

CHAPTER FOUR

4 FINAL FORMULATION AND STABILITY TESTING

4.1 INTRODUCTION

During the preformulation experiments a trial-and-error approach was followed to determine the influences that various thickening agents in varying concentrations and added to the basic Pheroid® formula through different mixing procedures had on the physical and chemical stability. The influence of these variables on the physical and chemical stability was tested via pH measurements, particle size analysis, viscosity measurement, light microscopy and CLSM as well as visible evaluation. The importance of preservation of the final formula led to a preservative efficacy test performed on the Pheroid® formula with various preservatives added.

4.2 FORMULATION FACTORS

When formulating emulsions, an important factor to consider is the rate in which the creaming in emulsions is affected by the size of the globules of the dispersed phase, the smaller the better. The viscosity of a product can also be affected by the size of these globules. In general a mean globule diameter of between 0.5 and 2.5 µm has been found to give the best emulsions with respect to physical stability and texture. The optimum particle size can be achieved with the suitable intensity of shearing delivered by equipment for the emulsification process. Other considerations, however, include the presence of surfactants, which reduce interfacial tension and aid in the process of emulsification and promoting emulsion stability, the volume and viscosity of the emulsion and the interfacial tension between the oil and the water. Homogenizers are often used after initial mixing to enable smaller globule sizes to be produced. They all work on the principle of forced discharge of the emulsion under pressure through fine interstices, formed by closely packed metal surfaces, in order to provide an intense shearing action (Billany, 2002:357).

It is important to ensure that methods of manufacture developed on a laboratory scale can be easily extended to large-scale production without any change in the quality of the product. During manufacture it is common to add the dispersed phase

to the continuous phase during the initial mixing. The other ingredients are dissolved, prior to mixing, in the phase in which they are soluble. Should any of the oily ingredients be of solid or semisolid consistency they must be melted before mixing. It is also essential the aqueous and continuous phases be heated to the same temperatures to avoid premature solidification of the oil phase by the colder water on mixing, but before emulsification has taken place. This also has the advantage of reducing the viscosity of the system, thereby enabling shear forces to be transmitted through the product more easily. Because of the increased kinetic motion of the emulgent molecules at the oil/water interface, however, it is necessary to continue stirring during the cooling process to avoid demulsification (Billany, 2002:358).

Stability testing entails the use of exaggerated conditions such as temperature and humidity to test the stability of drug formulations. Accelerated temperature stability studies, for example, may be conducted for six months at 40°C with 75% relative humidity. If a significant change in the product occurs under these conditions, lesser temperature and humidity may be used, such as 30°C and 60% relative humidity. Short-term accelerated studies are used to determine the most stable of the proposed formulations for a drug product. In stress testing, temperature elevations, in 10°C increments higher than used in accelerated studies, are employed until chemical or physical degradation occurs. Once the most stable formulation is ascertained, its long-term stability is predicted from the data generated from ongoing stability studies. Depending on the types and severity of conditions employed, it is fairly common to maintain samples under exaggerated conditions of both temperature and varying humidity for six to 12 months. Such studies are used to predict the shelf life for a drug product (Allen *et al.*, 2005:123).

4.2.1 STABILITY TESTS CONDUCTED

In this chapter, two formulations of the Pheroid® emulgel were tested over a three month accelerated storage period compared to the period of two years for formal stability testing. The storage temperatures used were 5°C and 25°C at 60% relative humidity, 30°C and 65% relative humidity and 40°C at 75% relative humidity. Stability intervals were time zero (48 hours after manufacturing), one month, two months and three months after manufacture.

The manufacturing sheets of the two final formulas are shown in Annexure J. In this chapter, two different formulations of the emulgel were compared. F1 was prepared with Carbopol® 934P, 0.2% w/v and PG, 10% w/v. F2 has XG, 1.50% w/v, as

thickening agent with added PG, 10% w/v prepared according to method A. In both formulas Nipastat[®], 0.175% w/v, was the preservative of choice (see Table 4.1).

Table 4.1: Outline of formula 1 and formula 2 for accelerated stability study.

<i>Formula 1</i>	<i>Composition</i>	<i>% w/v</i>	<i>Formula 2</i>	<i>Composition</i>	<i>% w/v</i>
Oil phase	Vit F Ethyl Ester	2.800	Oil phase	Vit F Ethyl Ester	2.800
	Cremophor [®] EL	1.000		Cremophor [®] EL	1.000
	dL- α -Tocopherol	0.200		dL- α -Tocopherol	0.200
	Butylated hydroxyanisole	0.040		Butylated hydroxyanisole	0.040
	Butylated hydroxytoluene	0.200		Butylated hydroxytoluene	0.200
	Tert-Butylhydroquinone	0.200		Tert-Butylhydroquinone	0.200
Water phase	Nipastat [®]	0.175	Water phase	Nipastat [®]	0.175
	Sodium hydroxide (18%)	0.460		-	-
	Propylene glycol	10.000		Propylene glycol	10.000
	Carbopol [®] 934P	0.200		Xanthan gum	1.500
	N ₂ O.H ₂ O	84.725		N ₂ O.H ₂ O	83.885

4.2.2 PACKAGING MATERIAL

The role of the pack and the packaging operation needs emphasis as the shelf life of all pharmaceutical products is largely dependent on certain functions of the pack. Profitability is one of the key issues. Presentation will contribute to or enhance product confidence while maintaining adequate identification and information and lastly, it must contribute in terms of convenience and compliance. Each of these aspects has to be considered against the total (shelf) life of the product, possible display and finally use or administration, directly by a patient or indirectly by a healthcare professional. The primary pack consists of those packaging components which form the part of the pack directly containing the product (i.e. bottle, cap, cap liner, label, etc.) The main functions of the primary pack are to contain and to restrict any hazards that may cause or lead to product deterioration (Dean, 2002:555).

A further stability variable tested was the type of packaging material used to store the two emulgel formulations under investigation. Type 1 was an amber glass container and Type 2 was foil sachets.

Glass has had a successful history for pharmaceutical products in that it offers transparency, sparkle, easy cleaning, effective closure and reclosure where applicable and depending on the selection of the correct type of glass, is generally inert.

Films, foils and laminates have been used in various combinations, e.g. sachets, diaphragm seals for bottles, strip packs, blister packs, liners for large containers, overwraps, flow wraps and liners for boxes. The material used to pack the Pheroid[®] formulas is High Density PolyEthylene foil (HDPE-foil).

4.2.2.1 pH

Formula 1 contains the polymer Carbopol[®] 934P on which pH can affect the gel character adversely by either insufficient neutralisation or an excessively high pH values during emulgel formulation. Nipastat[®] was used as a preservative in both formulas containing Carbopol[®] 934P and XG. Paraben preservatives are pH sensitive and the effect of changes in storage times and temperatures was monitored. Figures 4.1 and 4.2 display the effect of storage materials and storage temperatures over time on the pH of the manufactured formulas.

4.2.2.2 Results & Discussion

In Table 4.2 the average pH values for Carbopol[®] 934P measured over the three month storage period were neutral and between 7.166 (5°C), 7.165 (25°C + 60%RH),

7.132 (30°C + 40%RH), 7.113 (40°C + 75%RH). It appears that Carbopol® 934P is uninfluenced by the various storage temperatures and humidity levels applied over the three months of accelerated stability test.

Table 4.2: pH values measured for Carbopol® 934P (0.2% w/v) and XG (1.5% w/v) over a period of three months stored in amber glass bottles.

<i>Time points</i>	<i>Initial</i>	<i>1 Month</i>	<i>2 Months</i>	<i>3 Months</i>	<i>AVE</i>
CAR 0.2(PG) 5°C	7.1900	7.1650	7.1600	7.1500	7.17
CAR 0.2(PG) 25°C + 60%RH	7.1900	7.1740	7.1380	7.1560	7.16
CAR 0.2 (PG) 30°C + 65%RH	7.1900	7.1220	7.1290	7.0880	7.13
CAR 0.2 (PG) 40°C + 75%RH	7.1900	7.1240	7.0860	7.0530	7.11
XG 1.5A (PG) 5°C	6.9360	6.9320	6.8960	6.9060	6.92
XG 1.5A (PG) 25°C + 60%RH	6.9360	6.8630	6.6160	6.6420	6.76
XG 1.5A (PG) 30°C + 65%RH	6.9630	6.6670	6.5290	6.3900	6.64
XG 15.A (PG) 40°C + 75%RH	6.9630	6.2070	5.9230	5.6680	6.19

Figure 4.1 shows the influence of temperature and prolonged storage period on the XG formulations. The average pH values obtained from Table 4.2 varied between 6.910 (5°C), 6.764 (25°C + 60% RH), 6.637 (30°C + 65% RH), 6.190 (40°C + 75% RH). The pH of the XG gradually lowered as the storage temperature and humidity levels increased.

The effect of the higher temperatures on the pH of the XG formula can be explained when you understand the influence of temperature on the structure of XG (see section 1.5.3.1). Since no electrolytes were added at the preparation of the XG formula the structure was altered by the higher temperatures and the influence is seen in the values measured over time.

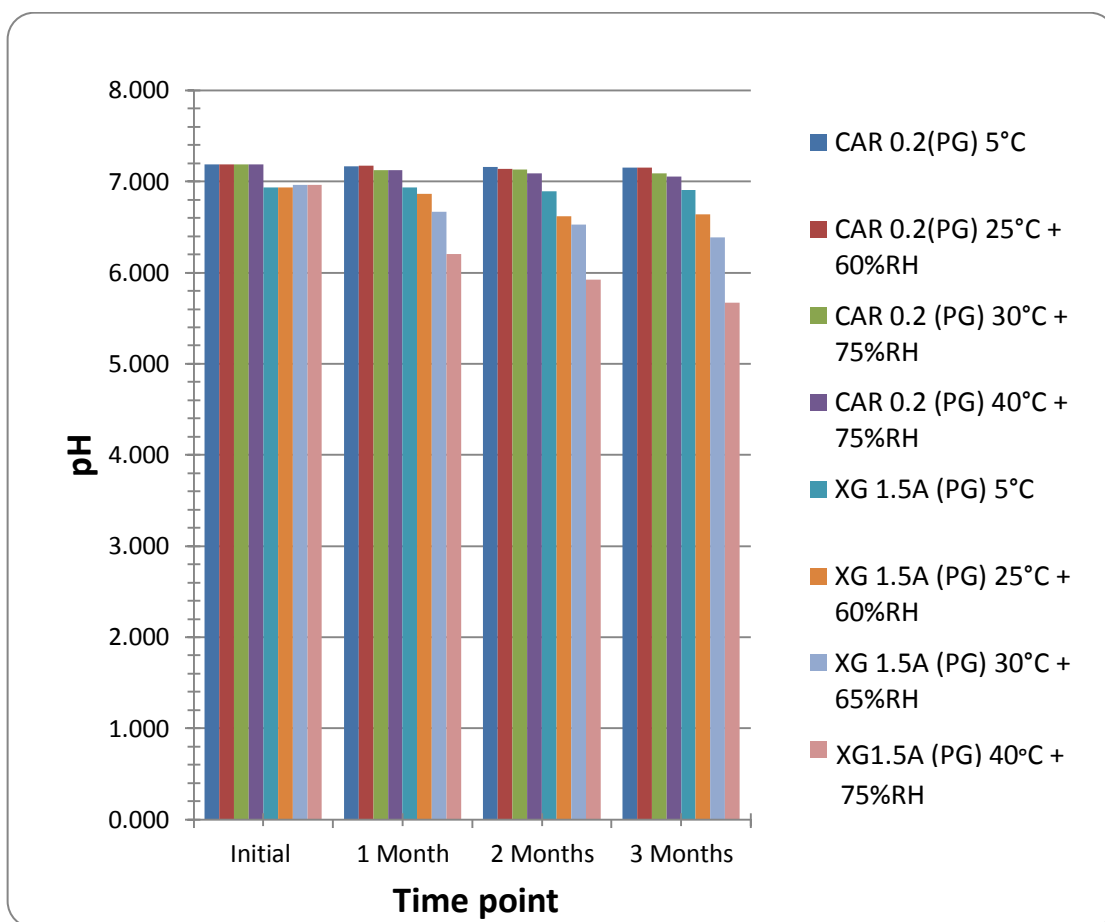


Figure 4.1: Graph indicating the influence of temperature and humidity on the pH of emulgel formulations stored in amber glass bottles over a period of three months.

