summary, having no more than 250 words, and including the explicit sub-headings (as in-line or run-in headings in bold). Use of abbreviations should be avoided and the references should not be cited in the abstract. Ideally, each abstract should include the following sub-headings, but these may vary according to requirements of the article.

- Background
- Objective

- Method
- Results
- Conclusion

B.6.5 Graphical abstract

A graphic must be included with each manuscript for use in the Table of Contents (TOC). This must be submitted separately as an electronic file (preferred file types are EPS, PDF, TIFF, Microsoft Word, PowerPoint and CDX etc.). A graphical abstract, not exceeding 30 words along with the illustration, helps to summarize the contents of the manuscript in a concise pictorial form. It is meant as an aid for the rapid viewing of the journals' contents and to help capture the readers' attention. The graphical abstract may feature a key structure, reaction, equation, etc. that the manuscript elucidates upon. It will be listed along with the manuscript title, authors' names and affiliations in the contents page, typeset within an area of 5 cm by 17 cm, but it will not appear in the article PDF file or in print.

Graphical Abstracts should be submitted as a separate file (must clearly mention graphical abstract within the file) online *via* Bentham's Content Management System by selecting the option "supplementary material".

B.6.6 Keywords

6 to 8 keywords must be provided.

B.6.7 Text organization

The main text should begin on a separate page and should be divided into title page, abstract and the main text. The text may be subdivided further according to the areas to be discussed, which should be followed by the Acknowledgements and Reference sections. For Research Articles the manuscript should begin with the title page and abstract followed by the main text, which must be structured into separate sections as Introduction, Materials and Methods, Results, Discussion, and Conclusion, List of Abbreviations, Conflict of Interest, Acknowledgements, and References. The Review article should mention any previous important old and recent reviews in the field and contain a comprehensive discussion starting with the general background of the field. It should then go on to discuss the salient features of recent developments. The authors should avoid presenting material which has already been published in a previous review. The authors are advised to present and discuss their observations in brief. The manuscript style must be uniform throughout the text and 10 pt Times New Roman fonts should be used. The full term for an abbreviation should precede its

first appearance in the text unless it is a standard unit of measurement. The reference numbers should be given in square brackets in the text. Italics should be used for Binomial names of organisms (Genus and Species), for emphasis and for unfamiliar words or phrases. Non-assimilated words from Latin or other languages should also be italicized e.g. *per se, et al. etc.*

B.6.7.1 Standard protocol on approvals, registrations, patient consents & animal protection

All clinical investigations must be conducted according to the Declaration of Helsinki principles. For all manuscripts reporting data from studies involving human participants, formal review and approval by an appropriate institutional review board or ethics committee is required. For research involving animals, the authors should indicate whether the procedures followed were in accordance with the standards set forth in the eighth edition of Guide for the Care and Use of Laboratory Animals (grants.nih.gov/grants/olaw/Guide-for-the-care-and-use-of-Laboratory-animals/; published by the National Academy of Sciences, The National Academies Press, Washington, D.C.).

A specific declaration of such approval must be made in the copyright letter and in a stand-alone paragraph at the end of the Methods section especially in the case of human studies where inclusion of a statement regarding obtaining the written informed consent from each subject or subject's guardian is a must. The original should be retained by the guarantor or corresponding author. Editors may request to provide the original forms by fax or email.

B.6.7.2 Authentication of cell lines

The NIH acknowledges the misidentification and/or cross-contamination of cell cultures e.g. HeLa cells being used in a research study as a serious problem. In order to ensure the validation of the work and proper utilization of resources, it is a prerequisite that correct reagents be used in studies dealing with established human (tumor) cell lines that have been cultured for more than 4 years up to the date of submission of the manuscript. Cell lines such as short-term cultures of human tumors, murine cell lines (as a catalog of DNA profiles is not yet available) and tumor cell lines established in the course of the study that is being submitted, are presently exempt from this rule. To minimize the risk of working with misidentified and/or contaminated cell lines, tests such as isoenzyme analysis, karyotyping/cytogenetic analysis and, more recently, molecular techniques of DNA profiling may be carried out to authenticate cell cultures. These tests may help confirm or establish the identify profile for a cell line. Bentham Science recommends that all cell lines be authenticated prior to submitting a paper for review. Authors are therefore required to provide authentication of the origin and identity of the cells by performing cell profiling either in their own laboratory or by outsourcing an approved

laboratory or cell bank. Authentication is required when a new line is established or acquired, before freezing a cell line, if the performance of the line is not consistent or results are unexpected, if using more than one cell line, and before publication of the study.

The cell lines profile should be cross-checked with the profile of the donor tissue of other continuous cell lines such as provided by the authentic data bank such as www.dsmz.de/fp/cgi-bin/str.html, ATCC®, etc.

B.6.7.3 Greek symbols and special characters

Greek symbols and special characters often undergo formatting changes and get corrupted or lost during preparation of manuscript for publication. To ensure that all special characters used are embedded in the text, these special characters should be inserted as a symbol but should not be a result of any format styling (Symbol font face) otherwise they will be lost during conversion to PDF/XML comprising a list of items relevant to their specific research design. Chemical equations, chemical names, mathematical usage, unit of measurements, chemical and physical quantity & units must conform to SI and Chemical Abstracts or IUPAC.

All kinds of measurements should be reported only in International System of Units (SI).

B.6.8 Conclusion

A small paragraph summarizing the contents of the article, presenting the final outcome of the research or proposing further study on the subject, may be given at the end of the article under the Conclusion section.

B.6.9 List of abbreviations

If abbreviations are used in the text either they should be defined in the text where first used, or a list of abbreviations can be provided.

B.6.10 Conflict of interest

Financial contributions and any potential conflict of interest must be clearly acknowledged under the heading 'Conflict of Interest'. Authors must list the source(s) of funding for the study. This should be done for each author.

B.6.11 Acknowledgements

All individuals listed as authors must have contributed substantially to the design, performance, analysis, or reporting of the work and are required to indicate their specific contribution. Anyone

(individual/company/institution) who has substantially contributed to the study for important intellectual content, or who was involved in the article's drafting the manuscript or revising must also be acknowledged.

Guest or honorary authorship based solely on position (e.g. research supervisor, departmental head) is discouraged.

The specific requirements for authorship have been defined by the International Committee of Medical Journal Editors (ICMJE; www.icmje.org). Examples of authors' contributions are: 'designed research/study', 'performed research/study', 'contributed important reagents', 'collected data', 'analyzed data', 'wrote paper' etc. This information must be included in the submitted manuscript as a separate paragraph under the heading 'Acknowledgements'. The corresponding author is responsible for obtaining permission from all co-authors for the submission of any version of the manuscript and for any changes in the authorship.

B.6.12 References

References must be listed in the numerical system (Vancouver). All references should be numbered sequentially [in square brackets] in the text and listed in the same numerical order in the reference section. The reference numbers must be finalized and the bibliography must be fully formatted before submission.

