CHAPTER TWO

2 PREFORMULATION STUDY

2.1 INTRODUCTION

Various challenges are involved in manufacturing a proper dosage form. Preformulation is the early stages of any new formulation. Basic information is

collected and compared from previous studies on chemically similar drugs. The physical, chemical, biological and pharmaceutical sciences all play an integral part. The early stages of any new formulation revolve around interpreting all the basic information of the drug substance.

Formulation development is required at various stages during drug development. As we have discussed earlier, drugs are rarely administered alone. Incorporation of the drug into a formulation provides various advantages such as ease of handling, ease of administration, better stability or better bioavailability.

Preformulation studies are an essential component of drug development wherein it supports development of formulations. It provides the scientific basis for formulation development. Efforts spent on preformulation provide cost savings in the long run, by reducing challenges during formulation development.

Preformulation studies can be broadly classified into two classes (i) fundamental properties and (ii) derived properties. Fundamental preformulation properties are specific to the drug molecule and are dependant on the chemical structure of the drug molecule. In contrast, derived preformulation properties are carries out to learn about the issues related to development of a particular dosage form such as solids, liquids or parenteral (Arbro analytical division, 2011).

2.2 STRUCTURAL CHARACTERISTICS OF PHEROID®

The reason for using colloidal systems as carriers of API's or cosmetic compounds is to enhance the efficacy of the administered compounds while reducing the unwanted side effects. The bio-distribution of colloidal particles is dependent on the route of administration and the physicochemical properties of the system, including the particle size and surface characteristics (e.g., surface charge and surface affinity), as well as the inherent particle characteristics, such as elasticity (Grobler, 2009:149).

The Pheroid® is a fatty acid-based delivery system and a number of the terms used in colloidal drug delivery is applicable and will be used. Originally a triangular concept was hypothesised with the fatty acids as one corner, nitrous oxide the second corner and the API as the third corner of the triangle. Clearly, this was an oversimplification, as the presence of tocopherol is required and the amount of tocopherol may change the morphology of the vesicle. The unsaturated fatty acid component of the Pheroid® received much attention since it gave the Pheroid® the added dimension of inherent therapeutic qualities. In addition, changes in the fatty acid component by the addition of fatty acids not present in Vitamin F ethyl ester, led to changes in the morphology of the particles. However, the use of anti-oxidants and preservatives may have an impact on the size of the particle formed and contribute to the stability of the vesicles (Grobler, 2009:120)

2.3   MANUFACTURING PROCESS OF PHEROID®

Several problems are generally applicable to the production of any lipid-based system. These may include the cost of production, the poor quality of the raw material (phospholipids) and the poor characterisation of the physicochemical properties of the produced lipid particles. Specific problems encountered were low entrapment efficiencies, problems with upscalling and lack of stability with resultant short shelf life of the product (Grobler, 2009:127).

Most of the stability, entrapment and production problems associated with liposomal formulations are not applicable to the patented Pheroid® technology with its simple method of preparation in a vessel adapted for the purpose. The procedure for the preparation of Pheroid® has been progressively simplified since the original batch production in 2000; the current procedure is presented in Figure 2.1 (Grobler, 2009:12).
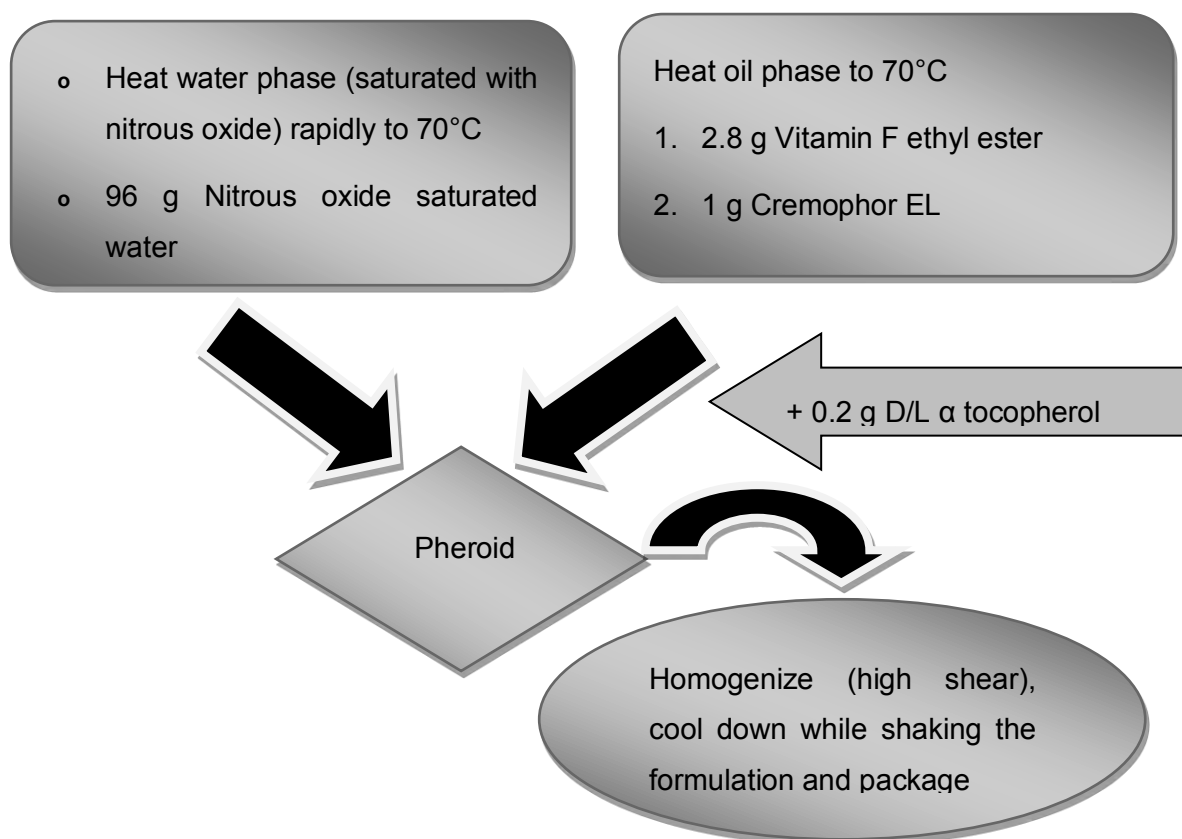
Figure 2.1: Schematic representation of the manufacturing process of the Pheroid® (Grobler, 2010:129).

2.4 METHODS AND APPARATUS

2.4.1 PRESSURE VESSEL

Initially, water was saturated with nitrous oxide by placing a beaker containing the desired water phase in a pressure cooker. The pressure cooker was modified to house a gas inlet. Using a regulator, nitrous oxide was supplied through the inlet until a pressure of between 160 to 200 kPa was reached. The water phase was left under pressure for four days to achieve saturation. However, the air present in the vessel was also forced into the water phase, diminishing the amount of nitrous oxide. For that reason a manifold, with an escape or purging valve, was introduced to the vessel to allow the replacement of most if not all air by nitrous oxide, which is heavier than air. With this system, batches of up to 5 litres can be prepared and the system is still used to prepare small research scale batches (Grobler, 2009:131).

Figure 2.2: The pressure vessel used to prepare the nitrous oxide water phase.

2.4.2   HOMOGENIZER

A WiseStir® MSH-20D (Celsius Scientific, Johannesburg) digital hot plate stirrer with a stirring capacity of 20ℓ water, a speed range between 80 – 1500 rpm and a maximum temperature range of 380°C was used during the manufacturing process of the Pheroid® preparation. This homogenizer has the advantage of a temperature probe which can accurately measure temperature to ± 0.3°C accuracy. A digital screen gives feedback of the temperature, the timer, stirring speed and the power bar graph.

A more intense rate of shearing can be achieved using a turbine mixer such as the Heidolph® Diax 600 homogenizer (Labotec® (Pty) Ltd., Johannesburg). In this type of machine the short, vertical or angled rotor blades are enclosed within a stationary perforate ring and connected by a central rod to a motor. The liquids are therefore subjected to intense shearing, caused initially by the rotating blades, and then by the forced discharge through the perforated ring. Homogenizers are often used after initial mixing to enable smaller globule sizes to be produced (Billany, 2002:357).

2.4.3   PARTICLE SIZE

Particle size analysis was performed by means of lazer diffraction using a Malvern Mastersizer 2000 (Malvern™ Instruments Ltd., Malvern, Worcestershire, UK). The apparatus was switched on half an hour before starting the measurements to allow the laser beam to warm up and stabilise. The emulsion samples were mixed moderately to ensure uniform distribution of droplets before addition to the Mastersizer cell for measurement. Care was taken to eliminate bubbles in the dispersion fluid since they are also detected and may cause variation in the data obtained.

 A dispersion of 2 ml of the sample was added to 800 ml of distilled water at a pump speed of 2500 rpm until an obscuration rate of 10 – 20% was obtained. Background and samples were measured for 12 seconds, with a delay of 20 seconds between measurements. The parameters used to analyse the droplet size distribution were defined by the presentation code 4NHD. Optical properties of the sample were defined as follows: refractive index 1.4564 for the emulsion droplets and 1.3300 for the dispersion medium used (distilled water). The absorbance value of the emulsion droplets was 0.1000 (similar to the particles of olive oil in water in the Malvern™ software). This setting was maintained for all evaluations. Each sample was measured in duplicate, 24 hours after preparation of the emulsion.

2.4.4   RHEOLOGY

The flow behaviour of the emulsions was determined by rotational viscometry using a Brookfield® DV-II+ viscometer (Brookfield Engineering Laboratories, Inc., Middleboro, U.S.A.) The standard test methods for rheological properties of non-Newtonian materials were followed.