Table 4.3 contains the pH values measured for the two test formulas stored in foil sachets at various temperatures and humidity values are depicted. In Figure 4.2, no visual difference in results could be seen compared to the formulas stored in amber glass bottles.

Table 4.3: pH values measured for Carbopol® 934P (0.2% w/v) and XG (1.5%) over a period of three months stored in foil sachets.

<i>Time points</i>	<i>Initial</i>	<i>1 Month</i>	<i>2 Months</i>	<i>3 Months</i>	<i>AVE</i>
CAR 0.2(PG) 5°C	7.2140	7.1850	7.1510	7.1780	7.18
CAR 0.2(PG) 25°C + 60%RH	7.2140	7.0920	7.1460	7.1370	7.15
CAR 0.2 (PG) 30°C + 65%RH	7.2140	7.1060	7.1180	7.1390	7.14
CAR 0.2 (PG) 40°C + 75%RH	7.2140	7.1330	7.0570	7.0580	7.12
XG 1.5A (PG) 5°C	6.9170	6.9000	6.8130	6.8260	6.86
XG 1.5A (PG) 25°C + 60%RH	6.9170	6.7700	6.6480	6.6330	6.74
XG 1.5A (PG) 30°C + 65%RH	6.9170	6.6660	6.1590	6.5740	6.58
XG 15.A (PG) 40°C + 75%RH	6.9170	6.3510	6.4270	5.6360	6.33

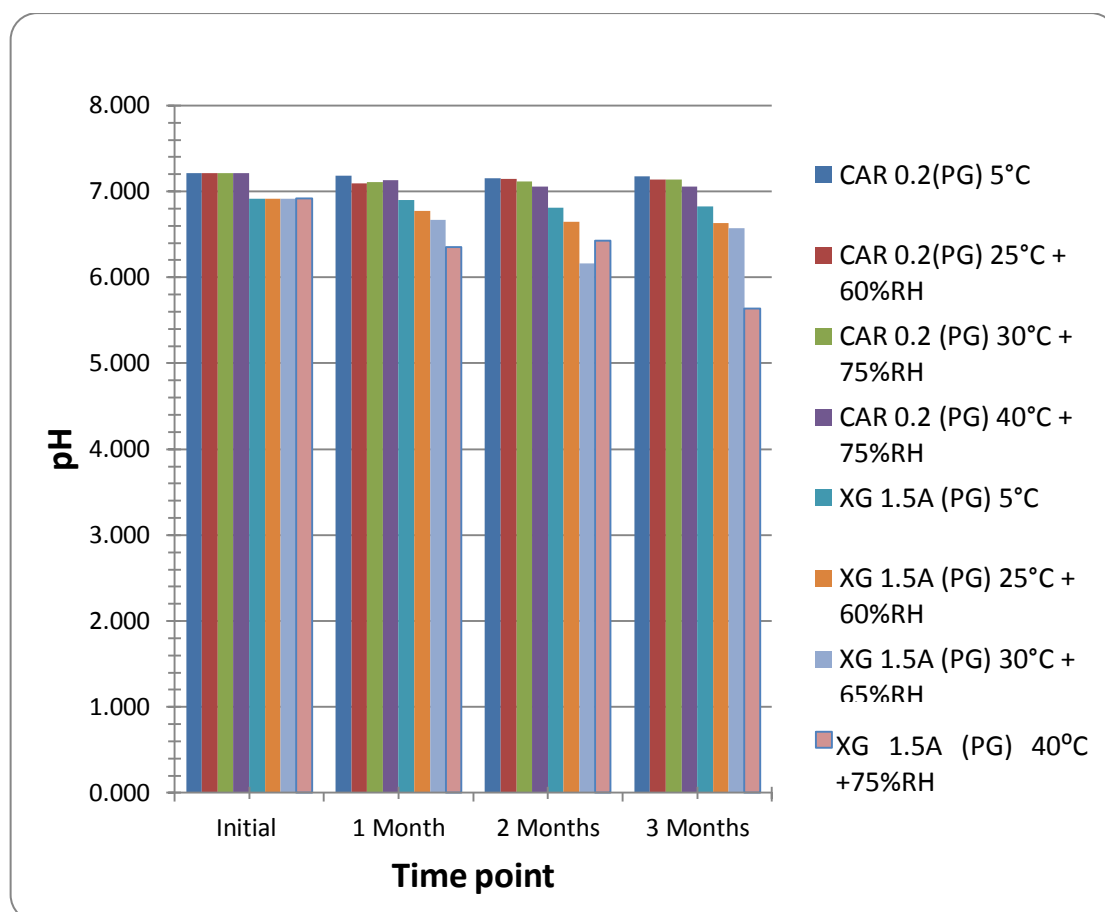


Figure 4.2: Graph indicating the influence of temperature and humidity on the pH of emulgel formulations stored in foil sachets over a period of three months.

4.2.3 VISCOSITY

Certain factors such as alternations in the viscosity-building agents or interaction between components in the system and particle growth may be responsible for changes in dispersion viscosity over time (Zatz *et al.*, 1996:290).

Processing under high shear can be the cause of depolymerisation. Decreases in the average molecular weight, therefore causes a decrease in viscosity. Further viscosity changes would not be expected after manufacture unless chemical degradation of the polymer has taken place. Degradation of cellulose derivatives by cellulase is an example of such a process. Chemical changes in the system over time, producing a drift in pH or generating ionic products, may alter viscosity by virtue of the effect of these environmental alterations. Time-dependent hydration of polymers or other viscosity-building agents can also result in a change in measured viscosity over time. In contrast with many of the other influences, continuing hydration results in an increase in viscosity after manufacture. Typically, viscosity reaches a plateau value after one or two weeks (Zatz *et al.*, 1996:291).

Changes in storage conditions may also cause emulsion instability. An increase in temperature will cause an increase in the rate of creaming, owing to a fall in apparent viscosity of the continuous phase. A temperature increase will influence kinetic motion of the dispersed droplets and of the emulsifying agent at the oil/water interface, the number of collisions between globules will increase, increased motion of the emulgent will result in a more expanded monolayer and coalescence is more likely. Certain macromolecular emulsifying agents may also be coagulated by an increase in temperature. At the other extreme, freezing of the aqueous phase will produce ice crystals that may exert unusual pressure on the dispersed globules and their adsorbed layer of emulgent. In addition, dissolved electrolyte may concentrate in the unfrozen water, thus affecting the charge density on the globules. Certain emulsifying agents may also precipitate at low temperatures (Billany, 2002:353).

4.2.3.1 Results & Discussion

The viscosity of the emulgel formulations, stored in either amber glass bottles or foil sachets for the duration of the accelerated stability test, are depicted in Figures 4.3 and 4.4, respectively. The effect of the three months storage of the Carbopol® 934P formulas and XG formulas at various temperatures are portrayed by a single value, plastic viscosity (η). The plastic viscosity of the Carbopol® 934P kept in amber glass bottles measured higher values than the formulas prepared with XG, which was also in amber bottles.

The Carbopol® 934P formula stored at 40°C + 75% RH had an initial viscosity of 1321 η . After one month of storage the plastic viscosity value measured was 1326 η . At two months of storage, the effect of temperature contributed to a drop in viscosity measured as 711.5 η . The third month of storage measured a viscosity of 678.4 η . One possible explanation for the lowering in viscosity due to storage at 40°C for extended periods is the cross-linked bounds inside the gel, which can weaken because of temperature increases and the polymers chain can become partially degraded. When the strong bounds become weaker, liposomes incorporated between these cross-linked networks can sediment onto the bottom of the gel. With regard to stability of liposomal hydrogels, the research literature shows that stability depends on the type of gelling polymer and the composition of gel used. Some hydrophilic polymers, such as carboxymethylcellulose, are unaffected by the incorporation of liposomes in hydrogels. However, in some hydrogels, such as xanthan hydrogels, separation of API's from liposomes can be readily observed after 10 days (Kermany, 2010:59)

The XG formulations kept under the stress conditions of temperature and humidity over three month storage had no influence on the viscosity of the formulas as the values stayed stable over the time period.

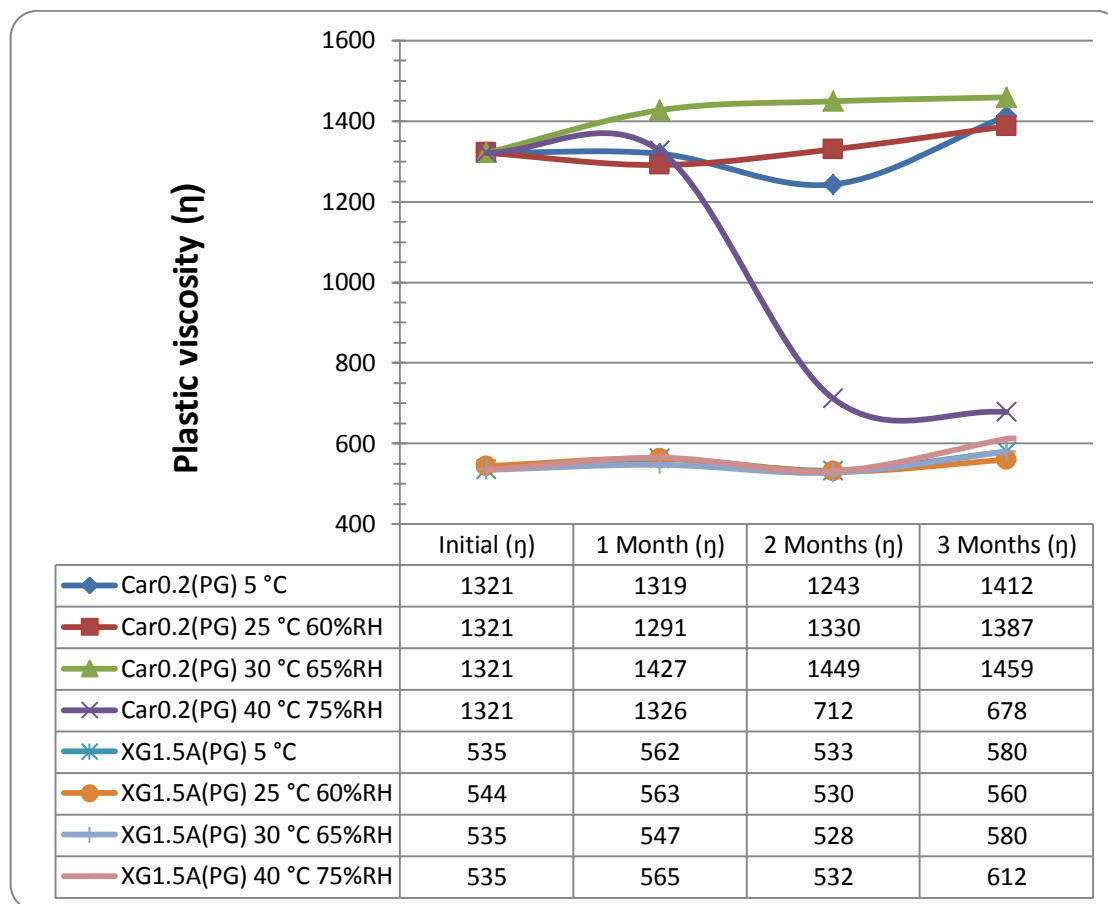


Figure 4.3: Graph indicating the influence of temperature and humidity on the viscosity of emulgel formulations stored in amber glass bottles over a period of three months.