See below few examples of references listed in the correct Vancouver style:

B.6.12.1 Typical paper reference

[1] Boehm M, Nabel EG. Angiotensin-converting enzyme 2-a new cardiac regulator. N Engl J Med 2002; 347: 1795-7.

[2] SoRelle R. Long reach of the N-terminal of B-type natriuretic peptide. Circulation 2002; 106: 9059-63.

[3] Leone A. Biochemical markers of cardiovascular damage from tobacco smoke. Curr Pharm Des 2005; 11: 2199-208.

[4] Meuillet EJ, Mahadevan D, Vankayalapati H, *et al.* Specific inhibition of the Akt1 pleckstrin homology domain by D-3-deoxyphosphatidyl- myo-inositol analogues. Mol Cancer Ther 2003; 2: 389-99.

B.6.12.2 Typical chapter reference

[5] Ban Y, Tomer Y. Endocrine diseases. Graves's and Hashimoto's diseases. In: Oksenberg J, Brassat D, Eds. Immunogenetics of autoimmune disease. Medical Intelligence Unit. New York: Landes Bioscience and Springer Science-Business Media 2006; pp. 41-58.

[6] Astrup P. The arterial wall in atherogenesis. In Cavallero Ed. Atherogenesis. Padua: Piccin Medical Books 1965: pp. 77-92.

B.6.12.3 Book reference

[7] Carlson BM. Human embryology and developmental biology. 3rd ed. St. Louis: Mosby 2004.

[8] Rosai J. Histological typing of tumours of the thymus. 2nd ed. Berlin and Heidelberg: Springer-Verlag 1999.

B.6.12.4 Edited book

[9] Brown AM, Stubbs DW, Eds. Medical physiology. New York: Wiley 1983.

B.6.12.5 Conference Paper and Proceedings:

[10] Kimura J, Shibasaki H, Eds. Recent advances in clinical neurophysiology. Proceedings of the 10th International Congress of EMG and Clinical Neurophysiology; 1995 Oct 15-19; Kyoto, Japan. Amsterdam: Elsevier 1996.

[11] Cooper R, Cutler J, Desvigne-Nickens P, et al. Disparities in coronary heart disease, stroke, and other cardiovascular diseases in the United States: findings of the National Conference on Cardiovascular Disease Prevention. Circulation 2000; 102: 3137-47.

[12] Chao JT, Theriault A, Gapor A. Delta-tocotrienol is a potent inhibitor of monocyte-endothelial cell adhesion. In: Cutting edge technologies For sustained competitiveness. Proceedings of the 2001 PI POC International palm Oil Congress, Food Technology and Nutrition Conference, Kuala Lumpur, Malaysia, Aug. 20-22, 2001; pp. 225-34.

B.6.12.6 Journal article on the internet

[13] Aylin P, Bottle A, Jarman B, Elliott P. Paediatric cardiac surgical mortality in England after Bristol: descriptive analysis of hospital episode statistics 1991-2002. BMJ [serial on the Internet]. 2004 Oct 9; [cited 2004 October 15]; 329: [about 10 screens]. Available from: (bmj.bmjjournals.com/cgi/content/full/329/7470/825)

[14] NCT01013350. Prospective observational long-term safety registry of multiple sclerosis patients who have participated in cladribine clinical trials (PREMIERE). Available at (clinicaltrials.gov/ct2/show/NCT01013350)

B.6.12.7 Book/monograph on the internet

[15] Donaldson MS, Ed. Measuring the quality of health care [monograph on the internet]. Washington: National Academy Press 1999 [cited 2004 Oct 8]. Available from: (legacy.netlibrary.com)

[16] Silva ATA. Ed. Development of compounds potentially active against Helicobacter pylori. [monograph on the internet]. Araraquara: School of Pharmaceutical Science, Universidade Estadual Paulista 2008 [cited 2010 July 10]. Available from: (www.fcfar.unesp.br/posgraduacao/cienciasfarmaceuticas/Disertacoes/2008/antonio_tavora-completo.pdf) [monograph in Portuguese].

B.6.12.8 Web site/homepage

[17] HeartCentreOnline [homepage on the Internet]. Boca Raton, FL: HeartCentreOnline, Inc.; c2000-2004 [updated 2004 May 23; cited 2004 Oct 15]. Available from: (www.heartcentralonline.com)

[18] Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF), Global discovery program for novel anti-tuberculosis drugs [homepage on the Internet]. The National Institute of Allergy and Infectious Diseases (NIAID) [cited 2011 Jan10]. Available from: (www.taacf.org)

B.6.12.9 Journal with part/supplement

If a journal carries continuous pagination throughout the volume, then the issue number can be omitted.

B.6.12.10 Issue with supplement

[19] Glauser TA. Integrating clinical trial data into clinical practice. Neurology 2002; 58(12 Suppl 7): S6-12.

[20] Durandy A, Kaveri SV, Kuijpers TW, *et al* . Intravenous immunoglobulins-- understanding properties and mechanisms. Clin Exp Immunol 2009; 158(Suppl 1): 2-13.

B.6.12.11 Volume with part

[21] Abend SM, Kulish N. The psychoanalytic method from an epistemological viewpoint. Int J Psychoanal 2002; 83(Pt 2): 491-5

[22] O'Riordan JI, Thompson AJ, Kingsley DP, *et al* . The prognostic value of brain MRI in clinically isolated syndromes of the CNS. A 10-year follow-up. Brain 1998; 121(Pt 3): 495-503.

B.6.12.12 Issue with part

[23] Ahrar K, Madoff DC, Gupta S, Wallace MJ, Price RE, Wright KC. Development of a large animal model for lung tumors. J Vasc Interv Radiol 2002; 13(9 Pt 1): 923-8.

[24] Bluestone JA. Costimulation and its role in organ transplantation. Clin transplant 1996; 10(1 Pt 2): 104-9.

B.6.12.13 Patent

Pagedas AC, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug.

B.6.12.14 E-citations

Citations for articles/material published exclusively online or in open access (free-to-view), must contain the accurate Web addresses (URLs) at the end of the reference(s), except those posted on an author's Web site (unless editorially essential), e.g. 'Reference: Available from: URL'.

Some important points to remember:

- All references must be complete and accurate.
- If the number of authors exceeds six then *et al*. will be used after three names (the term "*et al*." should be in italics).
- Date of access should be provided for online citations.
- Journal names should be abbreviated according to the Index Medicus/MEDLINE.
- Punctuation should be properly applied as mentioned in the examples given above.
- Superscript in the in-text citations and reference section should be avoided.
- Abstracts, unpublished data and personal communications (which can only be included if prior permission has been obtained) should not be given in the references section. The details may however appear in the footnotes.
- The authors are encouraged to use a recent version of End Note (version 5 and above) or Reference Manager (version 10) when formatting their reference list, as this allows references to be automatically extracted.