The rheological measurements were performed at 25.0 ± 0.1°C with a small sample adapter, an 18 spindle and a SC4-28 chamber. The sample volume used was 11 ml. The hysteresis loop was obtained by recording the stress values by increasing the viscometer speed gradually from 0 rpm to 100 rpm with 30 second intervals. The small sample adapter was connected to a water bath to regulate the temperature for the duration of the experiment.

The Wingather Brookfield® Software package (Monitoring & Control Laboratories (Pty) Ltd., Johannesburg) was used to interpret the data values obtained.

2.4.5   pH

A Mettler Toledo® pH-meter (Microsep™ (Pty) Ltd., Johannesburg) was used to measure the pH of the Pheroid® vesicles at room temperature (25 ± 2ºC).

The pH-meter was calibrated using pH 4.0, pH 7.00 and pH 10.00 buffer solutions (Microsep™ (Pty) Ltd., Johannesburg).   An Inolab® 410 glass probe was used for pH measurements of the emulgel preparations.   After each measurement the probe was thoroughly rinsed with distilled water and stored afterwards in a 3M potassium chloride (KCl) solution (Microsep™ (Pty) Ltd., Johannesburg).

2.4.6   MICROSCOPY AND CONFOCAL LASER SCANNING MICROSCOPY

The selection of the applicable objective depends on the magnification required for microscopy images.   If an oil objective is selected, put a drop of non-fluorescent microscope oil on the cover sip side of the slide.

To aid with visualisation of the formed Pheroid® vesicles, a confocal laser scanning microscope (CLSM) was used.   The advantage of this analytical method over and above normal light microscopy is the ability to be able to view the fluorescent stained vesicles at varying depths.   This is achieved by labeling the sample with a fluorescent marker, a molecule which enters an excited state during lazer exposure (excitation) that emits photons at a specific wavelength (emission).   The lazer used as an energy source enables the microscope to act as an optical knife.

The microstructure of the Pheroid® vesicles was determined by CLSM (Nikon™ PCM 2000 with digital camera DMX 1200, The Netherlands).   A He/Ne lazer was used with an objective of 60x, with an emission of 568 nm.   A volume of 100 µl of the Pheroid® vesicles were stained with 2 µl Nile Red (Molecular Probes, Inc., U.S.A.) for five to 10 minutes.   After this, 20 µl of the stained Pheroid® vesicles was placed on a glass slide and covered with a glass cover slip.   Excessive Pheroid® vesicles were removed by squeezing the cover slide on tissue paper.

2.5   MATERIALS USED IN THE FORMULATIONS

To produce a stable product the formulator must identify raw materials with the desired functionality and be able to combine these materials in the proper ratios. The excipients used throughout this study are listed in Table 2.1. Each component has certain functionality within the preparation. The broad classes of these components will be discussed in further detail.

In the past, excipients would be used mainly as fillers, binders, diluents, lubricants, coatings, solvents and dyes in the manufacture of drug products. Over time, advances in pharmaceutical science and technology have produced a range of novel excipients. In many cases interactions can occur between an excipient, biological surroundings or container closure systems. It is recognised that not all excipients are inert substances and some may be potential toxins. In the United States, the Food and Drug Administration (FDA) has published listings in the Code of Federal Regulations (CFR) for Generally Recognized As Safe (GRAS) substances. Over the years, the Agency also maintained a list entitled Inactive Ingredient Guide (IIG) for excipients approved and incorporated into marketed products. This guide is helpful in that it provides the database of approved excipients and the dosage level by route of administration for each excipient (Chen, 2007:769).

Table 2.1: List of excipients used indicating the supplier and function in the preparation.

Excipient	Supplier	Function
Vitamin F Ethyl Ester	Chemisches Laboratorium, Germany	Fatty acid building blocks
Cremophor® EL	Chemisches Laboratorium, Germany	Emulsifying agent
DL-α-Tocopherol	Chempure (Pty) Ltd., South Africa	Anti-oxidant
Butylated hydroxyanisole (BHA)	Sigma-Aldrich (Pty) Ltd., South-Africa	Anti-oxidant
Butylated hydroxytoluene (BHT)	Sigma-Aldrich (Pty) Ltd. South-Africa	Anti-oxidant
tert-Butylhydroquinone (TBHQ)	Sigma-Aldrich (Pty) Ltd., South-Africa	Anti-oxidant
Propylene glycol (PG)	Merck, South Africa	Co-solvent
Xanthan gum (XG)	Savannah Fine Chemicals, South Africa	Viscosity-increasing agent
Hydroxypropylmethylcellulose (HPMC)	Fluka, South Africa	Viscosity-increasing agent
Carbopol® 934P	Noveon, Inc. United States of America	Viscosity-increasing agent
Nipagin™ M sodium	Sample received from Clariant™,	Preservative
Nipagin™ A sodium	Sample received from Clariant™,	Preservative
Nipasol™ M	Sample received from Clariant™,	Preservative
Nipastat™	Sample received from Clariant™,	Preservative
Nipasept™	Sample received from Clariant™,	Preservative
Butylparaben	Sigma-Aldrich (Pty) Ltd., South-Africa	Preservative
NaOH (Sodium hydroxide)	Merck, South Africa	Buffer
Distilled water	Adcock Ingram, South Africa	Solvent
Nitrous oxide (N$_2$O gas)	Afrox, South Africa	Anaesthetic

2.5.1   FATTY ACIDS

One of the primary excipients in the Pheroid® is ethylated and pegylated polyunsaturated fatty acids such Vitamin F Ethyl Ester. The molecular stacking of the fatty acids in the membrane is extremely specific in two aspects — the sizes of the Pheroid® and the pores or channels built into the membranes of the Pheroid®. For each type of Pheroid®, the sizes formed are pre-determined by the specific fatty acids used, the ratios of the fatty acids used, the saturation state of the fatty acids used, the modification state of the fatty acids and to a lesser extent by the manufacturing procedure (Grobler, 2009:155).

The design of the Pheroid® allows for manipulation of both its structural and functional features. The surface charge of the Pheroid® can be adapted by the degree of hydrogenation of the fatty acids. The mean particle size can be reproducibly manipulated by changing the composition and ratio of the fatty acids. For example, an increase in the pegylated ricinoleic acids leads to smaller particles with thicker membranes and smaller interior spaces whilst hydrogenation of the fatty acids increases the molecular radius of the fatty acids but results in less elasticity of the membranes. However, the relationships between ratios and sizes and other characteristics are not simple and direct and are generally determined empirically. The same principle is used in changing the phase behaviour of the system. The modifications of the fatty acids are an inherent feature of the Pheroid® and not an outer coating of the structure, as is the case in stealth liposomes or pegylation of API's or delivery structures (Grobler, 2009:155).

2.5.2   EMULISIFIERS

Cremophor® EL is the registered trademark of BASF Corporation for its version of polyethoxylated castor oil. It is prepared by reacting 35 moles of ethylene oxide with each mole of castor oil. The resulting product is a mixture; the major component is the material in which the hydroxyl groups of the castor oil triglyceride have ethoxylated with etlene oxide to form polyethylene glycol esters. Cremophor® EL is a synthetic, non-ionic surfactant. Its utility comes from its ability to stabilise emulsions of non-polar materials in aqueous systems (Cremophor® EL, 1997).

Cremophor® EL is a pale yellow, oily liquid with a characteristic odour. At temperatures above 26°C the liquid is clear and completely liquified. The HLB (hydrophilic – lipohilic balance) is between 12 and 14. With cationic emulsifiers, the solubility in water decreases with rising temperature. Cremophor® EL is miscible with all other Cremophor® grades and on heating with fatty acids, fatty alcohols and

certain animal and vegetable oils. It is thus miscible with oleic and stearic acids, dodecyl and octadecyl alcohols, castor oil and a number of lipid-soluble substances. Cremophor[®] EL can be sterilised in an autoclave for 30 minutes at 120°C. When used as oral application in human medicine the hydrogenated and thus tasteless form of Cremophor[®] EL must be chosen (Cremophor[®] EL, 1997).

2.5.3   ANTI-OXIDANTS

Polyunsaturated fatty acids are very unstable and are prone to oxidation, leading to the development of rancid, fishy off-flavours due to formation of aldehydes, alkanes and ketones, with time. Oxidation of fish oil may be preventable by excluding oxygen, light and metal ions (especially iron and copper), operating at low temperatures and/or using antioxidants and metal ion scavengers. Proper handling and storage of the materials are also of utmost importance (Zuidam & Beindorff, 2007:22).

The use of antioxidants (especially lipophilic ones such as tocopherols), shielding from light and packaging of the fish oil under nitrogen, or vacuuming in metalised packaging material might enhance their stability (Zuidam & Beindorff, 2007:23).

Natural fats and oils and certain emulsifying agents are susceptible to oxidation by atmospheric oxygen, these materials therefore require the addition of one or more antioxidants to prevent decomposition. The particular antioxidant system should be chosen with regard to factors such as colour, odour, potency, irritancy, toxicity, stability and compatibility (Lund, 1994:151).