The formulas stored in foil sachets had variations in viscosity measured for the formulas prepared with Carbopol® 934P and stored at 5°C and 30°C + 60% RH after one month of storage. All the Carbopol® 934P formulas retained their viscosity after three months, with the exception of the formula kept at 40°C + 75% initially measuring a viscosity of 1321 η , then at 3 months a viscosity of 687.7 η (see Figure 4.4).

The XG formulations stored in foil sachets had an initial plastic viscosity value of 535 η . After one month of storage, at various temperatures and humidity levels, the XG preparation stored at 40°C + 75% RH measured a lowered value of 349.4 η . After two months the XG formulations stored at 5°C and 30°C + 65% RH measured

viscosity values of 403.4 η and 321.2 η . After three months, the four samples kept at various temperatures and humidity levels all retained their viscosity values.

For the purpose of the accelerated stability study performed the XG samples stayed relative stable for future product development.

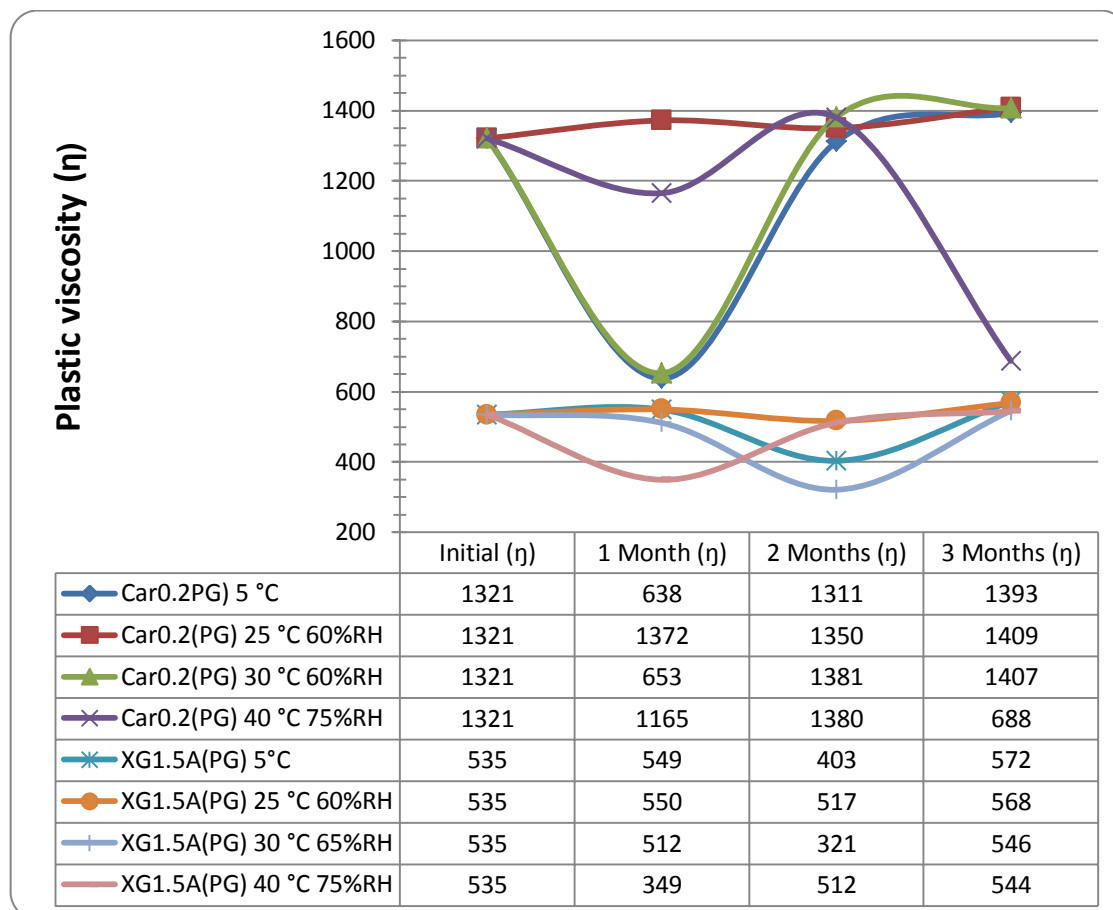


Figure 4.4: Graph indicating the influence of temperature and humidity on the viscosity of emulgel formulations stored in foil sachets over a period of three months.

4.2.4 ORGANOLEPTIC ASSESSMENT

The emulgel formulations stored in amber bottles were evaluated for their appearance, colour and odour. These are criteria that need to be met to determine consumer approval.

4.2.4.1 Results & Discussion

The visual assessment of the emulgels was performed once a month for three months as described in Tables 4.4 and 4.5. The emulgel formulations stored in the foil sachets did not show any discolouring and did not present with an excessive odour in comparison to the samples stored in amber bottles. This proves the type of

storage material is of utmost importance in protecting the emulgel formulations against chemical and physical instabilities.

Table 4.4: Comparison of the organoleptic assessment of the emulgel formulations with Carbopol® 934P as thickening agent stored in amber glass bottles for three months.

<i>Formulation</i>	<i>Initial</i>	<i>1 Month</i>	<i>2 Months</i>	<i>3 Months</i>
Car0.2%(PG) 5°C	*White colour *Homogenous *Slight oily smell	*No discolouring *Slight oily smell	*No discolouring *Slight oily smell	*No discolouring *Slight oily smell
Car0.2%(PG) 25°C + 60%RH	*White colour *Homogenous *Slight oily smell	*Light discolouring to pink on top layer *Slight oily smell	*Light discolouring to pink on top layer with yellow oil patches *Slight oily smell	*Discolouring to pink on the top layer with yellow oil patches *Strong oily smell
Car0.2%(PG) 30°C + 65%RH	*White colour *Homogenous *Slight oily smell	*Light discolouring to pink on the top layer *Strong oily smell	*Light discolouring to pink on top layer with yellow oil patches *Slight oily smell	*Discolouring to pink on the top layer with yellow oil patches *Strong oily smell
Car0.2%(PG) 40°C + 75%RH	*White colour *Homogenous *Slight oily smell	*Discolouring to pink on the top layer with yellow oil patches *Strong oily smell	*Discolouring to pink on the top layer with yellow oil patches *Strong oily smell	*Discolouring to pink on the top layer with yellow oil patches *Strong oily smell

Table 4.5: Comparison of the organoleptic assessment of the emulgel formulations with XG as thickening agent stored in amber glass bottles for three months.

<i>Formulation</i>	<i>Initial</i>	<i>1 Month</i>	<i>2 Months</i>	<i>3 Months</i>
XG1.50A(PG) 5°C	*White colour *Homogenous *Slight oily smell	*No discolouring *Slight oily smell	*No discolouring *Slight oily smell	*No discolouring *Slight oily smell
XG1.50A(PG) 25°C + 60%RH	*White colour *Homogenous *Slight oily smell	*Light discolouring to pink on top layer *Slight oily smell	*Light discolouring to pink on top layer with yellow oil patches *Slight oily smell	*Light discolouring to pink on top layer with yellow oil patches *Slight oily smell
XG1.50A(PG) 30°C +65%RH	*White colour *Homogenous *Slight oily smell	*Light discolouring to pink on the top layer *Strong oily smell	*Discolouring to pink on the top layer with yellow oil patches *Strong oily smell	*Light discolouring to pink on top layer with yellow oil patches *Strong oily smell
XG1.50A(PG) 40°C +75%RH	*White colour *Homogenous *Slight oily smell	*Discolouring to pink on the top layer with yellow oil patches *Strong oily smell	*Discolouring to pink on the top layer with yellow oil patches *Strong oily smell	*Discolouring to pink on the top layer with yellow oil patches *Strong oily smell

A significant stability difference was detectable between the contents of the foil sachets and the amber bottles. For both formulations of Carbopol® 934P and XG stored in the foil sachets no visible signs of degradation of the oil phase could be

detected over the three month storage period. The preparations stayed uniform and white in colour over the whole range of storage conditions it was exposed to. The oil had the advantage of sealing and preventing possible oxygen exposure of the contents of the pouch which could contribute to degradation (see Figures 4.5 to 4.7 and Figures 4.11 to 4.13).



Figure 4.5: Carbopol® 934P 0.2% w/v after one month of storage in foil sachets. Temperature ranges are 5°C in top left corner; 25°C + 60% RH in top right corner; 30°C + 65% RH in bottom left corner; 40°C + 75% RH in bottom right corner.



Figure 4.6: Carbopol® 934P 0.2% w/v after two months of storage in foil sachets. Temperature ranges are 5°C in top left corner; 25°C + 60% RH in top right corner; 30°C + 65% RH in bottom left corner; 40°C + 75% RH in bottom right corner.



Figure 4.7: Carbopol® 934P 0.2% w/v after three months of storage in foil sachets. Temperature ranges are 5°C in top left corner; 25°C + 60% RH in top right corner; 30°C + 65% RH in bottom left corner; 40°C + 75% RH in bottom right corner.

No physical changes were detectable in the batches prepared with Carbopol® 934P or XG, when stored at 5°C in amber bottles. The other batches kept at room temperature (25°C) and higher (30°C and 40°C) showed signs of phase separation with slight discolouring to pink and yellow oil patches forming on the top of the formulation. A strong fish oil smell was distinguishable in most of the batches stored at 30°C and 40°C, which is due to oxidation and physical degradation of the individual components (see Figures 4.8 to 4.10 and 4.14 to 4.16).



Figure 4.8: Carbopol® 934P 0.2% w/v after one month of storage in amber bottles. Temperatures ranged from left to right from 5°C through to 40°C.



Figure 4.9: Carbopol® 934P 0.2% w/v after two months of storage in amber bottles. Temperatures ranged from left to right from 5°C through to 40°C.



Figure 4.10: Carbopol® 934P 0.2% w/v after three months of storage in amber bottles. Temperatures ranged from left to right from 5°C through to 40°C.



Figure 4.11: XG 1.50% w/v after one month of storage in foil sachets. Temperature ranges are 5°C in top left corner; 25°C + 60% RH in top right corner; 30°C + 65% RH in bottom left corner; 40°C + 75% RH in bottom right corner.



Figure 4.12: XG 1.50% w/v after two months of storage in foil sachets. Temperature ranges are 5°C in top left corner; 25°C + 60% RH in top right corner; 30°C + 65% RH in bottom left corner; 40°C + 75% RH in bottom right corner.



Figure 4.13: XG 1.50% w/v after three months of storage in foil sachets. Temperature ranges are 5°C in top left corner; 25°C + 60% RH in top right corner; 30°C + 65% RH in bottom left corner; 40°C + 75% RH in bottom right corner.



Figure 4.14: XG 1.50% w/v after one month of storage in amber bottles. Temperatures ranged from left to right from 5°C through to 40°C.



Figure 4.15: XG 1.50% w/v after two months of storage in amber bottles. Temperatures ranged from left to right from 5°C through to 40°C.