B.6.13 Appendices

In case there is a need to present lengthy, but essential methodological details, use appendices, which can be a part of the article. An appendix must not exceed three pages (Times New Roman, 12 point fonts, 900 max. words per page). The information should be

provided in a condensed form, ruling out the need of full sentences. A single appendix should be titled APPENDIX, while more than one can be titled APPENDIX A, APPENDIX B, and so on.

B.6.14 Figures/illustrations

All authors must strictly follow the guidelines below for preparing illustrations for publication in ***Current Pharmaceutical Design***. If the figures are found to be sub-standard, then the manuscripts will be rejected and the authors offered the option of figure improvement professionally by. The costs for such improvement will be charged to the authors.

Illustrations should be provided as separate files, embedded in the text file, and must be numbered consecutively in the order of their appearance. Each figure should include only a single illustration which should be cropped to minimize the amount of space occupied by the illustration.

If a figure is in separate parts, all parts of the figure must be provided in a single composite illustration file.

Photographs should be provided with a scale bar if appropriate, as well as high-resolution component files.

B.6.14.1 Scaling/resolution

Line Art image type is normally an image based on lines and text. It does not contain tonal or shaded areas. The preferred file format should be TIFF or EPS, with the color mode being Monochrome 1-bit or RGB, in a resolution of 900-1200 dpi.

Halftone image type is a continuous tone photograph containing no text. It should have the preferred file format TIFF, with color mode being RGB or Grayscale, in a resolution of 300 dpi.

Combination image type is an image containing halftone, text or line art elements. It should have the preferred file format TIFF, with color mode being RGB or Grayscale, in a resolution of 500-900 dpi.

B.6.14.2 Formats

Illustrations may be submitted in the following file formats:

- **Illustrator**
- **EPS** (preferred format for diagrams)
- **PDF** (also especially suitable for diagrams)

- **PNG** (preferred format for photos or images)
- **Microsoft Word** (version 5 and above; figures must be a single page)
- **PowerPoint** (figures must be a single page)
- **TIFF**
- **JPEG** (conversion should be done using the original file)
- **BMP**
- **CDX** (ChemDraw)
- **TGF** (ISISDraw)

Bentham Science does not process figures submitted in GIF format.

For TIFF or EPS figures with considerably large file size restricting the file size in online submissions is advisable. Authors may therefore convert to JPEG format before submission as this results in significantly reduced file size and upload time, while retaining acceptable quality. JPEG is a 'lossy' format, however. In order to maintain acceptable image quality, it is recommended that JPEG files are saved at High or Maximum quality.

Zipit or Stuffit tools should not be used to compress files prior to submission as the resulting compression through these tools is always negligible.

Please refrain from supplying:

1. Graphics embedded in word processor (spreadsheet, presentation) document.
2. Optimized files optimized for screen use (like GIF, BMP, PICT, WPG) because of the low resolution.
3. Files with too low a resolution.
4. Graphics that are disproportionately large for the content.

B.6.14.3 Image conversion tools

There are many software packages, many of them freeware or shareware, capable of converting to and from different graphics formats, including PNG.

General tools for image conversion include Graphic Converter on the Macintosh, Paint Shop Pro, for Windows, and ImageMagick, available on Macintosh, Windows and UNIX platforms.

Bitmap images (e.g. screenshots) should not be converted to EPS as they result in a much larger file size than the equivalent JPEG, TIFF, PNG or BMP, and poor quality. EPS should only be used for images produced by vector-drawing applications such as Adobe Illustrator or CorelDraw. Most vector-drawing applications can be saved in, or exported as, EPS format. If the images were originally prepared in an Office application, such as Word or PowerPoint, original Office files should be directly uploaded to the site, instead of being converted to JPEG or another format of low quality.

B.6.14.4 Color figures/illustrations

- The cost for each individual page of color figures/plates/illustrations is **US$ 950**.
- Color figures should be supplied in CMYK and not RGB colors.

B.6.15 Chemical structures

Chemical structures must be prepared in ChemDraw/CDX and provided as separate file.

B.16.15.1 Structure drawing preferences

[As according to the ACS style sheet]

<u>Drawing Settings:</u>	
Chain angle	120°
Bond spacing	18% of width
Fixed length	14.4 pt (0.500cm, 0.2in)
Bold width	2.0 pt (0.071cm, 0.0278in)
Line width	0.6 pt (0.021cm, 0.0084in)
Margin width	1.6 pt (0.096cm)
Hash spacing	2.5 pt (0.088cm, 0.0347in)
<u>Text settings:</u>	
Font	Times New Roman
Size	8 pt
<u>Under the Preference Choose:</u>	
Units	points
Tolerances	3 pixels
<u>Under Page Setup Use:</u>	
Paper	US letter
Scale	100%

B.6.16 Tables

- Data Tables should be submitted in Microsoft Word table format.
- Each table should include a title/caption being explanatory in itself with respect to the details discussed in the table. Detailed legends may then follow.
- Table number in bold font *i.e*. Table **1**, should follow a title. The title should be in small case with the first letter in caps. A full stop should be placed at the end of the title.
- Tables should be embedded in the text exactly according to their appropriate placement in the submitted manuscript.
- Columns and rows of data should be made visibly distinct by ensuring that the borders of each cell are displayed as black lines.
- Tables should be numbered in Arabic numerals sequentially in order of their citation in the body of the text.
- If a reference is cited in both the table and text, please insert a lettered footnote in the table to refer to the numbered reference in the text.
- Tabular data provided as additional files can be submitted as an Excel spreadsheet.

B.6.17 Supportive/supplementary material

We do encourage to append supportive material, for example a PowerPoint file containing a talk about the study, a PowerPoint file containing additional screenshots, a Word, RTF, or PDF document showing the original instrument(s) used, a video, or the original data (SAS/SPSS files, Excel files, Access Db files etc.) provided it is inevitable or endorsed by the journal's Editor.

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Any additional files will be linked to the final published article in the form supplied by the author, but will not be displayed within the paper. They will be made available in exactly the same form

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All manuscripts submitted for publication will be immediately subjected to peer-reviewing, usually in consultation with the members of the Editorial Advisory Board and a number of external referees. Authors may, however, provide in their Copyright Letter the contact details (including e-mail addresses) of four potential peer reviewers for their paper. Any peer reviewers suggested should not have recently published with any of the authors of the submitted manuscript and should not be members of the same research institution.

All peer-reviewing will be conducted *via* the Internet to facilitate rapid reviewing of the submitted manuscripts. Every possible effort will be made to assess the manuscripts quickly with the decision being conveyed to the authors in due course. Papers which are delayed by authors in revision for more than 30 days will have to be re-submitted as a new submission.

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The text of every submitted manuscript is checked using the Content Tracking mode in iThenticate. The Content Tracking mode ensures that manuscripts with an overall low percentage similarity (but which may have a higher similarity from a single source) are not overlooked. The acceptable limit for similarity of text from a single source is 5%. If the similarity

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B.20.2 High text similarity

There may be some manuscripts with an overall low similarity percentage, but a higher percentage from a single source. A manuscript may have less than 20% overall similarity but there may be 15% similar text taken from a single article. The similarity index in such cases is higher than the approved limit for a single source. Authors are advised to thoroughly rephrase the similar text and properly cite the original source to avoid plagiarism and copyright violation.