Butylated hydroxianisole (BHA) is used in a wide range of cosmetics, foods and pharmaceuticals. When used in foods, it is used to delay or prevent oxidative rancidity of fats and oils. Butylated hydroxyanisole (BHT) is frequently used in combination with other antioxidants, particularly butylated hydroxytoluene (Rowe *et al*., 2003:61). These two components are practically insoluble in water but freely soluble in PG, peanut oil etc. Alpha tocopherol is primarily recognised as a source of vitamin E. Alpha tocopherol also exhibits antioxidant properties. This agent is highly lipophilic and is an excellent solvent for many poorly soluble drugs. Tocopherols (also known as vitamin E) are of value in oil- or fat-based pharmaceutical products and are normally used in a concentration range of 0.001 – 0.05% v/v. Tocopherol is practically insoluble in water but freely soluble in acetone, ethanol, ether and vegetable oils (Rowe *et al*., 2003:27). Another subsidiary function ascribed to vitamin E is its ability to act as a membrane stabiliser by forming complexes with products of membrane lipid hydrolysis such as free fatty acids and lysophospholipds, thereby

counteracting their disruptive effects (Grobler, 2009:156). Tert-Butylhydroquinone (TBHQ) is a synthetic food grade antioxidant, which is used to stabilise various vegetable oils, fats and food against oxidative breakdown. This extends the products storage life and prevents development of rancidity. TBHQ exhibits an outstanding stabilising effect in unsaturated fats, particularly in polyunsaturated vegetable oils, and inedible animal fats. TBHQ can be used with essential oils, nuts, butter fat and food packaging material (TBHQ, 2008).

2.5.4   HUMECTANTS OR CO-SOLVENT

Glycerol, polyethylene glycol and PG (PG) are examples of suitable humectants, which can be incorporated into aqueous suspensions for external application in concentrations of about 5% v/v. They can prevent the product from drying out after application to the skin and when added to an emulsion formulation can reduce the evaporation of water (Billany, 2002:351).

The advantages of PG will be discussed in Chapter 3, however its use in many pharmaceuticals is as a solvent including oral, injectable and topical formulations, for example, PG is used in the injectable form of diazepam. PG will be added to increase the solubility of the preservatives used in the Pheroid® formulation and at a concentration of 10 - 20% can also function as a preservative (Lund, 1994:152).

2.5.5   VISCOSITY-INCREASING AGENTS

Emulgels are emulsions, either of the o/w or w/o type, which are gelled by mixing with a gelling agent. Many auxiliary emulsifying agents, in particular the hydrophobic colloids, are viscosity enhancers and this property is part of their emulsifying capability; for example, inclusion of methylcellulose will reduce the mobility of the dispersed droplets in an o/w emulsion. Storage of emulsions at a low temperature (but above freezing point) will increase the viscosity of the continuous phase and also reduce the kinetic energy of the system. This will decrease the rate of migration of the globules of the dispersed phase, but it is unwise to rely solely on this method of controlling creaming, as storage conditions following the sale of the product are outside the control of the manufacturer (Billany, 2002:353).

The HPMC's are polymers with methyl and hydroxypropyl groups added to the anhydroglucose backbone. Along the cellulose backbone, methyl substitutes constitute hydrophobic zones whereas hydroxypropyl groups are more hydrophilic. The introduction of these groups allows HPMC to behave as a surfactant. Thus HPMC's are absorbed at fluid interfaces lowering the surface tension. As for HPMC, if the hydrophobicity and molecular weight increases, the intermolecular association

starts at lower concentrations because the hydrophobic groups are closer. The results of present work have shown the behaviour of HPMC's in solution and at the oil – water interface reveals a significant effect on pH, indicating the pH has a strong impact on the occurrence of hydrophobic interactions amongst HPMC molecules (Camino *et al.*, 2011:10).

Tritt-Goc and Pislewski (2002:85), in their study of HPMC hydration at pH 2 and pH 6, found a lower pH predominates a higher number of hydrogen bonds between the protons of the solvent and the HPMC's molecules. Due to the interactions of the molecules with the surrounding medium, the hydrophobic interactions (responsible for aggregate formation) would be impeded at pH 3. This is the reason why at lower pH's, a predominant HPMC monomeric form is found in solution.

Sawyer and Reed (2001:1245) also pointed out that the change in pH influences the properties of the HPMC's molecules when adsorbed into solid particles. They observed the hydrogen bonding to be pH dependent, with reduced adsorption to the solid interface at higher pH's, indicating a higher interaction between HPMC's molecules and not with the solid particle.

Three classes of viscosity-increasing agents were tested in the Pheroid® formula. The agents used were XG, Carbopol® 934P and HPMC. The effect of each of these agents in varying concentrations will be given in detail in the remainder of this chapter.

2.5.6   PRESERVATIVES

Microbial contamination is inevitable, during the period of use of an oral liquid preparation, when the container is opened for the removal of doses more than once in one day. As most oral liquids, especially aqueous preparations, provide suitable conditions for the growth of micro-organisms, an effective antimicrobial preservative should be included in formulations. Only a limited number of antimicrobial preservatives are appropriate for oral administration and few of these are active at an alkaline pH. Antimicrobial preservatives used in oral liquid preparations include chloroform, ethanol, benzoic acid, sorbic acid, the hydroxybenzoate esters and syrup simplex. Disadvantages of benzoic acid and sorbic acid are that they are only active at a low pH. Microbial growth in syrups with a sucrose concentration greater than 65% w/w is usually due to osmotic effects, at this strength. However, crystallisation of the sucrose may occur. Chloroform has several disadvantages such as loss of chloroform when the container is opened due to high volatility, permeation through

some plastics and reported carcinogenicity in animals led to concern about the safety of chloroform (Lund, 1994:43).

Nipastat® is the collective trade name for a product containing methylparaben, ethylparaben, propylparaben, buthylparaben and isobutylparaben in various concentrations (see table 2.2). During the early formulation studies, Nipastat® will be used. The combined combination of preservatives has the advantage of overcoming possible resistance of the organisms against single preservative components.

Parabens, esters of para-hydroxybenzoic acid, have been used as preservatives of cosmetics for over 60 years.   The methyl- and propylparabens are the most commonly used preservatives for cosmetics and they are widely used for pharmaceuticals as well. As has been demonstrated over their long history, the parabens are very safe however, they can sensitise the skin and cause contact dermatitis, although the incidence of this is low. The parabens are effective against moulds, yeasts and gram-positive bacteria, but can only be considered as *bacteriostatic* (a drug that prevents bacterial growth and reproduction but does not necessarily kill them) as when it is removed from the environment the bacteria start growing again. Combining parabens with *bactericidal* agents (an agent that kills bacteria), is a common means of ensuring complete microcidal activity in a formulation.   In addition, combinations of parabens are used if two different phases are present. For example, methylparaben will protect the aqueous phase, in which it is more soluble, whilst propylparaben and butylparaben will provide protection in the oil phase, in which they exhibit better solubility. The usefulness of parabens is often limited by their low water solubility. The presence of PG increases the activities of the parabens by increasing their solubilities. Parabens are subject to neutralisation by non-ionic surfactants as they can become bound, or trapped, in micelles. Increasing the length of the alkyl group generally increases antimicrobial activity, but decreases water which limits their application in certain formulations. Parabens are stable and effective in a pH range of 4 to 8. At pH 8.5, 50% of the compound is ionised, thus at the higher end of the effective pH range, the parabens ionise. The charged species cannot cross the microbial membrane and so the efficacy of the preservative is reduced (Anger *et al*., 1996:397). Tables 2.2 and 2.3 give more detail about the properties of the parabens.   Table 2.3 presenta the solubility of the parabens in different dispersion media; note the decrease in solubility in water with an increase in alkyl group length from methylparaben through to butylparaben.

Table 2.2 Physicochemical properties of the parabens (Anger *et al*., 1996:396).

Property	Methyl	Ethyl	Propyl	Butyl
Molecular weight	152.14	166.17	180.2	194.23
Melting point (°C)	125-127	116-118	96-98	69-71

Table 2.3: Solubility of the parabens in different solvents (Anger *et al*., 1996:396).

Solubility (g/100g)	Methyl	Ethyl	Propyl	Butyl
Water, (10°C)	0.2	0.07	0.025	0.005
Water, (25°C)	0.25	0.17	0.05	0.02
Water, (80°C)	2	0.86	0.3	0.15
Alcohol (25°C)	52	70	95	210
PG (25°C)	22	25	26	110
Glycerin (25°C)	1.7	0.5	0.4	0.3
Peanut oil (25°C)	0.5	1	1.4	5
Mineral oil, USP, (25°C)	0.01	0.025	0.03	0.1

2.5.7   BUFFER

Buffers are often added to solutions to prevent pronounced variations of pH during use and storage. Buffers dissolve in the solvent and then resist changes in pH should an acid or an alkali be added to the formulation. The pH and buffering capacity required will determine the choice of buffer. Most pharmaceutical buffers are based on carbonates, phosphates, citrates, lactates, gluconates and tartrates. Borates have been used for external products but not in those intended for abraded skin or membranes. The pH of most bodily fluid is 7.4 therefore this is the pH for which most products will be buffered (Marriott *et al*., 2006:82).

One of the viscosity-increasing agents, the acrylic polymer Carbopol® 934P, in its presolvated state is tightly coiled and its performance capabilities limited. Dispersed in water, the molecule is hydrated and uncoils to some extent. Even before neutralisation, the dispersion of the polymer can result in significant increase in

viscosity. Formulating and preparing a lotion, cream, gel or oral liquid with Carbopol® 934P will require only 10 to 20% w/v of a hydroxyl donor. The neutralisation ionises the polymer and generates negative charges along the backbone of the polymer. Repulsion of like charges then causes uncoiling of the molecule thereby extending the structure. This reaction is rapid and gives instantaneous thickening and emulsion formation/stabilisation. Inorganic bases, such as sodium hydroxide or potassium hydroxide, or low molecular weight amines and alkanol amines will provide satisfactory neutralisation.

For effective neutralisation, Lubrizol (2011) gives the following helpful tips:
- Prevent over neutralisation by pre-weighing the neutralising agent.
- Add strong inorganic bases as 10 to 20% w/v aqueous solutions.
- Use moderate agitation (800 to 1200 rpm) to avoid entrapping air bubbles.
- Allow sufficient time for reaction equilibrium when using pH measurements to determine degree of neutralisation.
- Do not use divalent inorganic bases such as calcium hydroxide to neutralise Carbopol® 934P.
- Let the acid dispersion stand to release entrapped air bubbles.
- Heating the dispersion hastens thickening but temperatures above 60°C should not be used.