Figure 4.16: XG 1.50% w/v after three months of storage in amber bottles. Temperatures ranged from left to right from 5°C through to 40°C.

4.2.5 LIGHT MICROSCOPY

The formulas prepared and stored in amber glass bottles were examined with a light microscope to see if the visual changes observed in Tables 4.4 and 4.5 can be related to physical changes in the formulations.

4.2.5.1 Results & Discussion

Figures 4.17 to 4.20 give the results for images taken of the formulas prepared with Carbopol® 934P at the initial time of 48 hours and repeated monthly for three months. The four storage temperatures and humidity levels used were 5°C, 25°C at 60% RH, 30°C at 65% RH and 40°C at 75% RH. The light microscopy photos taken for the Carbopol® 934P range showed initial particles of uniform size at 24 hours. After one month of storage at the various temperatures and conditions the particle droplets appeared to coalesce from the 25°C sample onwards. After two months of storage, oil droplets could be seen at the 30°C sample and what looks like the denaturation of the polymer at 40°C. After three months of storage, at elevated temperatures, the Carbopol® 934P showed denaturation of the polymer from 25°C onwards.

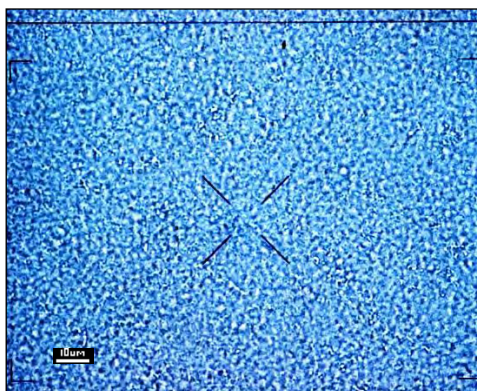


Figure 4.17: Light microscopy photo taken of CAR 0.2% w/v at time 48 hours.

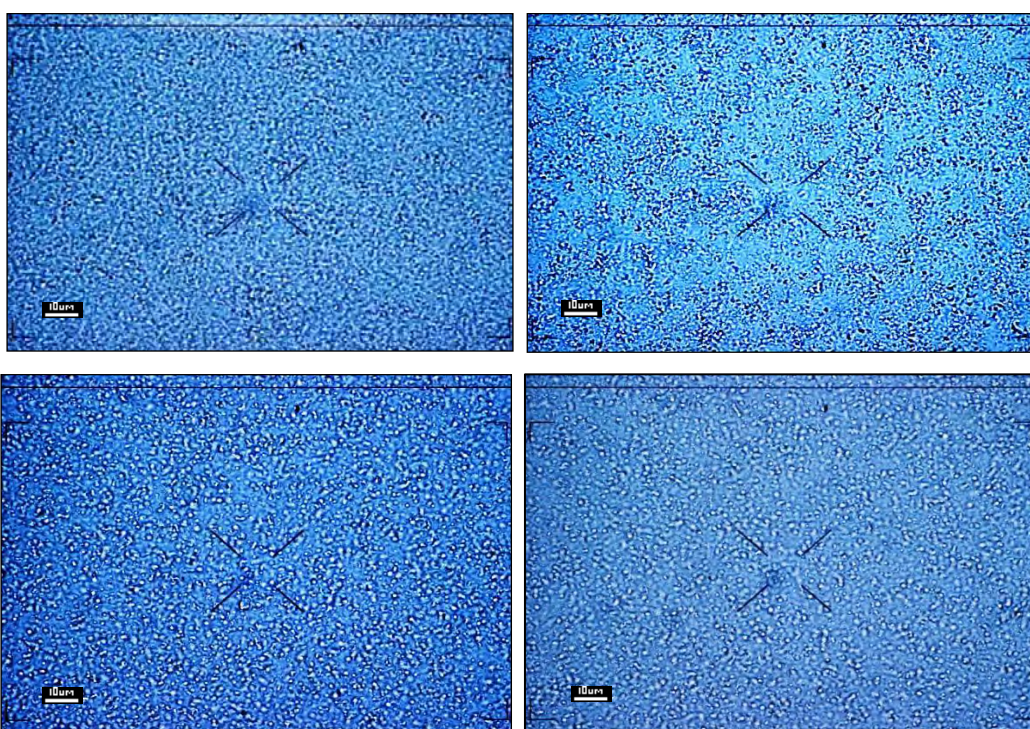


Figure 4.18: Light microscopy photos taken of CAR 0.2% w/v at time one month. Top left was stored at 5°C; top right stored at 25°C + 60% RH; bottom left was stored at 30°C + 65% RH and bottom right stored at 40°C at 75% RH.

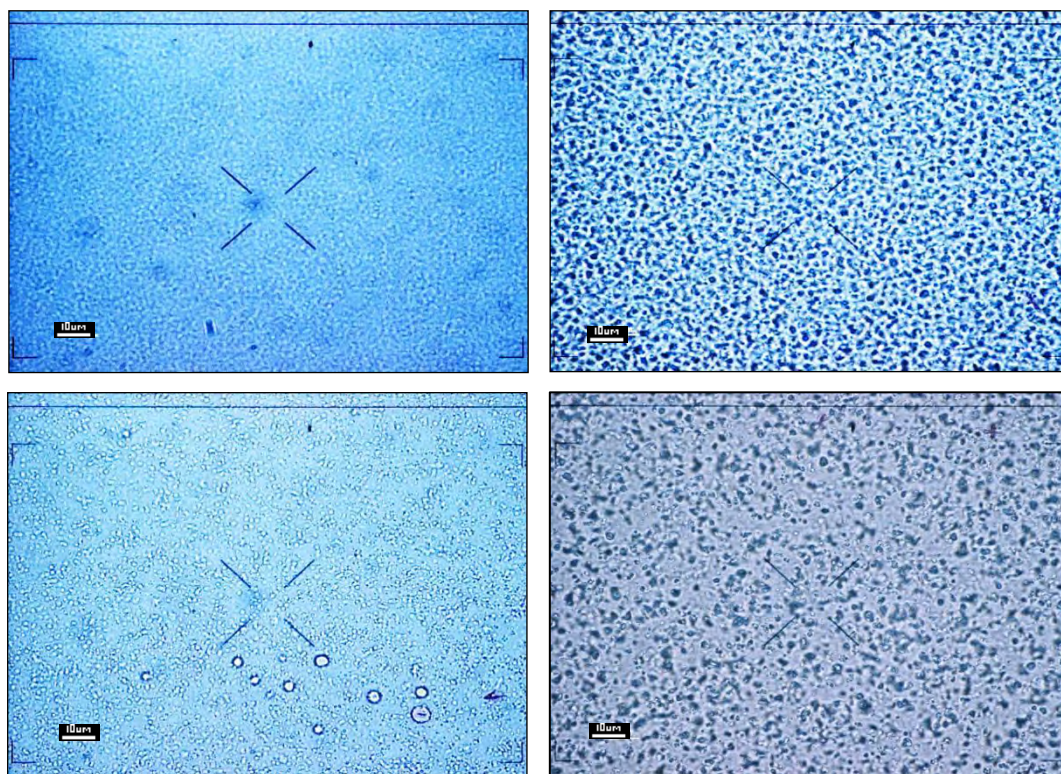


Figure 4.19: Light microscopy photos taken of CAR 0.2% w/v at time two months. Top left was stored at 5°C; top right stored at 25°C + 60% RH; bottom left was stored at 30°C + 65% RH and bottom right stored at 40°C at 75% RH.

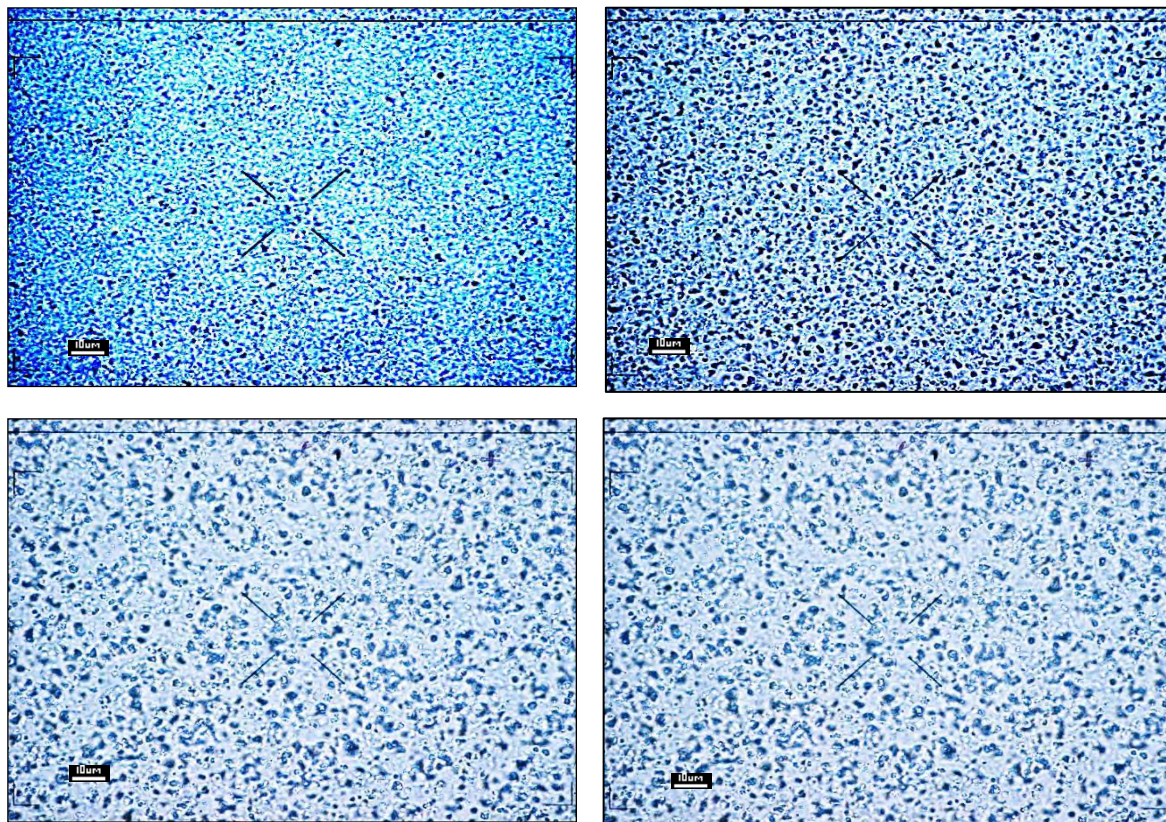


Figure 4.20: Light microscopy photos taken of CAR 0.2% w/v at time three months. Top left was stored at 5°C; top right stored at 25°C + 60% RH; bottom left was stored at 30°C + 65% RH and bottom right stored at 40°C at 75% RH.

In Figures 4.21 to 4.24, the images taken of the formulas prepared with XG at the initial time of 24 hours and repeated monthly for three months can be seen. The four storage temperatures used were 5°C, 25°C at 60% RH, 30°C at 65% RH and 40°C at 75% RH. At the initial 24 hour interval the XG formula was uniform with small particle size distribution. After one month of storage the influence of temperature and humidity could be seen on the 40°C sample with the formation of oil droplets. The second month the formation of oil droplets can be seen from 30°C sample onwards. The third month exhibited the extensive effect of storage at these high temperatures for extended time periods.