B.20.3 Types of plagiarism

We all know that scholarly manuscripts are written after thorough review of previously published articles. It is therefore not easy to draw a clear boundary between legitimate representation and plagiarism. However, the following important features can assist in identifying different kinds of plagiarized content. These are:

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B.20.4 Plagiarism in published manuscripts

Published manuscripts which are found to contain plagiarized text are retracted from the journal website after careful investigation and approval by the Editor-in-Chief of the journal. A 'Retraction Note' as well as a link to the original article is published on the electronic version of the plagiarized manuscript and an addendum with retraction notification in the journal concerned.

B.21 E-Pub ahead of schedule

Bentham Science Publishers are pleased to offer electronic publication of accepted papers prior to scheduled publication. These peer-reviewed papers can be cited using the date of access and the unique DOI number. Any final changes in manuscripts will be made at the time of print publication and will be reflected in the final electronic version of the issue. Articles ahead of schedule may be ordered by pay-per-view at the relevant links by each article stated *via* the **E-Pub Ahead of Schedule**

B.22 Disclaimer

Articles appearing in E-Pub Ahead-of-Schedule sections have been peer-reviewed and accepted for publication in this journal and posted online before scheduled publication. Articles appearing here may contain statements, opinions, and information that have errors in facts, figures, or interpretation. Accordingly, Bentham Science Publishers, the editors and authors and their respective employees are not responsible or liable for the use of any such inaccurate or misleading data, opinion or information contained in articles of the E-Pub Ahead-of-Schedule.

Member of Cope

Annexure C: Journal of Pharmaceutical Sciences: Author Guidelines

All submitted manuscripts should contain previously unpublished original research. Submitted manuscripts should not be under consideration for publication elsewhere.

C.1 Online submission and peer review

Authors should ensure that papers conform to the scientific and style instructions given below. In order to expedite the publication process the Journal requires that manuscripts be submitted online at http://mc.manuscriptcentral.com/jpharmsci.

Journal of Pharmaceutical Sciences has a completely digital submission, review, and production process. We therefore ask for production-quality files at the time of submission of your manuscript. This will speed the production and distribution of your work across a variety of print and electronic platforms. If you don't follow the simple guidelines given below, your submission will be returned to you for additional revision. This will of course delay review and, in the event that your work is accepted, would delay publication. Therefore we ask that you pay careful attention at this time and we thank you for your cooperation.

If you have not already done so, create an account for yourself in the system at the submission site, http://mc.manuscriptcentral.com/jpharmsci by clicking on the "Create an Account" button (you may check the progress of your manuscript throughout the review process by logging in and checking your Author Center). Please follow on-screen instructions and the system will guide you through the submission process. At the "File Manager" screen, you will be asked to upload manuscript files. Please designate the Peer-Review Version of your manuscript (incorporating all elements) as a "File For Review" (by selecting "Yes" in the drop-down box). Production-ready files (separate elements) should be designated as "Files Not For Review" (by selecting "No" in the drop-down box).

Online help is available to you at all times during the process. You are also able to exit/re-enter at any stage before finally "submitting" your work. All submissions are kept strictly confidential. You may contact the Journal's Editorial Assistant, Tammy Dunning, at tdunning@ku.edu, or at tel.785-864-5919, fax 785-864-5875.

C.1.1 Text

Submit your text in DOC format. Do not embed figures or tables in this document. These should be submitted as separate files.

C.1.2 Tables

Tables should be created with a word processor and saved in either DOC or RTF format. Do not embed tables in your text. Tables should be on separate pages and saved as one file in DOC format.

C.1.3 Figures

To ensure the highest print quality, your figures should be submitted in either TIF or EPS format at 300 dpi. For more instructions on preparing high quality figures, please see our Graphics FAQ.

C.1.4 Color figures

In addition to the above resolution guidelines, color figures must be submitted in a CMYK color. Do not submit color figures as RGB.

C.2 Scope of the *Journal of Pharmaceutical Sciences*

JPharmSci® focuses on two major questions of importance to pharmaceutical scientists: (i) What are the physical and biological barriers that limit the access of drugs to their therapeutic targets?; and (ii) How can drugs, excipients, traditional formulations, novel drug delivery systems and drug products be designed to maximize therapeutic efficacy? Answers to these questions have in the past and will in the future be forthcoming from research in a variety of scientific disciplines including but not limited to the following: physical pharmacy; pharmaceutics; pharmaceutical technology; drug delivery; pharmaceutical engineering; materials science; nanotechnology; animal, human, cellular and molecular biopharmaceutics; animal and human pharmacokinetics, pharmacodynamics and pharmacogenomics; drug metabolism and transport; biotechnology; medical chemistry, including drug design and prodrug strategies; biophysical chemistry; analytical and bioanalytical chemistry; physical organic, organic, and computational chemistry; molecular modeling; immunology; biochemistry; and cell and molecular biology. The scientific content of manuscripts submitted to *JPharmSci*® should fit into one of the following subject categories:

C.2.1 Drug discovery-development interface

Manuscripts in this scientific category should include descriptions of quantitative and mechanistic research in pharmaceutics, biopharmaceutics, pharmacokinetics, pharmacodynamics and drug metabolism and transport that are normally conducted during the discovery of organic chemistry-based and biotechnology-based hits, leads and potential drug candidates. Research results of particular interest to the readers of *JPharmSci*® would include those that afford valuable, new information about how a molecule's *in vitro* and *in vivo* behavior are influenced by its molecular and physico-chemical properties, traditional formulations and novel delivery systems used in lead optimization studies. This scientific category would also encompass manuscripts that describe: (i) new and novel analytical methodologies and that would facilitate and/or more accurately and completely characterize the physico-chemical and biological properties of hits, leads and potential drug candidates; and (ii) new and novel formulations strategies and drug delivery systems, including those built on bio-and nanotechnologies, that would enhance the delivery of these molecules to their pharmacological targets in animal models.

C.2.2 Pharmaceutical biotechnology

Manuscripts in this scientific category should include descriptions of quantitative and mechanistic research in pharmaceutics, drug delivery and pharmaceutical technology that are normally conducted during the preclinical and clinical drug development of biotechnology-based drug candidates and drugs (e.g. peptides, proteins, antibodies, vaccines, DNA, RNA). Research results of particular interest to the readers of *JPharmSci*® would include those that afford valuable, new information about how a molecule's *in vitro* and *in vivo* behavior is influenced by its molecular and physico-chemical properties, traditional formulations and novel drug delivery systems used in preclinical and clinical studies and the manufacturing processes that give rise to the final drug product. This scientific category would also encompass manuscripts that describe: (i) new and novel analytical methodologies that would facilitate and/or more accurately and completely characterize the physico-chemical and biological properties of biotechnology-based drug candidates and drugs; and (ii) new and novel formulations strategies and drug delivery systems, including those built on bio-and nanotechnologies, that would enhance the delivery of these types of molecules to their pharmacological targets in animals and humans.