2.5.8   SOLVENT

A number of liquids can act as pharmaceutical solvents, with water being the most commonly used in pharmacy. In the pharmaceutical context, there are a number of terms used to describe water, each reflecting the source, quality and purity of the water. Distilled water was used as the solvent in this study. Care must be taken to select an appropriate water type when preparing aqueous vehicles when compounding. Some drugs are insoluble or only poorly soluble in water and in such cases, it is necessary to employ an organic compound, either alone or as a co-solvent with water as the vehicle (Marriott *et al*., 2006:73).

2.5.9   NITROUS OXIDE

N_2O is a volatile anaesthetic compound that is both water- and fat-soluble. This characteristic enables the gas to move freely through the epidermal and dermal layers. N_2O has an oil/gas partition coefficient of 1.4, which indicates an average lipid solubility when compared with other volatile anesthetics. The lipid-rich Pheroid® membrane provides an ideal site in which N_2O can concentrate. When sufficient accumulation occurs, the membrane fluidity of specific cells is increased. The

cellular membranes are dynamic and constantly changing, with not only movement of proteins and lipids laterally on the membrane but molecules moving into and out of the membrane. The increase in fluidity brought on by N_2O and unsaturated fatty acids should increase the movement of hydrophobic molecules or hydrophilic compounds in association with essential fatty acids to move laterally in the membrane to the connecting cells. Molecular modelling indicates there is some interaction between the fatty acids and the nitrous oxide, resulting in stable vesicular Pheroid® structures. The Nitrous Oxide Essential Fatty Acid (NOEFA) matrix therefore provides a functional model for the transport of hydrophobic and hydrophilic API. It was noted in controlled experiments on various formulations that if either the N_2O or the Essential Fatty Acids (EFA's) were absent from the formulation, the efficacy and stability of the formulation was decreased dramatically (Grobler, 2009:153).

2.6   DEVELOPMENT PROGRAMME FOR PHEROID® EMULGEL

To develop a new product, a formulator must identify raw materials with the desired functionality and combine these materials in the proper ratios to yield an acceptable finished product that performs as intended and remains stable (Swart, 2002:15).

Since product development is so extensive, certain aspects of the process applicable to this study will be discussed next.

2.6.1   PREFORMULATION

Before the formulation of a drug substance into a dosage form, it must be chemically and physically characterised. Preformulation studies supply the information needed to define the nature of the drug substance. This information is then used as the framework for the drug's combination with pharmaceutical ingredients in the fabrication of a dosage form (Allen, 2008:2103).

2.6.2   EARLY FORMULATION

During early formulation a trial-and-error approach is followed. The basic Pheroid® formula as outlined in section 2.7.2.1 will be altered by adding varying concentrations of viscosity enhancers (outlay of formulas in table 2.5). Section 2.7.2.2 contains the stability tests performed on each formula to see the effect on the physical properties of each formula. The most suitable formulations will be identified and accelerated stability tests performed to obtain a formula for future development.

2.6.3 PRESERVATION OF THE PHEROID® FORMULA

It should be realised that the presence of microorganisms in a pharmaceutical preparation may have a variety of consequences, ranging from negligible to very serious. Apart from possible infection of patients, the other important effect of contamination of medicines is general spoilage. This may result in obvious changes, such as discolouration, breakdown of emulsions and the production of gas and various odours. Such comparatively dramatic effects of deterioration do have the virtue of directing the consumer's attention to the problem and discouraging their use of the medicine (Parker & Hodges, 2002:660). In Chapter 3 the results of a preservative efficacy test on a variety of Period® formulas with different kinds of preservatives will be presented.

2.6.4 FINAL FORMULATION

In Chapter 4, the best possible formulas, obtained during the early formulation tests, were manufactured in bulk for storage and testing purposes. A validated three month stability programme was followed at three storage temperatures.

2.7 FORMULATION OF A PHEROID® EMULGEL

2.7.1 INTRODUCTION

The inability of emulsion theory to predict the composition of appropriate emulsion systems is mitigated to some extent by the development of optimisation techniques that facilitate product development. The formulation of any product, even by a trial-and-error approach, involves an optimisation process: goals are defined, evaluation procedures are selected, initial compositions are defined, products are prepared and evaluated appropriately and the prospective formulation then modified until acceptable data are obtained. Presumably, a series of logical steps is taken by the experimenter who controls the variables until a satisfactory product results. Nonetheless, in the absence of a mathematically or statistically rigorous approach to optimisation, this satisfactory product is but a provisionally satisfactory product; it is not necessarily the optimal formulation. Subsequent experience with the less than optimal formulation during scale-up or processing or in the marketplace often demonstrates the formulation's suboptimal character whether by instability, poor performance or lack of acceptance by the consumer (Block, 1996:74).

2.7.2   MATERIALS AND METHODS

2.7.2.1 Materials

The following list of materials forms the basic Pheriod® formula (see Table 2.4).

Table 2.4: Composition of the basic Pheriod® formula.

	Composition	% w/v
Oil phase	Vit F Ethyl Ester	2.80%
	Cremophor® EL	1.00%
	DL-α-Tocopherol	0.20%
	Butylated hydroxyanisole (BHA)	0.04%
	Butylated hydroxytoluene (BHT)	0.20%
	Tert-Butylhydroquinone (TBHQ)	0.20%
Water phase	Nipastat®	0.175%
	$N_2O.H_2O$	to 100%

See Table 2.5 for experiment numbering. In the first six experiments Carbopol® 934 NF was added in three varying concentrations (0.10% v/w, 0.20% w/v and 0.30% w/v). Formulation F1 to F3 no PG was added and in formulation F4 to F6 PG (10.0% w/v) will be added.

Table 2.5:   The experimental outlay followed during the early formulation experiments.   (Propylene glycol = PG, XG = Xanthan gum, HPMC = Hydroxypropylmethylcellulose, A = method A, B = method B).

		Carbopol® 934P			XG			HPMC	
		0.10%	0.20%	0.30%	1.00%	1.50%	2.00%	2.00%	3.00%
PG	**0%**	F1	F2	F3	F7 A+B	F8 A+B	F9 A+B	F13 A+B	F14 A+B
	10%	F4	F5	F6	F10 A+B	F11 A+B	F12 A+B	F15 A+B	F16 A+B

Formulation F7 to F12 contained XG at different concentrations (1.00% w/v,1.50% w/v and 2.00% w/v) depending on the formula. Formulations F7 to F9 contained no PG while formulations F10 to F12 contained PG (10% w/v) as co-solvent.

A set of formulations, formulations F13 to F16 containing HPMC as viscosity enhancer was also prepared. HPMC was employed at two different concentrations, namely 2.00% w/v for formulations F13 to F15 and 3.00% w/v for formulations F14 to F16. Formulations F13 to F15 contained no PG, while formulations F14 to F16 contained PG at a concentration of 10% w/v. Two different methods of addition of XG and HPMC were also investigated:

1)   In method A, the XG and HPMC were dissolved in either the dinitrous oxide water or a mixture of dinitrous oxide water and PG.

2)   In method B, the XG and HPMC were dissolved in the oil phase. Method of formulation

2.7.2.2 Method of formulation

See Annexure B for the manufacturing sheets of all 17 experiments.

Pheroid® experimental batch manufacturing document for Carbopol® 934P, Formulations F1 to F6.

Step-wise weigh $N_2O.H_2O$ and Nipastat®.

- Add the Nipastat® to the water and stir well.
- Heat mixture to 60°C on hotplate with temperature probe.
- Weigh the Carbopol® 934 P and add it to the heated water mixture at 800 rpm.
- Add the sodium hydroxide buffer slowly to the mixture at 300 rpm while stirring continuously.
- Weigh and heat together Vit F Ethyl Ester, Cremophor® EL, butylated hydroxyanisole (BHA), butyl hydroxytoluene (BHT) and tert-Butylhydroquinone (TBHQ).
- Heat the oil mixture on hotplate to 60°C.
- Weigh the DL-α-tocopherol and add it to the heated oil phase.
- Immediately add the oil mixture to the water mixture and homogenise at 13500 rpm for 5 minutes.
- Add the cooled down mixture to clean amber bottles and shake on GFL shaker (GFL Gesellschaft für Labortechnik mbH, Germany) until room temperature (25 ± 2ºC) is reached and then store in a refrigerator at 6ºC for

24 hours before different tests for emulsion characteristics are performed (see section 2.7.2.3).

Formulations F4 to F6 with added PG, add the PG to the water mixture in step 1.

Pheroid® experimental batch manufacturing document for XG, Formulations F7 to F12, following method A

Method A is followed when the XG is dispersed in the water phase of the emulgel. Step-wise weigh $N_2O.H_2O$ and Nipastat®.

- Add the Nipastat® to the water. Stir well.
- Add the weighed XG to the water mixture while stirring with overhead stirrer at 1200 rpm for 10 minutes or until fully hydrated.
- Heat the water mixture to 70°C.
- Weigh and heat together Vit F Ethyl Ester, Cremophor® EL, butyl hydroxyanisole (BHA), butyl hydroxytoluene (BHT) and tert-Butylhydroquinone (TBHQ) on hotplate until 60°C.
- Weigh the DL-α-tocopherol and add it to the heated oil phase.
- Immediately add the oil mixture to the water mixture and homogenise at 13500 rpm for 5 minutes.
- Add the cooled down mixture to clean amber bottles and shake on GFL shaker (GFL Gesellschaft für Labortechnik mbH, Germany) until room temperature (25 ± 2ºC) is reached and then store in a refrigerator at 6ºC for 24 hours before different tests for emulsion characteristics are performed.