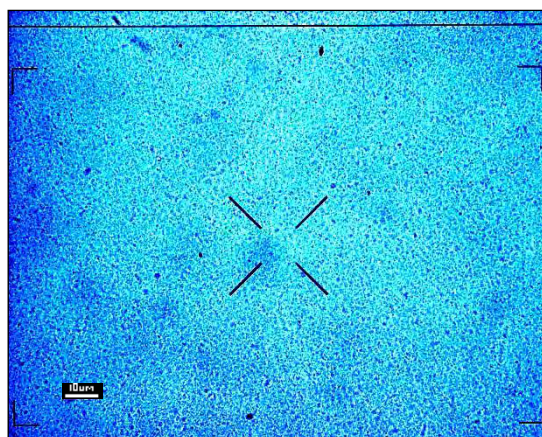


Figure 4.21: Light microscopy photo taken of XG 1.50% w/v at time 48 hours.

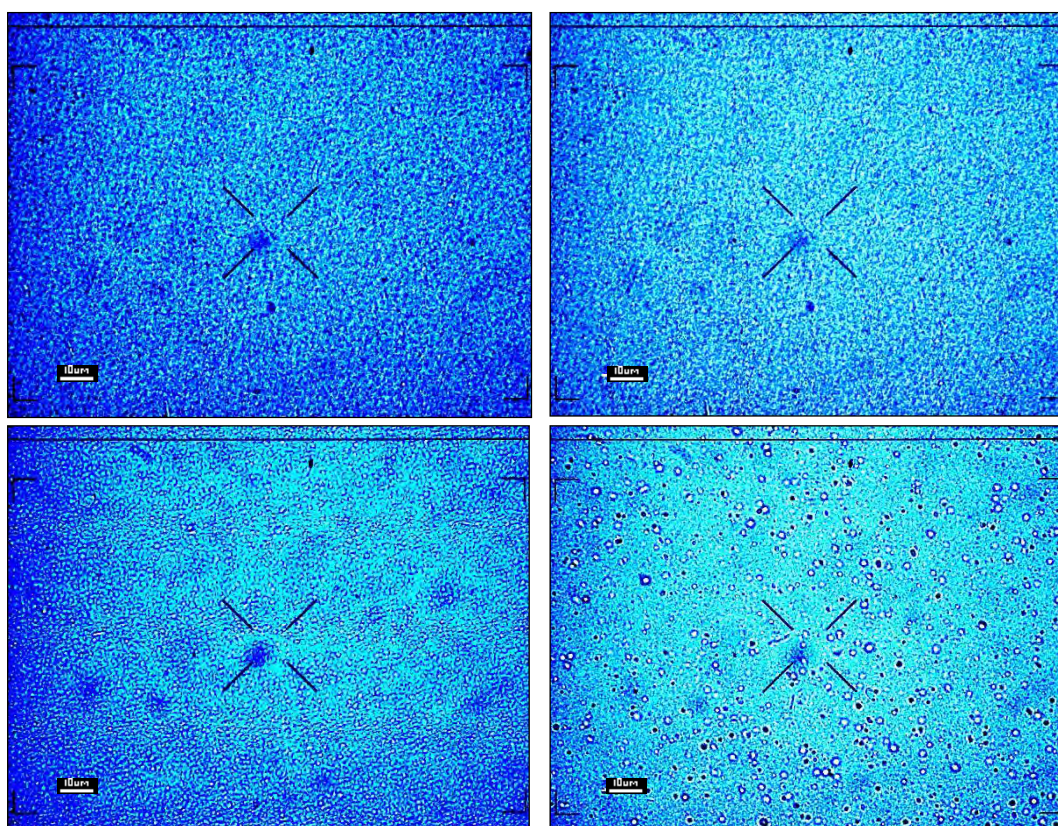


Figure 4.22: Light microscopy photos taken of XG1.50% w/v at time one month. Top left was stored at 5°C; top right stored at 25°C + 60% RH; bottom left was stored at 30°C + 65% RH and bottom right stored at 40°C at 75% RH.

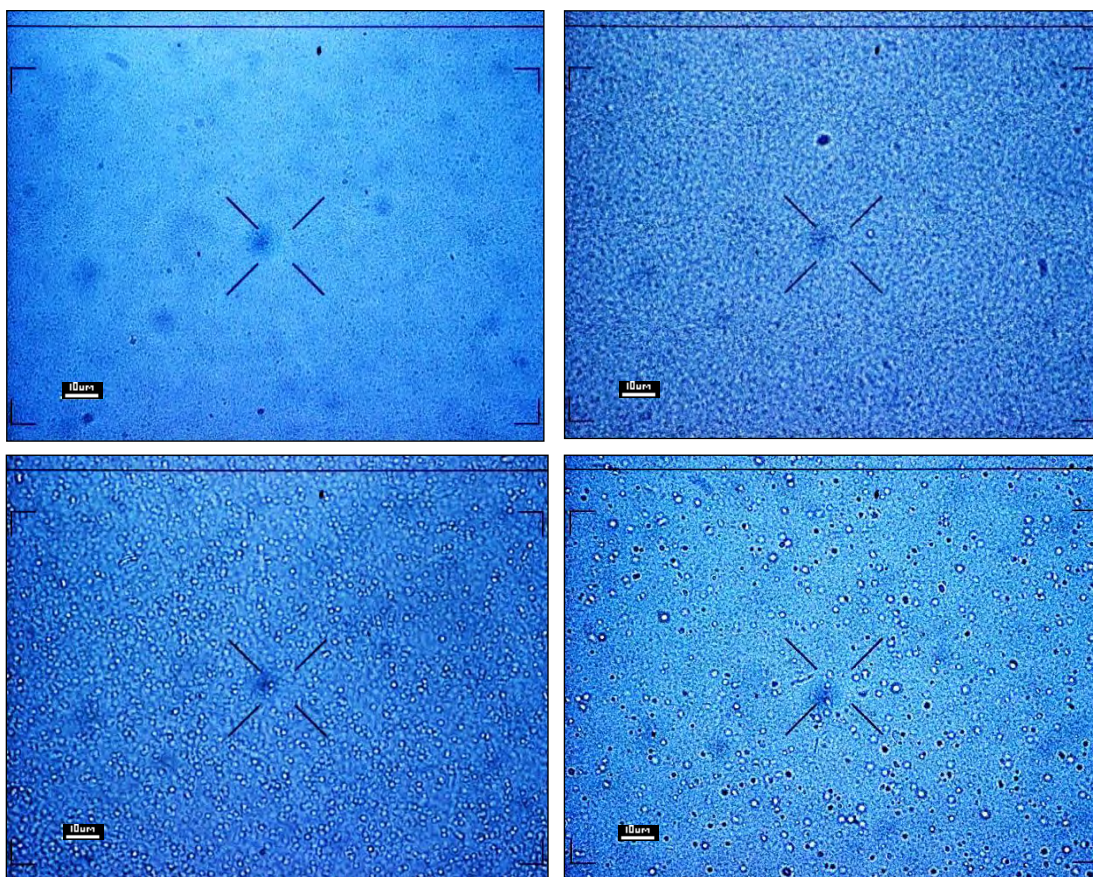


Figure 4.23: Light microscopy photos taken of XG1.50% w/v at time two months. Top left was stored at 5°C; top right stored at 25°C + 60% RH; bottom left was stored at 30°C + 65% RH and bottom right stored at 40°C at 75% RH.

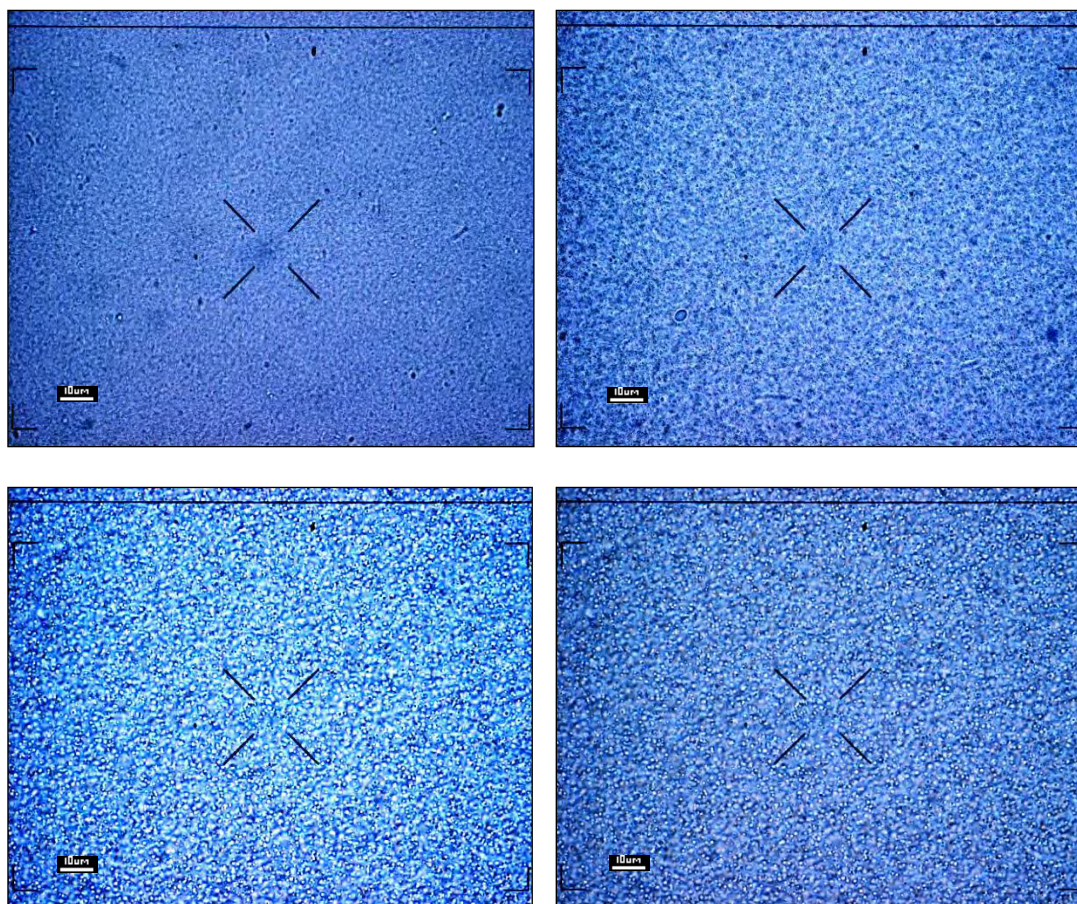


Figure 4.24: Light microscopy photos taken of XG 1.50% w/v at time three months. Top left was stored at 5°C; top right stored at 25°C + 60% RH; bottom left was stored at 30°C + 65% RH and bottom right stored at 40°C at 75% RH.

4.2.6 CONFOCAL LASER SCANNING MICROSCOPY

CLSM photos were taken of the Carbopol® 934P and XG formulas stored in amber glass bottles over the three month storage period. The influence of temperature and humidity over time was examined for each formula.

4.2.6.1 Results & Discussion

The original CLSM photo taken for Carbopol® 934P, at 48 hours, shows uniform droplets (see Figure 4.25). After one month of storage at various temperatures (Figure 4.26), the formula kept at 5°C showed a similar droplet diameter. At a temperature of 25°C, structural changes are noticeable in the oil phase of the Pheroid® with an increase in droplet diameter at 30°C and 40°C. At storage time of two months, the oil content in the formula increased and at 25°C, 30°C and 40°C patches of oil are visible (Figure 4.27). At three months, definite structural changes in the Carbopol® 934P structure is visible due to the prolonged storage of the Pheroid® at elevated temperatures (Figure 4.38).