C.2.3 Pharmaceutics, drug delivery and pharmaceutical technology

Manuscripts in this scientific category should include descriptions of quantitative and mechanistic research in pharmaceutics, drug delivery and pharmaceutical technology that are

normally conducted during the preclinical and clinical development of organic chemistry-based drugs based drugs or drug candidates. Research results of particular interest to the readers of *JPharmSci*® would include those that afford valuable, new information about how the *in vitro* and *in vivo* behavior of a drug molecule or formulation excipient is influenced by its molecular and physico-chemical properties, traditional formulations and novel drug delivery systems used in pre-clinical and clinical studies and the manufacturing processes that give rise to the final drug product. This scientific category also encompasses manuscripts that describe: (i) new and novel analytical methodologies that facilitate and/or more accurately and completely characterize physico-chemical and biological properties of biotechnology-based drugs and drug candidates; (ii) new and novel pro-drug strategies and formulation strategies and drug delivery systems, including those built on bio- and nanotechnologies, that enhance the delivery of these types of molecules to their pharmacological targets in animals and humans; and (iii) new and novel developments in manufacturing of drugs and drug delivery systems, including continuous manufacturing and the Quality by Design concept.

C.2.4 Pharmaceutical nanotechnology

Manuscripts in this scientific category should describe quantitative and mechanistic experimental or theoretical research in nanoscale-based pharmaceuticals or diagnostics in which the innovation resides specifically in the nanoscale aspects of the work. Manuscripts reporting advances in pharmaceutical nanotechnology that are being disclosed for the first time would be of particular interest. Suitable topics in this category include advances in the fabrication of nanoscale materials with demonstrably new or significant functionality potential for pharmaceutical applications. Additional topics include improved quantitative methods of characterization of nanoscale pharmaceutical materials and mechanistic studies that contribute to an improved understanding of functionality of nanoscale-based technologies with clear therapeutic implications. The manuscript's conclusions should be supported by relevant *in vitro* and/or *in vivo* experimenal data and appropriate statistical analysis. Notable exceptions to the requirement for appropriate physical and biological characterization are : (i) comprehensive and complete theoretical or computational studies; and (ii) meta-analyses of historical data or reviews of the existing literature.

C.2.5 Pharmacokinetics, pharmacodynamics and drug transport and metabolism

Manuscripts in this scientific category should encompass quantitative and mechanistic research normally conducted during the preclinical and clinical drug development of organic chemistry-based or biotechnology-based drug candidates or drugs that affords valuable, new information (e.g. drug-drug interactions) about the molecule's *in vitro* metabolism and/or *in vitro* absorption,

distribution, metabolism and excretion (ADME) and how these properties relate to the molecule's *in vivo* pharmacological and toxicological properties. This scientific category would also encompass manuscripts that describe new and novel analytical methodologies that would facilitate and/or more accurately and completely characterize the pharmacokinetics, pharmacodynamic and drug metabolism and transport properties of these types of drug candidates and drugs in animals and humans.

C.2.6 Global health

Manuscripts in this scientific category should encompass descriptions of quantitative and mechanistic research in pharmaceutics, biopharmaceutics, pharmacokinetics, pharmacodynamics, and metabolism and transport properties that are normally conducted during the discovery of organic chemistry-based and biotechnology-based hits, leads and potential drug candidates and the preclinical and clinical development of drug candidates and drugs targeting diseases common in developing countries. Research results of particular interest to the readers of *JPharmSci*® would include those that afford valuable, new information about how a molecule's *in vitro* and *in vivo* behavior are influenced by its molecular and physico-chemical properties, traditional formulations, novel drug delivery systems and manufacturing processes. The scientific category would also encompass manuscripts that describe new and novel analytical methodologies that would facilitate and/or more accurately and completely characterize the pharmaceutics, pharmacokinetics, pharmacodynamics, drug metabolism, drug delivery and manufacturing properties of these types of drug candidates and drugs in animals and humans.

C.3 Types of manuscripts

The Editor-in-Chief and one Editor, as well as members of the *Journal's* Editorial Advisory Board and independant experts, will review most manuscripts submitted to *JPharmSci*®. However, the Editor-in-Chief and the Editors reserve the right to reject a manuscript without conducting an in-depth review if they feel that the manuscript is "out of scope" or it does not meet the minimal acceptance criteria for publication in *JPharmSci*®.

C.3.1 Rapid Communications

Rapid Communications are preliminary accounts of significant and original experimental and/or theoretical results that fit within the scope of *JPharmSci*®. The results must be of sufficient significance, originality and general interest to justify accelerated publication. Authors are asked to write their manuscripts in a clear and concise manner and to include only data crucial to arriving at their final conclusions. Preferably manuscripts should not exceed 2,000 words of

text and a total of 4 figures and/or tables. Extra experimental and/or theoretical data in the form of figures and tables should be deposited under Supporting Information.

C.3.2 Research Articles

Research Articles are comprehensive accounts of significant and original experimental and/or theoretical results that fit within the scope of *JPharmSci*®. Authors are asked to write their manuscripts in a clear and concise manner and to include only data crucial to arriving at their final conclusions. Preferably manuscripts should not exceed, 5,500 words of text and a total of 8 figures and/or tables. Extra experimental and/or theoretical data in the form of figures and tables should be deposited under Supporting Information.

C.3.3 Notes

Notes differ from Rapid Communications in that they are final reports and from Research Articles in that they are limited in scope. Authors are asked to write their manuscripts in a clear and concise manner and to include only data crucial to arriving at their final conclusions. Preferably manuscripts should not exceed 2,000 words of text and a total of 4 figures and/or tables. Extra experimental and/or theoretical data in the form of figures and tables should be deposited under Supporting Information.

C.3.4 Lessons Learned

Lessons Learned are short articles (600 words) which provide authors with a means of informing other scientists about critical issues, experiences and observations, the descriptions of which would not be appropriate for a typical Research Article, Communication, Note, Commentary or Review. Examples include, but are not limited to, key insights into an unanticipated manufacturing problem, knowledge accumulated over a career of "tricks of the trade" for a given analytical or formulation method, how to avoid a mistake that is repeated over and over again by scientists in industry and academia. Each article will be reviewed directly by an Editor who has expertise in the relevant scientific area. Because each of these articles represents the personal opinion, experience and/or insights of the author(s), data are not required (but could be described) nor does the identity of a given drug need to be divulged. Articles may contain up to three key references.