Formulations F10 to F12 with added PG, add the PG to the water mixture in step 1.

Pheroid® experimental batch manufacturing document for XG, Formulations F7 – F12, following method B

Method B is followed when the XG is dispersed in the oil phase of the emulgel.
- Step-wise weigh Vit F Ethyl Ester, Cremophor® EL, butyl hydroxyanisole (BHA), butyl hydroxytoluene (BHT) and tert-Butylhydroquinone (TBHQ) and mix well with spatula.
- Weigh the XG and add slowly to the oil phase while stirring with spatula.
- Heat the mixture to 60°C.
- Weigh the DL-α-tocopherol and add it to the heated oil phase.
- Weigh Nipastat®, $N_2O.H_2O$ and mix together.
- Heat the water mixture on hotplate until 70°C.

- Immediately add the oil phase to the water phase while homogenising at 13500 rpm for 5 minutes.
- Continue stirring with overhead stirrer at 400 rpm until room temperature is reached.
- Add the cooled down mixture to clean amber bottles and shake on GFL shaker (GFL Gesellschaft für Labortechnik mbH, Germany) until room temperature (25 ± 2ºC) is reached and then store in a refrigerator at 6ºC for 24 hours before different tests for emulsion characteristics are performed.

Formulations F10 to F12 with added PG, add the PG to the water mixture.

Pheroid® experimental batch manufacturing document for HPMC, Formulations F13 to F16, following method A

Method A is followed when the HPMC is dispersed in the water phase of the emulgel. Step-wise weigh $N_2O.H_2O$ and Nipastat®.

- In a 400 ml glass beaker add the Nipastat® to the water.
- Stir well.
- Add the HPMC while stirring with overhead stirrer a 300 rpm for 10 minutes or until fully hydrated.
- Heat the water mixture to 70°C on hotplate.
- Weigh and heat together Vit F Ethyl Ester, Cremophor® EL, butyl hydroxyanisole (BHA), butyl hydroxytoluene (BHT) and tert-Butylhydroquinone (TBHQ) on hotplate until 60°C.
- Weigh the DL-α-tocopherol and add it to the heated oil phase.
- Immediately add the oil mixture to the water phase while homogenising at 13500 rpm for 2 minutes.
- Continue stirring with overhead stirrer at 300 rpm until room temperature is reached.
- Add the cooled down mixture to clean amber bottles and shake on GFL shaker (GFL Gesellschaft für Labortechnik mbH, Germany) until room temperature (25 ± 2ºC) is reached and then store in a refrigerator at 6ºC for 24 hours before different tests for emulsion characteristics are performed.

Formulations F15 F16 with added PG, add the PG to the water mixture in step 1.

Pheroid® experimental batch manufacturing document for HPMC, Formulations F13 – F16, following method B

Method B is followed when the HPMC is dispersed in the oil phase of the emulgel.

- Weigh and heat together Vit F Ethyl Ester, Cremophor® EL, butyl hydroxyanisole (BHA), butyl hydroxytoluene (BHT) and tert-Butylhydroquinone (TBHQ).
- Weigh the HPMC and add it slowly to the oil phase while stirring with spatula.
- Heat to 60°C.
- Weigh the DL-α-tocopherol and add it to the heated oil phase.
- Weigh Nipastat® and $N_2O.H_2O$ and mix together.
- Heat on hotplate until 70°C.
- Immediately add the heated oil phase to the water phase while homogenising at 13500 rpm for 3 minutes and a further 2 minutes at 1838 rpm.
- Continue stirring with overhead stirrer at 300 rpm until room temperature is reached.
- Add the cooled down mixture to clean amber bottles and shake on GFL shaker (GFL Gesellschaft für Labortechnik mbH, Germany) until room temperature (25 ± 2ºC) is reached and then store in a refrigerator at 6ºC for 24 hours before different tests for emulsion characteristics are performed.

Formulations F15 to F16 with added PG, add the PG to the water mixture.

2.7.2.3 Results

The influence of the varying concentrations of the three viscosity enhancers was tested over a 28 day period. The tests used to examine the stability of each formulation were pH measurements, viscosity measurements, light microscopy and CLSM as well as visual assessment. The stability intervals for each test are 24 hours, 72 hours, seven days and 28 days.

2.7.2.4 pH

The average pH value measurements obtained for Carbopol® 934P (see Table 2.6) in varying concentrations of 0.10% w/v, 0.20% w/v and 0.30% w/v with or without added PG varied with less than ± 0.32 pH units, therefore the pH remained similar over the 28 days stability period. The neutral values (pH ± 7), proved that total neutralisation of the carbomer was needed to obtain sufficient viscosity for the emulgel preparation.

Table 2.6: pH values measured for Carbopol® 934P in varying concentrations of 0.10% w/v, 0.20% w/v and 0.30% w/v with and without added PG (10% w/v). Prepared according to method A and method B over a 28-day stability period.

Time points	24 H	72 H	7 Days	28 Days	AVE	STDEV
0.10%	6.865	6.876	6.744	6.644	6.782	0.110
0.10% + 10% PG	7.092	6.964	7.010	6.834	6.975	0.108
0.20%	6.838	6.819	6.890	6.757	6.826	0.055
0.20% + 10% PG	6.845	6.861	6.883	6.763	6.838	0.052
0.30%	6.835	6.623	6.645	6.550	6.663	0.121
0.30% + 10% PG	6.819	6.796	6.819	6.781	6.804	0.019

On average the added PG did not increase the pH values for each formula compared to the formulas without PG (see Figure 2.3).
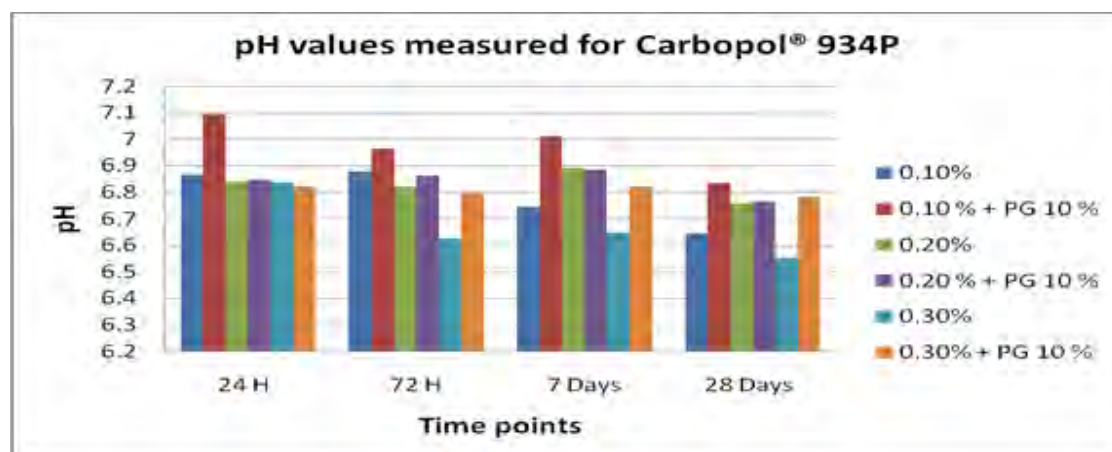
Figure 2.3: The influence of different concentrations of Carbopol® 934P on the pH values over a 28-day stability interval.

The average pH value obtained for XG (see Table 2.7) in varying concentrations of 1.0% w/v, 1.5% w/v and 2.0% w/v with or without added PG varied with less than ± 0.14 pH units, therefore the pH remained similar over the 28-day stability period. The added PG did not cause a pronounced change in the pH values for each formula compared to the formulas without PG (see Figure 2.4).
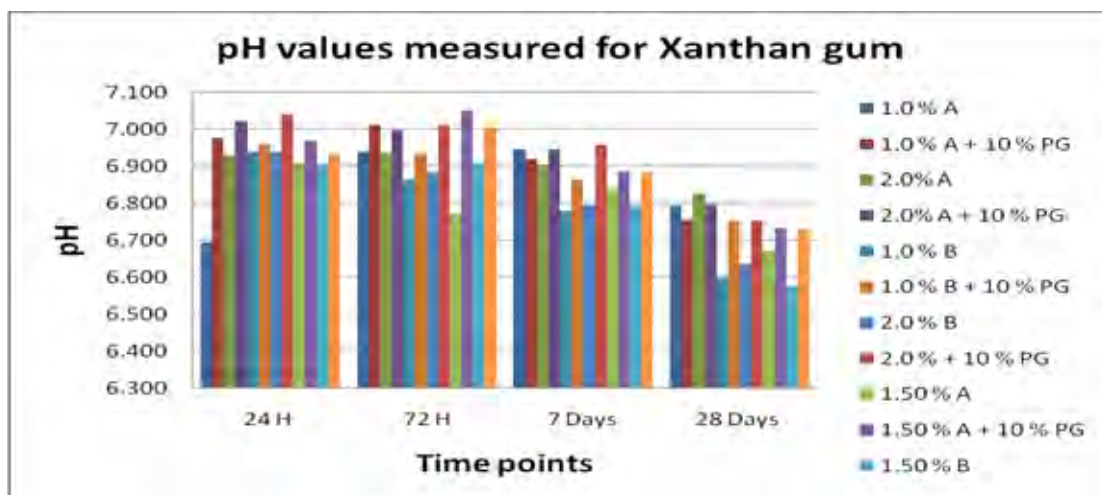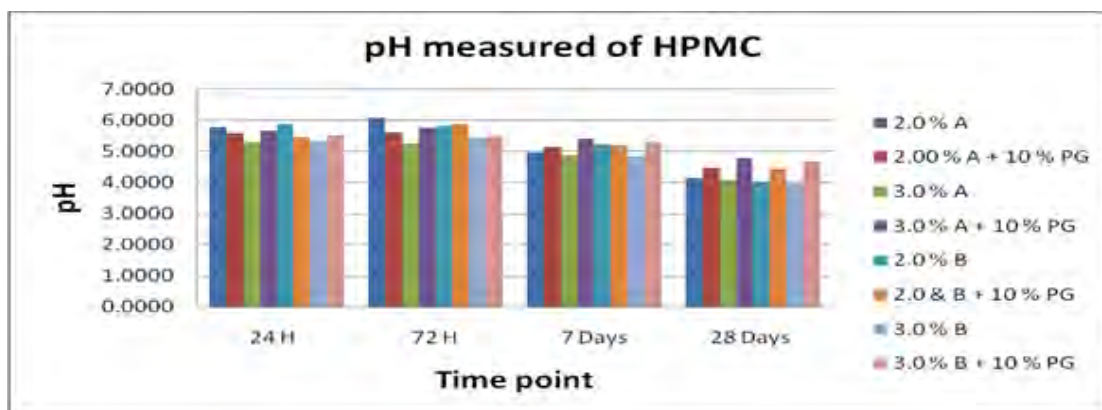
Figure 2.4: The influence of different concentrations of XG on the pH values over a 28-day stability interval.