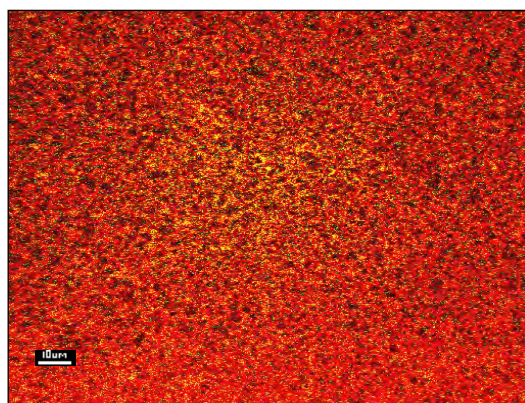


Figure 4.25: CLSM photo taken of CAR 0.2% w/v at time 48 hours.

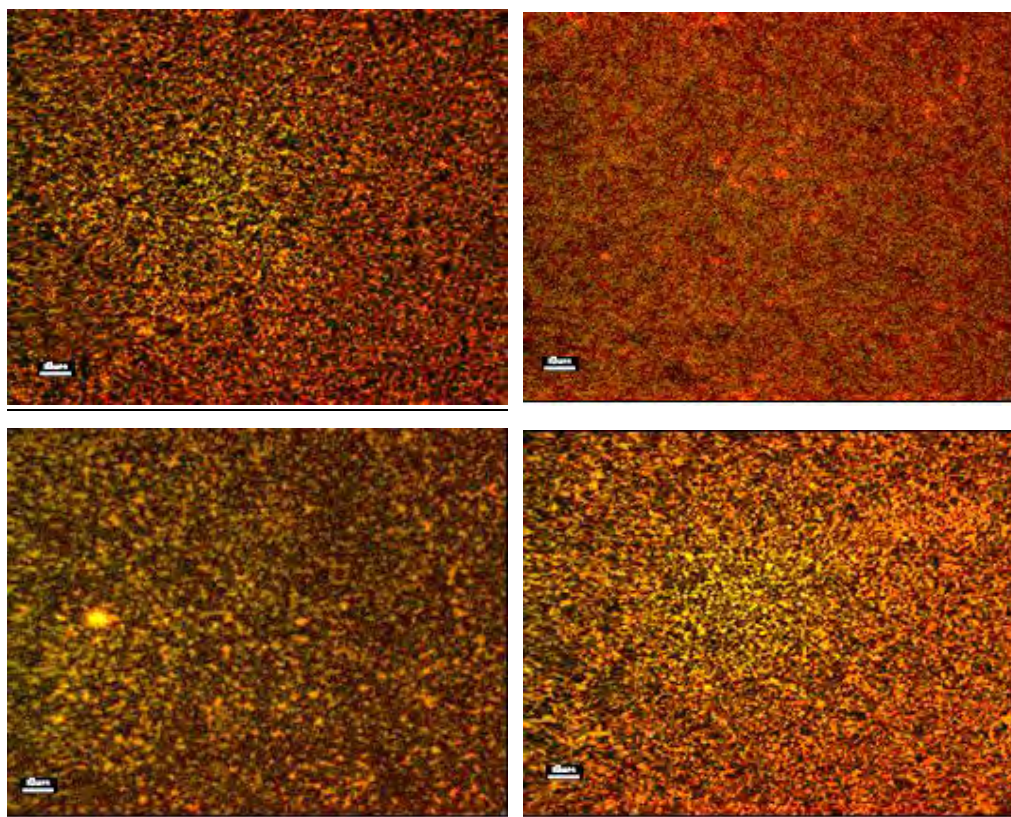


Figure 4.26: CLSM photos taken of CAR 0.2% w/v at time one month. Top left was stored at 5°C; top right stored at 25°C + 60% RH; bottom left was stored at 30°C + 65% RH and bottom right stored at 40°C at 75% RH.

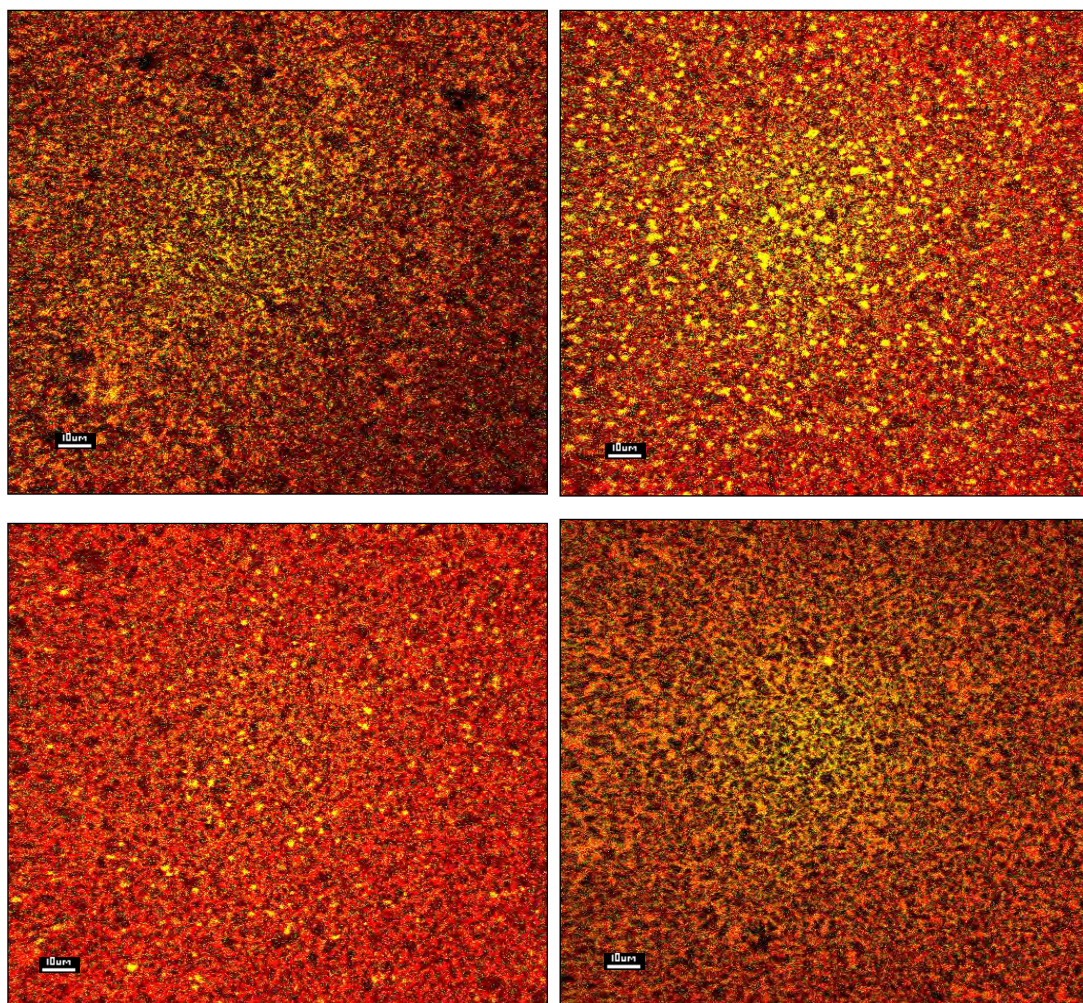


Figure 4.27: CLSM photos taken of CAR 0.2% w/v at time two months. Top left was stored at 5°C; top right stored at 25°C + 60% RH; bottom left was stored at 30°C + 65% RH and bottom right stored at 40°C at 75% RH.

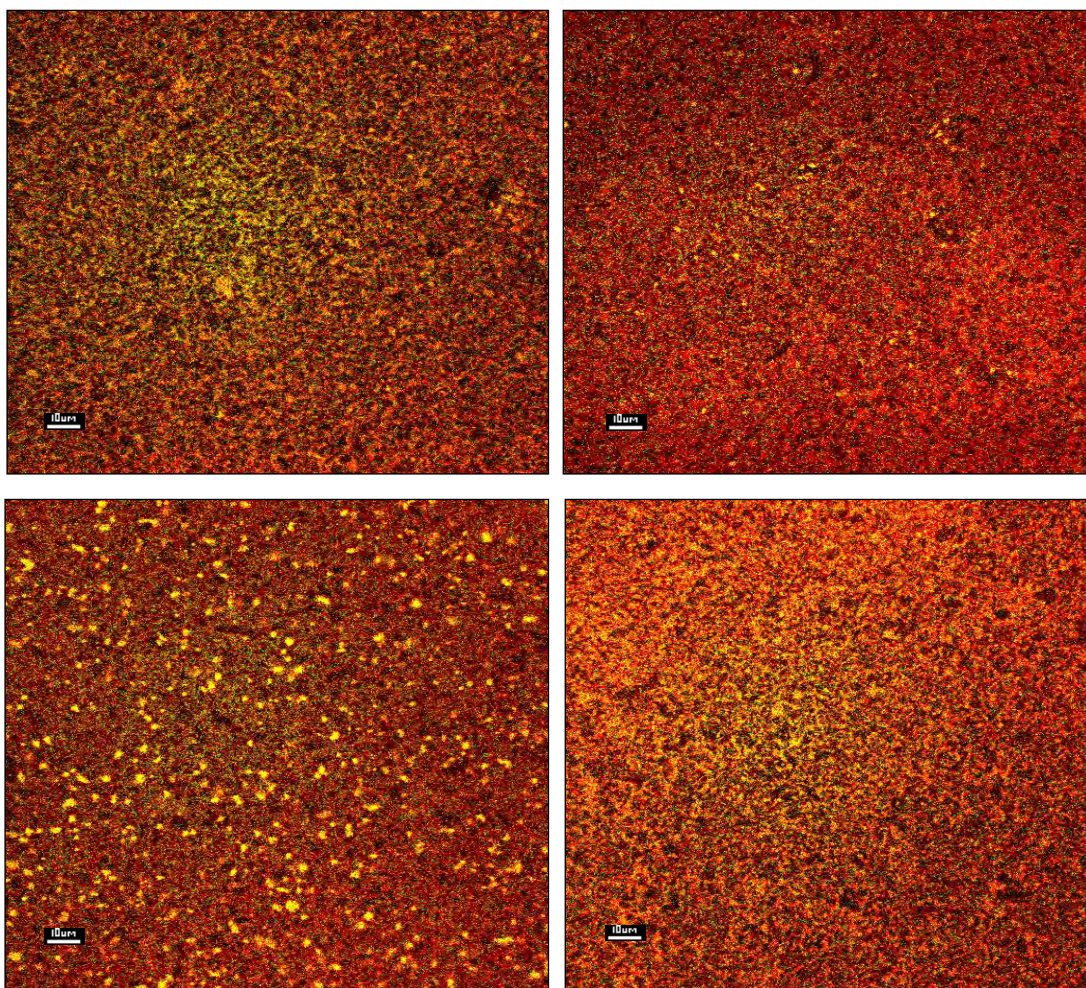


Figure 4.28: CLSM photos taken of CAR 0.2% w/v at time three months. Top left was stored at 5°C; top right stored at 25°C + 60% RH; bottom left was stored at 30°C + 65% RH and bottom right stored at 40°C at 75% RH.

Figures 4.29 to 4.32 show the CLSM photos taken of the XG formula after 48 hours and repeated monthly for three months. Compared to the photos taken for the Carbopol® 934P samples, the droplet diameter is smaller and uniform. After one, two and three months of storage at various temperatures the XG photos stayed similar with the exception of the 40°C sample, which has bigger oil droplets evident. This is confirmed with the visual assessment done on the sample.