C.3.5 General Commentaries, Global Health Commentaries, Clinical Trials and Translational Medicine Commentaries, and Special Topic Commentaries

General Commentaries, Global Health Commentaries, Clinical Trials and Translational Medicine Commentaries, and Special Topic Commentaries (by invitation only) present authors'

considered opinions on scientific or technical subjects within the scope of *JPharm Sci*®. If the Commentary is critical of the content of a Research Article, Note, or Rapid Communication published in the *JPharmSci*®, the authors of the original article will be given the opportunity to submit a "reply" Commentary and the "critical" Commentary and the "reply" Commentary will be published back-to-back in the same issue of *JPharmSci*®. Authors interested in preparing Commentaries for *JPharmSci*® should provide a brief outline to Editor John Carpenter (Editor in charge of General Commentaries and Special Topic Commentaries) or Editor Rodney Ho (Editor in charge of Clinical Trials and Translational Medicine, and Global Health Commentaries), requesting invitations to submit manuscripts in one of these categories.

C.3.6 Perspectives

Perspectives (by invitation only) articles summarize the viewpoints of distinguished pharmaceutical scientists with regard to the current status and future direction of the field. Perspectives are similar in length to Commentaries and Reviews, and may be submitted only by invitation. An author interested in preparing a Prespective for *JPharmSci*® should provide a brief outline to Professor John Carpenter requesting an invitation to submit a manuscript in this category.

C.3.7 Reviews

Reviews (by invitation only) provide a comprehensive summary of broadly-based topics of general interest to pharmaceutical scientists. Reviews are not limited as to the number of words, tables, figures and references that may be included. An author interested in preparing a Review for *JPharmSci*® should provide a brief outline to Professor John Carpenter requesting an invitation to submit a manuscript in this category.

C.3.8 Minireviews

Minireviews (by invitation only) are well-focused, well-documented examinations of timely issues in the pharmaceutical sciences. The issues may be of a controversial nature, or may address a more narrowly focused area than those typically covered in a Review. Minireviews are limited to approximately 3,000-4,000 words, including tables, figures and references. An author interested in preparing a Minireview for *JPharmSci*® should provide a brief outline to Professor John Carpenter requesting an invitation to submit a manuscript in this category.

C.4 Preparation of manuscripts

(a) General Considerations. In order to expedite peer review, authors are required to submit their manuscripts online at http://mc.manuscriptcentral.com/jpharmsci. (See Section 1 above for details about the on-line submission process.)

Authors should write manuscripts in clear, concise English. The responsibility for all aspects of manuscript preparation rests with the authors. Authors should note that extensive changes or rewriting of the manuscript will not be undertaken by the editors.

There are no page charges for publication in the Journal of Pharmaceutical Sciences.

(b) Suggested Reviewers. The Journal requires that submitting authors suggest at least four reviewers, up to a maximum of six reviewers, two of which must be Editorial Advisory Board (EAB) members; one must be a Scientific Advisor. Please include suggested reviewers' contact information. A list of Editorial Advisory Board (EAB) members and a list of Scientific Advisors can be found by clicking on the corresponding link.

Editorial Advisory Board Members Keywords List

Scientific Advisors Keywords List

(c) Title. Titles are of great importance for current awareness and for information retrieval. The wording of titles should be chosen carefully to provide information on the contents and to function as "points of entry" for information retrieval. Symbols, formulas, or arbitrary abbreviations should not be included in the title, except chemical symbols to indicate the structure of isotopically labeled compounds.

(d) Abstract. The abstract should briefly (80-200 words) present, in one paragraph, the problem and experimental approach and state the major findings and conclusions. It should be self-explanatory and suitable for reproduction without rewriting. Footnotes or undefined abbreviations may not be used. If a reference must be cited, complete publication data must be given.

(e) Keywords. Please provide up to 10 keywords that reflect the scientific content of your manuscript. These keywords must come from the Journal's official keyword list (**Official Keyword List (.pdf)**). In addition to facilitating indexing of articles, our keyword system assists in the assignment of qualified reviewers for your manuscript. In addition, each member of our Editorial Advisory Board has selected **keywords** that are applicable to their own work.

(f) Abbreviations. Abbreviations are used without periods. Standard abbreviations should be used throughout the manuscript. All nonstandard abbreviations should be kept to a minimum and must be defined in the text following their first use and in a footnote at the beginning of the manuscript.

(g) QSAR/QSPR. All manuscripts dealing with quantitative structure activity relationships (QSAR) and quantitative structure property relationships (QSPR) must identify individual chemical structures using Chemical Abstracts Service (CAS) SciFinder. To aid authors in the use of CAS SciFinder for structure searching, please click here for a Commentary written by Dr. Christopher Lipinski describing the procedure. This Commentary appears in Journal of Pharmaceutical Sciences 91(12): 2470-2472.

(h) Experimental Section. The experimental procedures should be described in sufficient detail to enable others to repeat the experiments. Names of products and manufacturers [with city, state, and country (if other than the U.S.)] should be included only if alternate sources are deemed unsatisfactory. Brand names may be used only once in the manuscript. For subsequent designation, use "formulation A", product B", etc. Novel experimental procedures should be described in detail, but published procedures should merely be referred to by literature citation of both the original and any published modifications. The purity of key compounds and descriptions(s) of the method(s) used to determine purity should be included in this section. For buffers, use terminology such as "20 mM potassium phosphate buffer (pH 7.7) containing...". Also, state w/v or v/v when appropriate.

Identification of and precautions for handling hazardous chemicals and dangerous procedures should be placed at the beginning of the section. An example would be "***Caution:*** *The following chemicals are hazardous and should be handled carefully; (list of chemicals and handling procedures or references)*".

Manuscripts containing data generated from animal and/or human studies must specify the committee and the institution that approved the experimental protocols used to generate these data.

(i) Results. The results should be presented concisely. Tables and figures should be designed to maximize the presentation and comprehension of the experimental data. Attention should be paid to the matter of significant figures (usually, no more than three). The same data should not be presented in more than one figure or in both a figure and a table. As a rule, interpretation of the results should be reserved for the discussion section of a **Research Article**, but under some circumstances it may be desirable to combine results and discussion in a single section.

(j) Discussion. The purpose of the discussion is to interpret the results and to relate them to existing knowledge in the field in as clear and brief a fashion as possible. Information given elsewhere in the manuscript should not be repeated in the discussion. Extensive reviews of the literature should be avoided.

(k) References and Notes. Literature references and notes must be numbered in one consecutive series by order of mention in the text, with numbers as unparenthesized superscripts. The accuracy of the references is the responsibility of the author. The complete list of references and notes should be typed double-spaced on separate page(s) at the end of the manuscript and follow the format shown.

All references should include titles.

For journals:

Yoneto K, Li SK, Higuchi WI, Jiskoot W, Herron JN 1996. Fluorescence probe studies of the interactions of 1-alkyl-2-pyrrolidones with stratum corneum lipid liposomes. J Pharm Sci 85:511-517.

For edited books:

Rall TW, Schleifer LS. 1985. Drugs effective in the therapy of the epilepsies. In Gilman AG, Goodman LS, Rall TW, Murad F, editors. The pharmacological basis of therapeutics, 7th ed., New York: Macmillan Publishing Co. p 446-472.