Table 2.7: pH values measured for XG in varying concentrations of 1.0% w/v, 1.50% w/v and 2.0% w/v with and without added PG (10% w/v). Prepared according to method A and method B over a 28-day stability period.

Time points	24 H	72 H	7 Days	28 Days	AVE	STDEV
1.0% A	6.696	6.942	6.945	6.795	6.845	0.121
1.0% A + 10% PG	6.978	7.014	6.920	6.757	6.917	0.114
2.0% A	6.931	6.936	6.905	6.828	6.900	0.050
2.0% A + 10% PG	7.024	6.998	6.946	6.798	6.942	0.101
1.0% B	6.940	6.863	6.780	6.598	6.795	0.147
1.0% B + 10% PG	6.961	6.935	6.864	6.753	6.878	0.093
2.0% B	6.939	6.885	6.794	6.637	6.814	0.132
2.0% + 10% PG	7.042	7.014	6.959	6.755	6.943	0.130
1.50% A	6.910	6.773	6.842	6.673	6.800	0.101
1.50% A + 10% PG	6.970	7.052	6.888	6.733	6.911	0.136
1.50% B	6.905	6.908	6.795	6.576	6.796	0.156

On average the HPMC pH values varied with ± 0.53 pH units between the various formulas prepared (see Table 2.8). After only seven days the pH values measured for all the formulas prepared showed a lowering in pH values from the initial values measured at 24 hours and 72 hours (see Figure 2.5). The pH measurements performed at 28 days showed an overall lowering in the values measured compared to the values measured at 24 hours, 72 hours and seven days.

Figure 2.5: The influence of different concentrations of HPMC on the pH values over a 28-day stability interval.

The addition of PG or the different methods used for preparing the formulas had no pronounced influence on the pH measurements.

Table 2.8: pH values measured for HPMC in varying concentrations of 2.0% w/v and 3.0% w/v with or without added PG (10% w/v). Prepared according to method A and method B over a 28-day stability period.

Time points	24 H	72 H	7 Days	28 Days	AVE	STDEV
2.0% A	5.799	6.086	4.962	4.134	5.245	0.881
2.0% A + 10% PG	5.584	5.611	5.145	4.484	5.206	0.527
3.0% A	5.288	5.265	4.877	4.088	4.880	0.560
3.0% A + 10% PG	5.662	5.765	5.410	4.785	5.406	0.440
2.0% B	5.869	5.816	5.225	4.013	5.231	0.863
2.0% B + 10% PG	5.467	5.884	5.197	4.434	5.246	0.610
3.0% B	5.328	5.446	4.847	3.984	4.901	0.664
3.0% B + 10% PG	5.527	5.500	5.282	4.690	5.2550	0.389

2.7.2.5 Viscosity

A gel formulation that is unstable or not suitable for marketing under normal circumstances, would exhibit some irreversible changes in its rheological properties of sufficient magnitude to cause it to be unacceptable for use. Examples of unstable gels include gels that ‑set-up" during storage and can no longer be squeezed from a tube, gels that undergo phase separation — either of the liquid (as in syneresis) or of the solid (as in particle sedimentation) — and gels that suffer a progressive loss of viscosity or consistency, changing from semisolids to viscous liquids. It is important to recall that rheological behaviour is complex. A change in rheological behaviour must be interpreted in the proper frame of reference — the desired properties of the final product. A small decrease in the viscosity of a clear hairdressing may be much less significant than the same change in a topical product (Zatz and Kushla, 1996:403).

See Annexure C for viscosity values obtained for all 17 test formulas. The Wingather Brookfield® Software package was used to interpret the data points of each test formula. A table with the viscosity (cP), speed (RPM), torque (%), shear stress (D/cm^2), shear rate (1/sec), temperature (°C) and time interval (mm.ss.t) was drawn. From these data points a histogram of shear stress vs. shear rate were drawn. As seen in Figure 2.6, the prepared emulgel formulation with added Carbopol® 934P as thickening agent exhibited shear-thinning flow behaviour since the viscosity (the slope of the curve) decreased with an increase in shear rate. As the shear rate increased, the normally disarranged molecules of the gelling material aligned along its axes in the direction of flow. Thus orientation reduced the internal resistance of the material and hence decreased the viscosity. The figure also showed the Carbopol® 934P emulgel formulation possessed thixotropic behaviour, where the descending curve was displaced with regard to the ascending curve, showing at any rate of shear on the down curve a lower shear stress than on the ascending curve. A hysteresis loop formed between the two curves. Thixotropy, or time-dependent flow, occurs because the gel requires an infinite time to rebuild its original structure that breaks down during continuous shear measurements. Carbopol-based formulations showed considerable changes in viscosity values with an increase in concentration used. The data obtained correlates well with experiments done by Mohamed (2004:3), where the influence of the type of gelling agent and concentration of both the oil and emulsifying agent on the drug release from the prepared emulgels were investigated. The Carbopol® 934P 0.30% w/v formulations gave viscosity values higher than could be measured accurately with the spindle set used throughout this

study. Carbopol® 934P 0.20% w/v with added 10% w/v of PG showed an initial lowering in viscosity after which it remained stable for the 28-day stability time. The Carbopol® 934P 0.20% w/v formulation, without added PG, showed a lowering in viscosity values throughout the 28 days testing period. This possibly indicates future instability for this formulation (see Table C1).
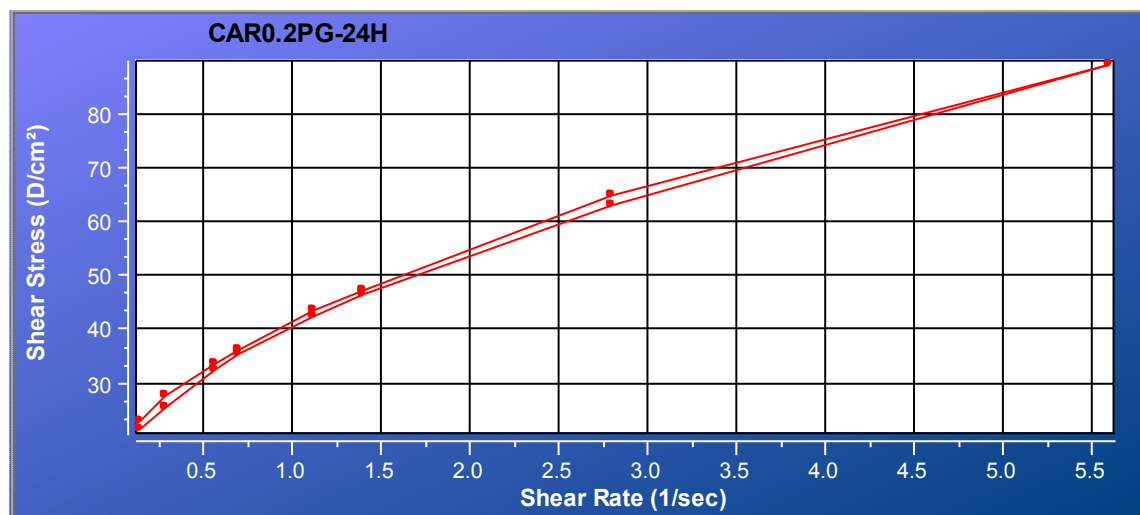
Figure 2.6: Rheogram of Carbopol®-based Pheroid® emulgel formulation with 0.20% w/v Carbopol® 934P and 10% w/v PG after an interval of 24 hours.