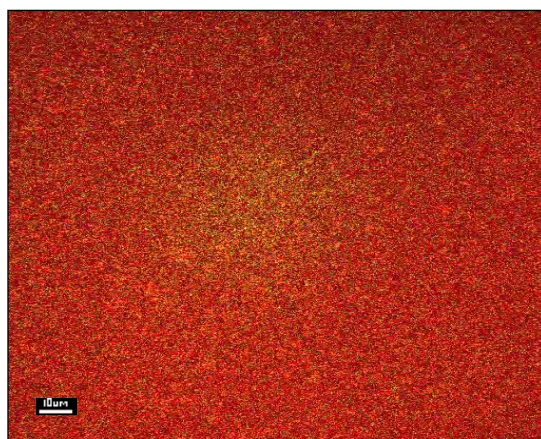


Figure 4.29: CLSM photo taken of XG 1.5% w/v at time 48 hours.

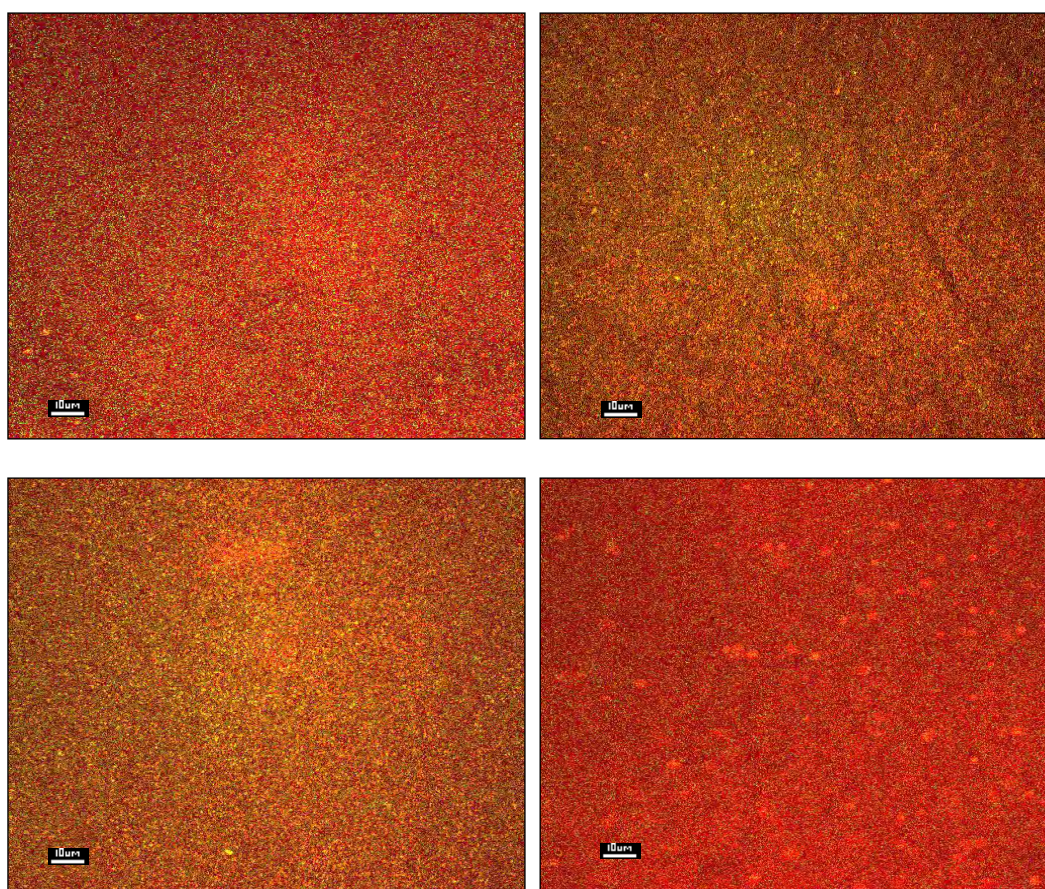


Figure 4.30: CLSM photos taken of XG 1.5% w/v at time one month. Top left was stored at 5°C; top right stored at 25°C + 60% RH; bottom left was stored at 30°C + 65% RH and bottom right stored at 40°C at 75% RH.

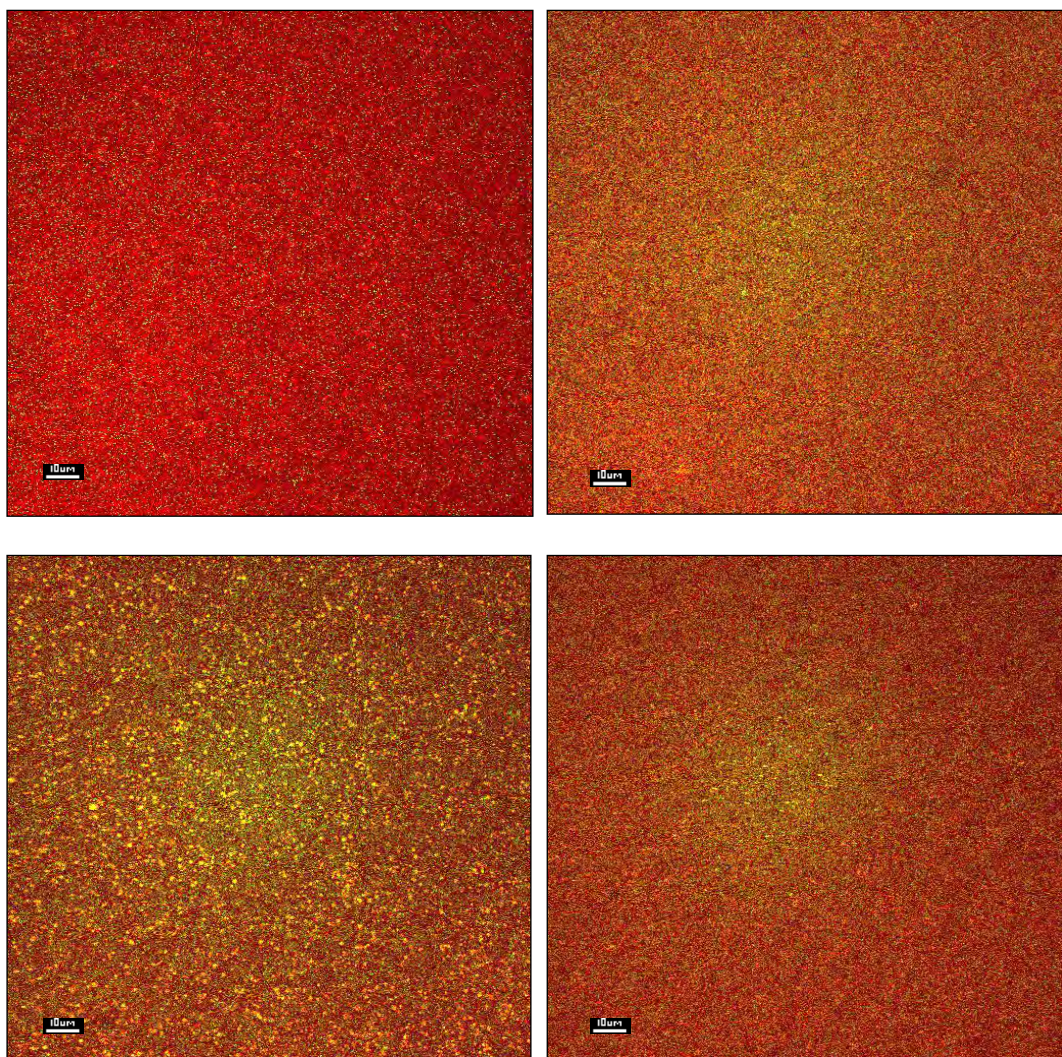


Figure 4.31: CLSM photos taken of XG 1.5% w/v at time two months. Top left was stored at 5°C; top right stored at 25°C + 60% RH; bottom left was stored at 30°C + 65% RH and bottom right stored at 40°C at 75% RH.

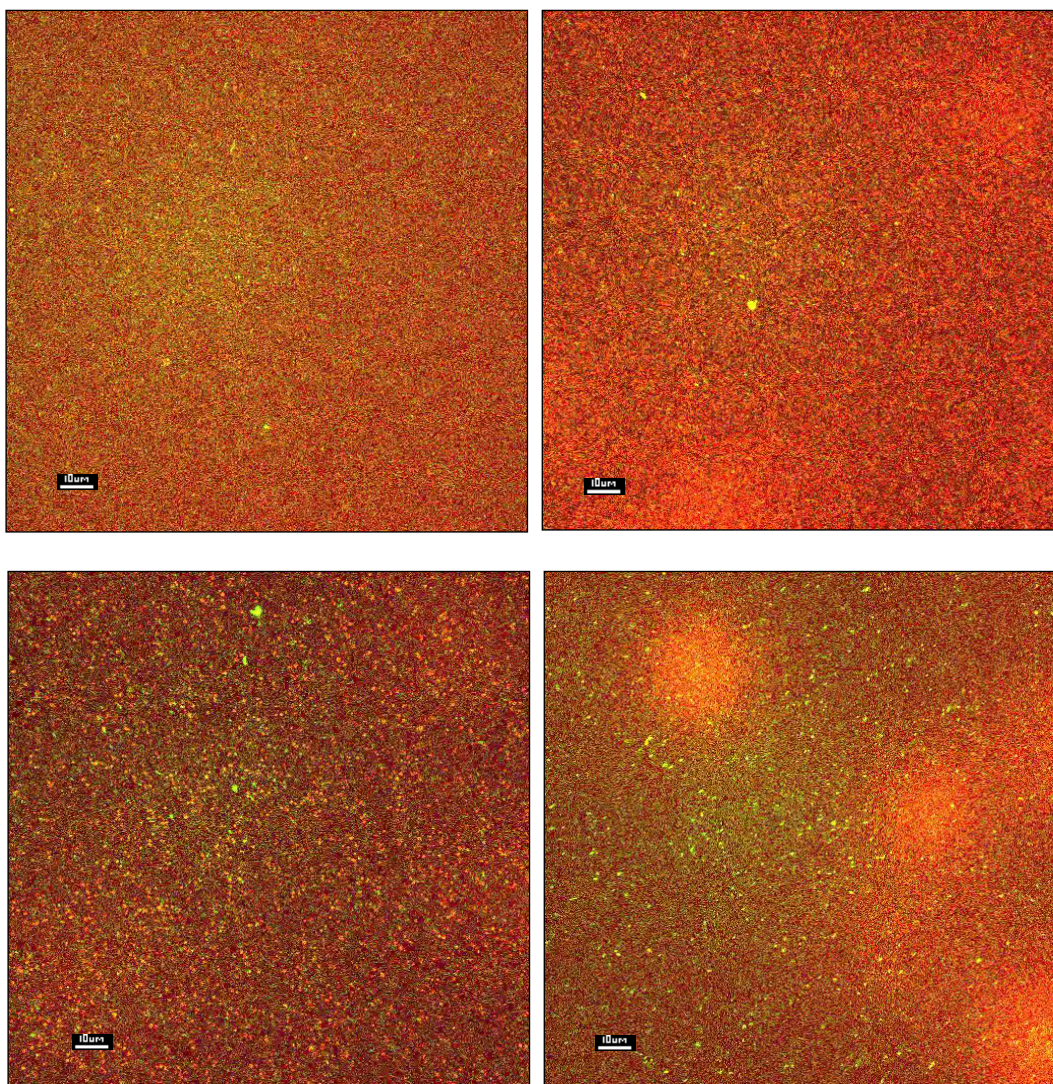


Figure 4.32: CLSM photos taken of XG 1.5% w/v at time three months. Top left was stored at 5°C; top right stored at 25°C + 60% RH; bottom left was stored at 30°C + 65% RH and bottom right stored at 40°C at 75% RH.