List submitted manuscripts as "in press" only if formally accepted for publication; otherwise, use "unpublished results" after the names of authors. Any footnotes to the text should be incorporated in the correct numerical sequence with the references.

(l) Supporting Information. The Supporting Information format of this journal can accommodate and make readily available almost any type of supplementary figures or data (e.g., reproductions of spectra, experimental procedures, tabulated data, expanded discussion of peripheral findings, etc.). The author should include a Supporting Information Available statement that describes the material at the end of the printed manuscript text. Consult a current issue of the Journal for the proper wording of this statement. Supporting Information should be clear and of high contrast (suitable for direct photo reproduction) and submitted in quadruplicate on 8.5- × 11-in. paper. All pages of Supporting Information must be consecutively numbered. Captions or legends for figures, spectra, etc., must appear directly on the figure. Supporting Information is available free of charge via the Internet (www.wileyonlinelibrary.com).

(m) Acknowledgments. This section should acknowledge financial support, technical assistance, advice from colleagues, gifts, etc. Permission must be received from persons whose contribution to the work is acknowledged in the manuscript.

(n) Spectral Data. It may be desirable to include such data for representative compounds in a series, for novel classes of compounds, and in structural determinations. Usually, it is not desirable to include routine spectral data for every compound in the manuscript. Papers where interpretations of spectra are critical to structural elucidation and those in which band shape or fine structure needs to be illustrated may be published with spectra included. When such presentations are deemed essential, only pertinent sections should be reproduced.

(o) Experimental Data. Experimental methods must be referenced or described in sufficient detail to permit the experiments to be repeated by others. Detailed descriptions of experimental methods should be placed in the experimental procedures section. Data may be presented as numerical expressions in tables or in graphical form with no duplication of information in the text. If tables or figures include a minimal number of experimental values (< four), the data should be presented in the text. Units should be abbreviated without punctuation and with no distinction between singular and plural forms (e.g., 1 mg, 25 mg). If possible, statistical significance of the experimental data should be provided. Statistical probability (p) in tables, figures, figure legends and text should be expressed as * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. For multiple comparisons within a table, footnotes italicized in lower case, superscript letters should be used and defined in the table legend. References to statistical methods of calculation should be provided. If statistical limits cannot be provided, the number of determinations and some indication of the variability and reliability of the results should be provided. For animal experimental data, doses and concentrations should be expressed as molar quantities (e.g., mmol/kg, mM) when comparisons are made between compounds having large differences in molecular weights. The routes of administration of test compounds and vehicles used should be indicated. **For animal and human studies, authors must specify the committee and the institution that approved the experimental protocols used to generate these data.**

If experimental data on proprietary compounds (i.e. compounds whose chemical structures are not available in the public domain) and/or using proprietary procedures (i.e. experimantal procedures and/or components of procedures that are not described in the public domain) are provided in a manuscript, authors should carefully read the next two paragraphs.

Traditionally, scientific papers must reveal sufficient information for the work to be repeated by others. That tradition led to the policy that *JPharmSci*™ has applied to manuscripts that contain

information on proprietary small molecules. This policy essentially states that information pertaining to proprietary (small molecule) compounds can be published providing that, in the opinion of the reviewers and editors, the paper would be publishable based solely on the information derived from studies of known compounds. Thus, information on proprietary compounds has been considered to be supplemental while the decision to publish or not has relied on compounds for which structures were disclosed.

Studies of proprietary proteins and other biologicals pose a difficult situation for *JPharmSci*™. In some cases, complete structures may not have been determined or, even if the structure is available, compounds having identical structures may be difficult for others to generate (e.g., the amino acid sequences of immunoglobulin hypervariable regions). Yet, interesting studies of proprietary biologicals can nevertheless be envisioned that may be deemed to have sufficient value that the failure to reveal detailed structural information should not be a deterrent to publication. Therefore, the journal will determine the acceptability of such papers on an individual basis. Decisions of acceptability will be made using the following criteria: (a) the structural information provided is adequate for the purpose of evaluating the paper using rigorous scientific standards; (b) the structural information provided is sufficient to enable others to verify the results by conducting essentially the same experiments; and (c) the work is judged to be of sufficient importance that a lack of complete structural information does not significantly detract from its scientific contributions.

(p) Tables. Tabulation of experimental results is encouraged when this leads to more effective presentation or to more economical use of space. Tables should be numbered consecutively with Arabic numerals. Provide a brief title with each table and a brief heading for each column. Clearly indicate the units of measure (preferably SI). Data should be rounded to the nearest significant figure. Explanatory material referring to the whole table is to be included as a footnote to the title (a). Footnotes in tables should be given lower case letter designations and cited in the tables as italicized superscripts. Tables that require special treatment, such as insertion of arrows or other special symbols under or over alphanumeric characters, or contain many structures should be submitted as camera-ready copy. All tables should be cited in the text.

(q) Illustrations. The quality of the illustrations printed in your paper depends on the quality of the originals you provide. Electronic submission of illustrations is required. Preferred formats for graphics and artwork are TIFF (tagged image file format) and EPS (Encapsulated PostScript). John Wiley & Sons, Inc. journal pages are now produced completely electronically. Please see our Graphics FAQ for more information.

In preparing illustrations, contrast is important. Use dark black ink on high quality, smooth, opaque white paper. Ordinary white bond paper works well. Avoid tracing paper or textured "artist" papers.

Illustrations must fit a one- or two-column format on the journal page: **For efficient use of journal space, single column illustrations are preferred.**

	Single (preferred)	Double
Minimum Width		9 cm (3.5 in)
Maximum Width	8 cm (3.125 in)	16.5 cm (6.5 in)
Maximum Depth	23 cm (9 in)	23 cm (9 in)

For best results, submit illustrations in the actual size at which they should appear in the journal. Original illustrations which do not need to be reduced to fit a single or double column will yield the best quality. Lettering should be no smaller than 6 points. (Helvetica type works well for lettering). Lines should be no thinner than 0.5 point. Lettering and lines should be of uniform density. If you must submit artwork that must be reduced, use larger lettering and thicker lines so that, when reduced, the artwork meets the above-mentioned parameters. Avoid using complex textures and shading to achieve a three-dimensional effect. To show a pattern, choose a simple cross-hatch design.

Color. All color figures will be reproduced in full color in the online edition of the journal at no cost to the authors. Authors are encouraged to submit color illustrations that highlight the text and convey essential scientific information. For best reproduction, bright, clear colors should be used. Dark colors against a dark background do not reproduce well; please place your color images against a white background whenever possible. Please email jpsprod@wiley.com for further information.

Chemical Structures. Structures should be produced with the use of a drawing program such as ChemDraw. Authors using the current versions of ChemDraw, ChemIntosh, and ChemWindows will find the necessary parameters incorporated into these programs ("JOC Document" under the Windows menus for ChemDraw and "Reduce 60% for JOC Style" under the Options menu for ChemIntosh/ChemWindows). In ChemDraw version 4.5, files should be saved in TIFF format to allow use of electronic files in production (see journal home page "Information for Authors" for further guidelines).