The XG formulations exhibited a shear-thinning behaviour with a definite yield point value. As the shear rate was sufficiently increased to overcome the Brownian motion, the emulsion droplets became more ordered along the flow field and offered less resistance to flow and hence the lower viscosity. Sun *et al* (2007:557) obtained similar results when they investigated the effect of XG on the physicochemical properties of an o/w emulsion. Another feature of XG solution is its viscoplasticity, which gives a high yield value even at low concentrations. The yield value is the minimum shear stress required for a solution to flow. The yield value is difficult to measure because it is necessary to work at very low shear rates and frequently this value is extrapolated with different rheological models, such as those of Bingham and Herschel-Buckley (Urlacher and Noble, 1997:289). The XG contributed to a yield value which appreciably inhibited creaming. Higher concentrations of XG resulted in higher viscosity values. The added PG further increased the viscosity of the preparations. With regard to dispersion method, method A resulted in higher viscosity values compared to method B. The formulations with 2.00% w/v added XG exhibited higher values than could be measured with the spindle set used. Formulation F11A prepared with 1.50% w/v XG, 10% w/v PG and prepared

according to method A had the most consistent viscosity measured over the 28-day period (see Table C2).
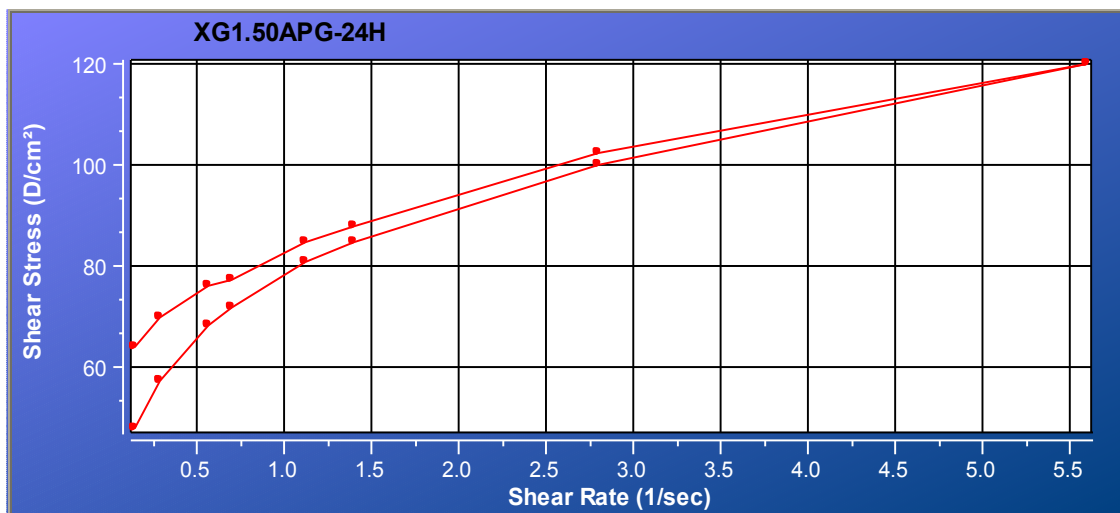
Figure 2.7: Rheogram of XG-based Pheroid® emulgel formulation with 1.50% w/v XG and 10% w/v PG manufactured according to method A after an interval of 24 hours.

The HPMC gels exhibited shear thinning and did not exhibit significant yield or hysteresis in their rheograms. The viscosity increased with an increase in the concentration of the polymer. The HPMC gels are viscoelastic and exhibited greater degrees of elasticity with an increase in PG concentration. Formulations formulated with method B proved to have higher viscosity although this was still not enough to help maintain the stability of the emulgel for the whole duration of the stability test. As will be seen with the visual assessment, the HPMC gels disintegrated into their basic oil and water phases. No viscosity values were obtainable at the 28-day interval (see Table C3).
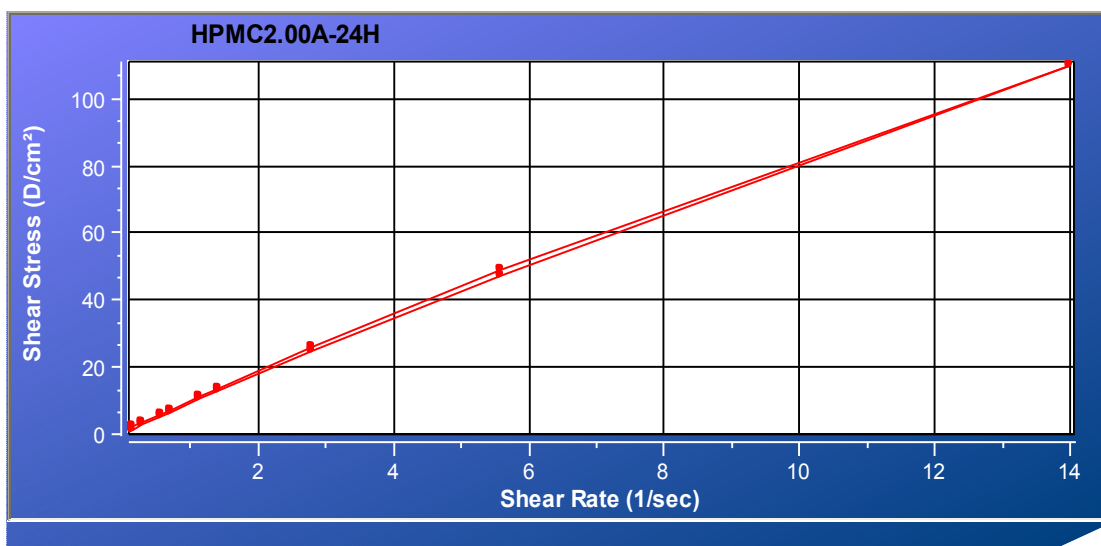
Figure 2.8: Rheogram of HPMC-based Pheroid® emulgel formulation with 2.00% w/v HPMC manufactured according to method A after an interval of 24 hours.

2.7.2.6 Visual assessment

Images of each formulation were taken at 24 hours, 72 hours, seven days and 28 days. Figure 2.9 shows the concentration range prepared for Carbopol® 934P (0.10% w/v, 0.20% w/v and 0.30% w/v) each prepared with PG 10% w/v and without PG. The lowest concentration (0.10% w/v) formed a thin layer of cream on top, proving the viscosity was not high enough to maintain a stable preparation. Both the formulations prepared with 0.20% w/v and 0.30% w/v overcame this problem. All six preparations displayed a slight discolouring to pink, which may be due to oxidation. For further stability studies, amber bottles were used.

Figure 2.9: Formulations F1 to F6 prepared with varying concentrations of Carbopol® 934P after an interval of 28 days.

According to the viscosity values measured for the Pheroid® range thickened and stabilised with XG the most stable formulation proved to be F11A. This formula contained 1.50% w/v XG along with 10% w/v PG and method A was used to prepare the formula. In Figure 2.10 the concentration range of 1.50% w/v XG can be seen with no visual difference between the formulas prepared with the PG or without the PG. No creaming could be seen and no discolouring took place over the 28-day time interval.

Figure 2.10: XG emulgel prepared with 1.50% w/v XG, with and without PG and according to method A and B after an interval of 28 days.

The HPMC formulations showed extensive stability problems. In Figure 2.11 the HPMC formulations with 2.00% w/v and 3.00% w/v HPMC each with and without added PG and manufactured according to method A is shown. See-through mixtures with pronounced creaming and discolouring to yellow made these formulations unfit for further preparation work. Possible reasons for this instability might be contributable to the use of a medium high viscosity range of HPMC. Light microscopy and CLSM will give a better insight to the particle size and the degree of depolymerisation of the polymer chain in the dispersed phase.

Figure 2.11: HPMC emulgel prepared with 2.00% w/v and 3.00% w/v HPMC, with and without PG, according to method A after an interval of 28 days.

2.7.2.7 Light microscopy and confocal laser scanning microscopy (CLSM)

The light microscopy and CLSM images gave a better understanding of the droplet formation in each formula over time and showed the distribution of oil particles and thickening agents used in the formula.

Figure 2.12 shows the light microscopy photo taken of the Pheroid® formula thickened with 0.2% w/v Carbopol® 934NF. At time interval 24 hours, an even distribution of oil droplets were seen. Compared to time interval at day 28 of the same formula, it is clear the droplet diameter had increased with bigger oil droplets seen in the figure on the right side. This increase in droplet diameter still did not influence the stability of the formula negatively and no creaming was found with this formula.
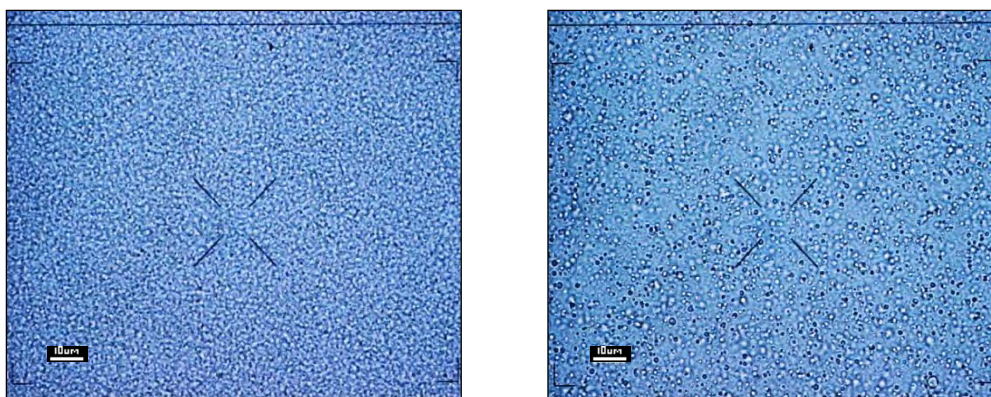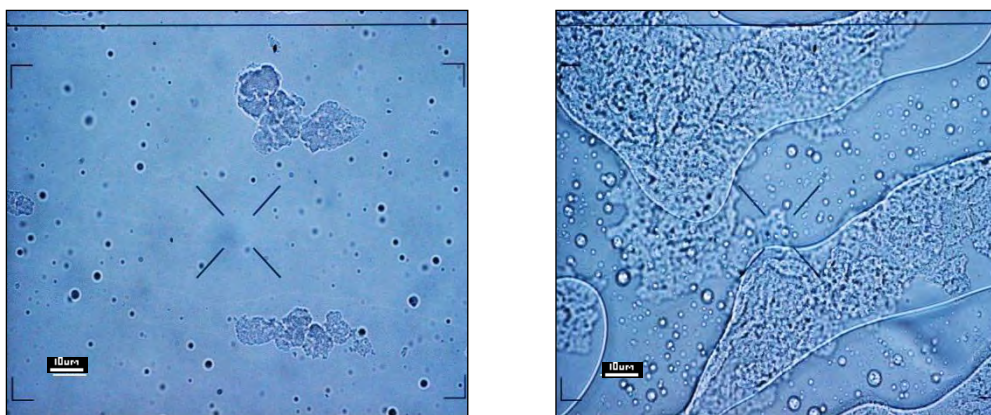
Figure 2.12: Light microscopy photos taken of CAR 0.20% w/v (PG) at time 24 hours and CAR 0.20% w/v (PG) after an interval of 28 days.

Figure 2.13 depicts the formulations prepared with 1.50% w/v XG and 10% w/v PG. The XG formulations did not show any instabilities and the droplet diameter stayed constant over the 28-day interval.
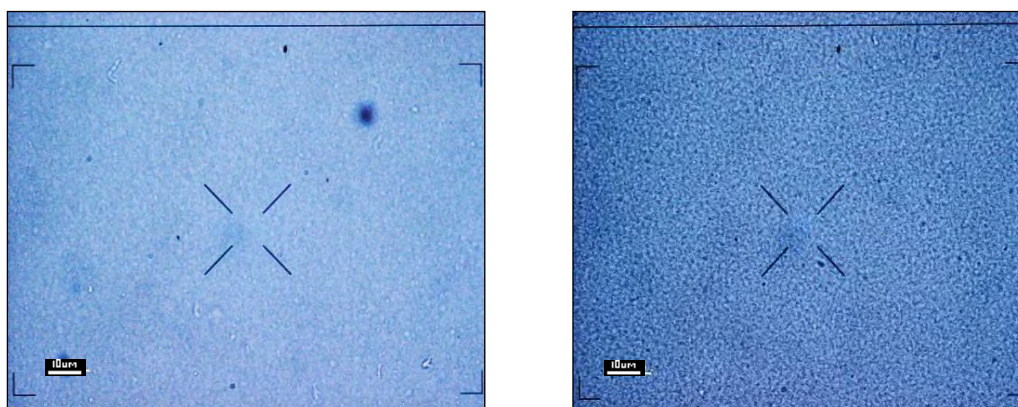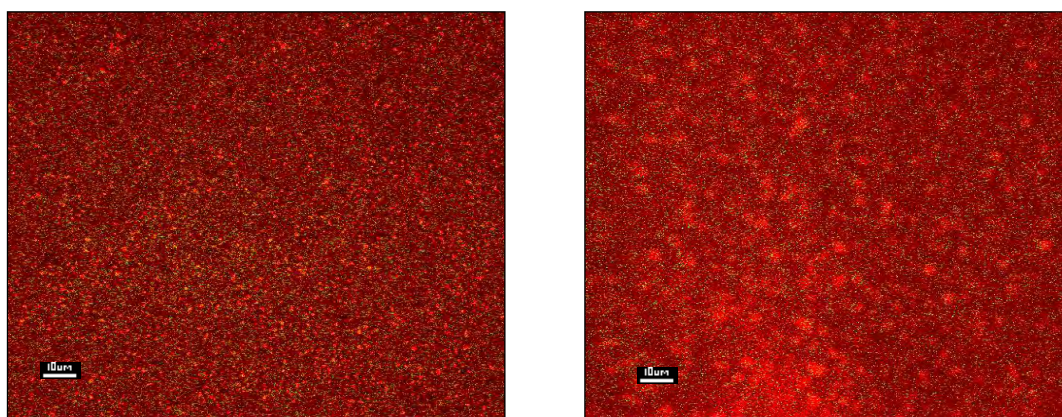

Figure 2.13: Light microscopy photos taken of XG 1.50% w/v A (PG) at time 24 hours and XG 1.50% w/v A (PG) after an interval of 28 days.

Figure 2.14 gave a better understanding of why the formulations prepared with HPMC did not form a gel network. At time 24 hours, undissolved pieces of the polymer were noticeable; at day 28, big oil patches and lumps of the HPMC were detected. This explains the two layers of oil and water formed over the 28-day interval of testing.
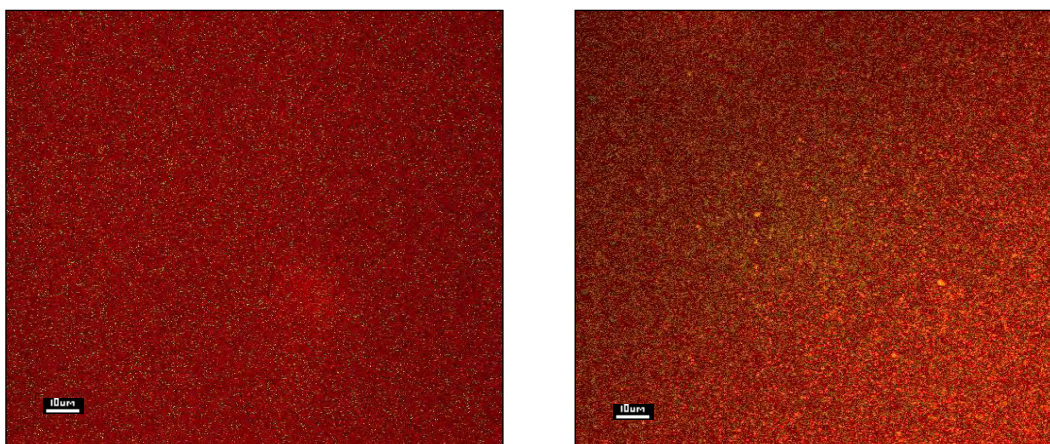
Figure 2.14: Light microscopy photos taken of HPMC 2.00% w/v A (PG) at time 24 hours and HPMC 2.00% w/v A (PG) after an interval of 28 days.

The CLSM gave a better indication of the oil versus polymer dispersion. The oil-phase is represented by the red fluorescent colour. The Carbopol® formulation shows a flocculation of the Pheroid® vesicles. The flocculate was not sufficient to cause sedimention and thus the formula stayed stable in viscosity over the test period. The XG formulation stayed stable with no obvious increase in droplet diameter. Figure 2.15 shows the formula with Carbopol® 934NF; no extensive differences were noticeable over the 28-day interval. Figure 2.16 shows the even fine distribution of the XG formula. Over the 28-day interval, no visual changes could be seen with the CLSM microscope, which shows great potential for XG as a thickener and stabiliser for the Pheroid® formula.
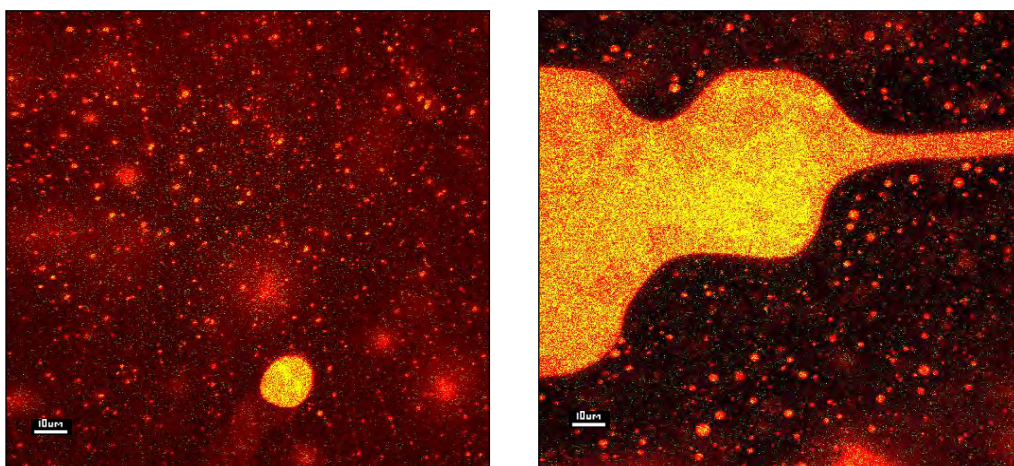

Figure 2.15: CLSM photos taken of CAR 0.20% w/v (PG) at time 24 hours and CAR 0.20% w/v (PG) after an interval of 28 days.

Figure 2.16: CLSM photos taken of XG 1.50% w/v A (PG) at time 24 hours and XG 1.50% w/v A (PG) after an interval of 28 days.

Figure 2.17 shows the cause of the instability observed with the HPMC formulations. After 24 hours, the oil droplets were small with a few patches of bigger droplets forming. Over time, the oil droplets coagulated and formed bigger oil patches. The oil, which has a lower density than the water, moved to the upper surface and formed a layer on top of the water phase.

Figure 2.17: CLSM photos taken of HPMC 2.00% w/v A (PG) at time 24 hours and HPMC 2.00% w/v A (PG) after an interval of 28 days.

2.7.2.8 Conclusion

As a formulator of a pharmaceutical product it is important to be able to foresee possible problems or shortcomings in your formulation. The building blocks should be fitted together to form a stable platform with certain repeatable results. It is a trial-and-error process with a lot to learn and a lot of satisfaction if a positive result is obtained.

Certain tests were identified to evaluate the stability features of the emulgel formulations. Time-dependant changes were recorded for pH, viscosity, visual observations, light- and confocal microscopy. After interpreting these values, two formulations were identified with potential for further development.

These two formulations consisted of the basic Pheroid® formulation and a viscosity enhancer namely Carbopol® 934NF (0.20% w/v) along with the co-solvent PG (10% w/v). The second formulation under further investigation was XG (1.50% w/v) along with PG (10% w/v) manufactured according to method A.

Further data will be obtained when an accelerated stability test is performed (see Chapter 4).