(r) Nomenclature. It is the responsibility of the authors to provide correct nomenclature. All nomenclature must be consistent and unambiguous and should conform with current American

usage. Insofar as possible, authors should use systematic names similar to those used by Chemical Abstracts Service, the International Union of Pure and Applied Chemistry, and the International Union of Biochemistry and Molecular Biology.

The chemical names for drugs should be used. If the terminology is unwieldy, nonproprietary names of drugs may be used throughout the manuscript after the first mention and identification. Formally adopted nonproprietary names listed in *United States Adopted Names(USAN)* should be used. In cases in which a name has not been assigned by *USAN, the International Nonproprietary Names (INN)*, approved by the World Health Organization, should be used. Trade names and laboratory codes should not be used except as additional information.

(s) Analyses. Adequate evidence to establish identity and purity should be provided for new compounds. When possible, this should include elemental analysis. The purity of compounds used for biological testing should be stated with a description of the method used to evaluate it.

(t) Hazardous Materials. All hazardous chemicals should be clearly identified as such. Precautions for handling dangerous materials or for performing hazardous procedures should be explicitly stated and referenced.

C.5 Proofs and reprints

Proofs are sent to the author who submitted the papers. Authors are directed via email to download proofs from Wiley Author Services. Proofs should be verified against the manuscript and appropriate corrections made. Substantial changes in a manuscript after type has been set require editorial approval and in some cases may be cause for re-reviewing. An order form for reprints will be sent with the proofs. Please return the reprint order form, along with a purchase order or check, to John Wiley & Sons, Inc. Reprints will be shipped within 2 weeks after the printed journal date.

C.6 Publication Online

Articles accepted for publication in the Journal of Pharmaceutical Sciences will be posted in Early View and then into the Online version of the journal (www.wileyonlinelibrary.com) as soon as author corrections to proofs are received and incorporated. This can occur anywhere from 2 to 6 weeks well in advance of the cover date of the printed issue. Authors should take this into account when planning their intellectual and patent activities related to an article. The actual date on which the article is posted online is recorded in a separate line at the bottom of the first page of the article in the printed issue.

C.7 Corrections

If errors of consequence are found in the published paper, a correction of the error should be sent by the author to the Editor-in-Chief for publication in the journal's Errata Section.

C.8 Copyright Transfer Agreements (CTA)

Copyright Transfer Agreements are located in Wiley Author Services. You will be notified by email after acceptance on how to access and submit your CTA for the Journal.

C.9 Confirmation of manuscript content must accompany initial submission

Manuscripts submitted to the Journal of Pharmaceutical Sciences should contain significant, unpublished and original data not being considered simultaneously for publication elsewhere. All authors should be aware of and in agreement with the submission of this manuscript and share responsibility for its content. The manuscript should provide full and appropriate credit to those who contributed to the underlying hypothesis and the generation and interpretation of the experimental data. Related research in the field should be acknowledged in the manuscript through appropriate literature citations. Manuscripts should be devoid of any forms of plagiarism with respect to ideas, data, words, graphic materials or other forms of communication. All authors should be aware that this manuscript will be checked for plagiarism using CrossCheck anti-plagiarism software.

C.10 Scientific Misconduct Issues

An alleged violation of any of these basic rules of scientific ethics will be investigated confidentially in accord with the procedures set forth in the American Medical Association Manual of Style: A guide for Authors and Editors (10th Edition). If the violations are deemed to be sufficiently serious, the Editor-in-Chief will request that the authors provide a written explanation. If the authors do not provide an explanation or the explanation is unsatisfactory, such that the Journal's Editorial Team believes that the evidence clearly shows that scientific misconduct did occur, the Editor-in-Chief would promptly reject the manuscript or proceed to retract a published manuscript. In addition, the Editor-in-Chief reserves the right to notify the authors's institution for the violation of the Journal's scientific ethics policy. The Editor-in-Chief also reserves the option to request the author's institution initate a formal investigation into the alleged violation of scientific ethics and to report back to the Journal in a timely manner. If the formal institutional investigation confirms scientific misconduct, the Editor-in-Chief will promptly reject a pending manuscript or proceed to retract a published manuscript. Further,

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C.11 Conflicts of Interest Guidelines: Authors

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Annexure E: Pharmaceutical Statistics: Author Guidelines

E.1 General

Pharmaceutical Statistics is an industry-led initiative, tackling real problems in statistical applications. The Journal publishes papers that share experiences in the practical application of statistics within the pharmaceutical industry. It covers all aspects of pharmaceutical statistical applications from discovery, through pre-clinical development, clinical development, post-marketing surveillance, consumer health, production, epidemiology, and health economics.

The Journal is both international and multidisciplinary. It includes high quality practical papers, case studies and review papers.

The journal accepts high quality papers in the following formats:

- Main Paper (Original Article)
- Viewpoint
- Teacher's Corner
- Book Review
- Literature Review

The aims of the Journal are to:

1. Disseminate information and practical examples of the full range of statistical methods and statistical thinking in all stages of drug development, from discovery to production;
2. Debate the current practice and application of statistics within the pharmaceutical industry and how this can best be developed;
3. Discuss and bring awareness to regulatory guidance documents;
4. Provide a vehicle for communication between practitioners, researchers, educators and policy makers concerned with the application of statistics within drug development; and
5. Provide guidance and tutorials to statisticians working within the pharmaceutical area.

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E.2 Manuscript Submission

All papers must be submitted via the online system. *Pharmaceutical Statistics* operates an online submission and peer review system that allows authors to submit articles online and track their progress via a web interface. Please read the remainder of these instructions to authors and then click http://mc.manuscriptcentral.com/pst-wiley to navigate to the *Pharmaceutical Statistics* online submission site. Full instructions and support are available on the site and a user ID and password can be obtained on the first visit. If you require assistance then click the Get Help Now link which appears at the top right of every ScholarOne Manuscripts page. If you cannot submit online, please contact Priscilla Goldby in the Editorial Office by telephone (01865 765933) or by e-mail (p.goldby@btopenworld.com).

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1. An introduction to the work. This should be accessible by scientists in any field and express the necessity of the experiments executed
2. Some scientific detail regarding the background to the problem
3. A summary of the main result
4. The implications of the result
5. A broader perspective of the results, once again understandable across scientific disciplines

It is crucial that the abstract conveys the importance and novelty of the work and be understandable without reference to the rest of the manuscript to a multidisciplinary audience. Abstracts should not contain any citation to other published works.

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2. Kuo W, Chien WTK, Kim T. *Reliability, Yield and Stress Burn-in* (2nd edn), Vol. 1. Kluwer: Norwell, MA, 1998; 35-70.
3. Engel KH, Heidlas J, Tressl R. Food is wonderful, I can't get enough. In *Food Flavours Part C. The Flavour of Fruit*, Morton ID, MacLeod AJ (eds). Elsevier: Amsterdam; 1990; pp. 190-195.
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