4.2.7 MICROBIAL PRESERVATIVE EFFICACY TEST

4.2.7.1 Results & Discussion

Tables 4.6 to 4.11 present the PET results obtained for the initial, one month, two months and three months stability points. The first formula under investigation is Carbopol® 934P 0.2% w/v with Nipastat® 0.175% w/v and PG 10% v/v. After the initial test, the Carbopol® 934P formula passed the PET for all five organisms under investigation. The criteria determined a 3 log reduction in growth of the bacteria and a 1 log reduction of the yeast and mould with no further increase after day 14. The one-month stability data obtained at the 5°C, 25°C, 30°C and 40°C stability intervals passed the PET for all five organisms. After two months of storage at the four temperature and humidity ranges, all the samples passed the criteria for the PET. After the third month of storage the PET criteria was passed for all the formulas and all the organisms.

A second formula containing XG 1.50% w/v with Nipastat® 0.175% w/v and PG 10% v/v w was also tested. The initial PET done for this formula proved fatal for all five organisms as all the criteria were met. After one month of storage the formulas stored at 5°C, 25°C, 30°C and 40°C all failed to preserve the product against *Candida albicans*. After the second month of the PET for XG formulations the 5°C, 25°C and 30°C samples preserved the formula, but at 40°C + 75%RH the *Candida albicans* strain increased in growth on the 14th day of investigation and thus failed the PET. In the third month of the PET of the XG formulas the growth of the organisms were inhibited from 5°C to 40°C. *Candida albicans* were inhibited. The overall impression is the XG formula failed the PET for oral products due to the growth of *Candida albicans* in some of the formulas.

Table 4.6: Preservative efficacy test results of Carbopol® 934P 0.2% w/v with Nipastat® 0.175% w/v and PG 10% w/v (Initial and month one). *ND = None detected

<i>Test organism</i>	<i>Total viable cell count (Cfu/ml)</i>				
Initial	Test dates			Log reduction	
	0 hours	14 days	28 days	0h - 14 days	0h - 28 days
<i>S. aureus</i>	1.6 x 10 ⁶	ND	ND	>3	>3
<i>P. aeruginosa</i>	2.4 x 10 ⁶	ND	ND	>3	>3
<i>E. coli</i>	2.3 x 10 ⁶	ND	ND	>3	>3
<i>A. brasiliensis</i>	3.5 x 10 ³	ND	ND	>1	>1
<i>C. albicans</i>	3.9 x 10 ³	ND	ND	>1	>1
One Month	Test dates			Log reduction	
	0 hours	14 days	28 days	0h - 14 days	0h - 28 days
5°C					
<i>S. aureus</i>	5.5 x 10 ⁵	ND	ND	>3	>3
<i>P. aeruginosa</i>	1.7 x 10 ⁶	ND	ND	>3	>3
<i>E. coli</i>	1.1 x 10 ⁶	ND	ND	>3	>3
<i>A. brasiliensis</i>	1.8 x 10 ³	ND	ND	>1	>1
<i>C. albicans</i>	4.0 x 10 ³	ND	ND	>1	>1
25°C + 60% RH					
<i>S. aureus</i>	6.2 x 10 ⁵	ND	ND	>3	>3
<i>P. aeruginosa</i>	1.7 x 10 ⁶	ND	ND	>3	>3
<i>E. coli</i>	1.8 x 10 ⁶	ND	ND	>3	>3
<i>A. brasiliensis</i>	1.5 x 10 ³	ND	ND	>1	>1
<i>C. albicans</i>	2.9 x 10 ³	ND	ND	>1	>1
30°C + 65% RH					
<i>S. aureus</i>	3.1 x 10 ⁵	ND	ND	>3	>3
<i>P. aeruginosa</i>	3.1 x 10 ⁵	ND	ND	>3	>3
<i>E. coli</i>	6.1 x 10 ⁶	ND	ND	>3	>3
<i>A. brasiliensis</i>	2.0 x 10 ³	ND	ND	>1	>1
<i>C. albicans</i>	1.9 x 10 ⁴	ND	ND	>1	>1
40°C + 75% RH					
<i>S. aureus</i>	6.8 x 10 ⁵	ND	ND	>3	>3
<i>P. aeruginosa</i>	4.4 x 10 ⁵	ND	ND	>3	>3
<i>E. coli</i>	2.4 x 10 ⁵	ND	ND	>3	>3
<i>A. brasiliensis</i>	9.3 x 10 ²	ND	ND	>1	>1
<i>C. albicans</i>	1.3 x 10 ²	ND	ND	>1	>1

Table 4.7: Preservative efficacy test results of Carbopol® 934P 0.2% w/v with Nipastat® 0.175% w/v and PG 10% w/v (month two). *ND = None detected

Test organism	Total viable cell count (Cfu/ml)				
Two months	Test dates			Log reduction	
	0 hours	14 days	28 days	0h - 14 days	0h - 28 days
5°C					
<i>S. aureus</i>	2.4 x 10 ⁵	ND	ND	>3	>3
<i>P. aeruginosa</i>	7.1 x 10 ⁵	ND	ND	>3	>3
<i>E. coli</i>	2.2 x 10 ⁵	ND	ND	>3	>3
<i>A. brasiliensis</i>	5.0 x 10 ³	ND	ND	>1	>1
<i>C. albicans</i>	2.0 x 10 ²	ND	ND	>1	>1
25°C + 60% RH					
<i>S. aureus</i>	6.1 x 10 ⁵	ND	ND	>3	>3
<i>P. aeruginosa</i>	5.2 x 10 ⁴	ND	ND	>3	>3
<i>E. coli</i>	4.3 x 10 ³	ND	ND	>3	>3
<i>A. brasiliensis</i>	3.5 x 10 ²	ND	ND	>1	>1
<i>C. albicans</i>	1.8 x 10 ⁶	ND	ND	>1	>1
30°C + 65% RH					
<i>S. aureus</i>	1.4 x 10 ⁶	ND	ND	>3	>3
<i>P. aeruginosa</i>	4.2 x 10 ⁵	ND	ND	>3	>3
<i>E. coli</i>	4.4 x 10 ⁵	ND	ND	>3	>3
<i>A. brasiliensis</i>	1.5 x 10 ⁴	ND	ND	>1	>1
<i>C. albicans</i>	5.0 x 10 ²	ND	ND	>1	>1
40°C + 75% RH					
<i>S. aureus</i>	8.2 x 10 ⁵	ND	ND	>3	>3
<i>P. aeruginosa</i>	1.2 x 10 ⁶	ND	ND	>3	>3
<i>E. coli</i>	2.4 x 10 ⁶	ND	ND	>3	>3
<i>A. brasiliensis</i>	9.0 x 10 ³	ND	ND	>1	>1
<i>C. albicans</i>	5.3 x 10 ²	ND	ND	>1	>1

Table 4.8: Preservative efficacy test results of Carbopol® 934P 0.2% w/v with Nipastat® 0.175% w/v and PG 10% w/v (month three). *ND = None detected

Test organism	Total viable cell count (Cfu/ml)				
Three months	Test dates			Log reduction	
	0 hours	14 days	28 days	0h - 14 days	0h - 28 days
5°C					
<i>S. aureus</i>	7.5 x 10 ⁵	ND	ND	>3	>3
<i>P. aeruginosa</i>	11.4 x 10 ⁶	ND	ND	>3	>3
<i>E. coli</i>	20 x 10 ⁵	ND	ND	>3	>3
<i>A. brasiliensis</i>	2.3 x 10 ³	ND	ND	>1	>1
<i>C. albicans</i>	5.5 x 10 ³	ND	ND	>1	>1
25°C + 60% RH					
<i>S. aureus</i>	6.2 x 10 ⁵	ND	ND	>3	>3
<i>P. aeruginosa</i>	2.1 x 10 ⁵	2.6 x 10 ¹	ND	>3	>3
<i>E. coli</i>	2.9 x 10 ⁶	ND	ND	>3	>3
<i>A. brasiliensis</i>	7.6 x 10 ³	ND	ND	>1	>1
<i>C. albicans</i>	3.5 x 10 ²	18 x 10 ¹	ND	>1	>1
30°C + 65% RH					
<i>S. aureus</i>	8.5 x 10 ⁶	ND	ND	>3	>3
<i>P. aeruginosa</i>	1.1 x 10 ⁷	ND	ND	>3	>3
<i>E. coli</i>	1.7 x 10 ⁶	8.6 x 10 ¹	ND	>3	>3
<i>A. brasiliensis</i>	3.0 x 10 ³	ND	ND	>1	>1
<i>C. albicans</i>	2.0 x 10 ³	ND	ND	>1	>1
40°C + 75% RH					
<i>S. aureus</i>	2.7 x 10 ⁶	ND	ND	>3	>3
<i>P. aeruginosa</i>	4.0 x 10 ⁶	ND	ND	>3	>3
<i>E. coli</i>	1.6 x 10 ⁶	ND	ND	>3	>3
<i>A. brasiliensis</i>	4.6 x 10 ³	ND	ND	>1	>1
<i>C. albicans</i>	1.5 x 10 ⁴	ND	ND	>1	>1

Table 4.9: Preservative efficacy test results of XG 1.50% w/v with Nipastat® 0.175% w/v and PG 10% w/v (Initial and month one). *ND = None detected

<i>Test organism</i>	<i>Total viable cell count (Cfu/ml)</i>				
Initial	Test dates			Log reduction	
	0 hours	14 days	28 days	0h - 14 days	0h - 28 days
<i>S.aureus</i>	6.3×10^5	1.6×10^2	ND	>3	>3
<i>P. aeruginosa</i>	1.5×10^6	1×10^2	ND	>3	>3
<i>E. coli</i>	2.1×10^6	ND	ND	>3	>3
<i>A. brasiliensis</i>	$>10^3$	ND	ND	>1	>1
<i>C. albicans</i>	8.8×10^6	ND	ND	>1	>1
1 month	Test dates			Log reduction	
	0 hours	14 days	28 days	0h - 14 days	0h - 28 days
5°C					
<i>S. aureus</i>	5.7×10^5	ND	ND	>3	>3
<i>P. aeruginosa</i>	1.0×10^6	ND	ND	>3	>3
<i>E. coli</i>	2.8×10^6	ND	ND	>3	>3
<i>A. brasiliensis</i>	3.0×10^3	ND	ND	>1	>1
<i>C. albicans</i>	1.7×10^4	1.8×10^3	ND	>1	>1
25°C + 60% RH					
<i>S. aureus</i>	1.2×10^6	ND	ND	>3	>3
<i>P. aeruginosa</i>	1.6×10^6	ND	ND	>3	>3
<i>E. coli</i>	2.8×10^6	ND	ND	>3	>3
<i>A. brasiliensis</i>	1.2×10^3	ND	ND	>1	>1
<i>C. albicans</i>	7.0×10^3	4×10^3	ND	<1	>1
30°C + 65% RH					
<i>S. aureus</i>	7.7×10^5	ND	ND	>3	>3
<i>P. aeruginosa</i>	1.4×10^6	ND	ND	>3	>3
<i>E. coli</i>	1.3×10^6	ND	ND	>3	>3
<i>A. brasiliensis</i>	1.1×10^3	ND	ND	>1	>1
<i>C. albicans</i>	2.3×10^3	6×10^2	ND	<1	>1
40°C + 75% RH					
<i>S. aureus</i>	1.0×10^6	ND	ND	>3	>3
<i>P. aeruginosa</i>	1.7×10^6	ND	ND	>3	>3
<i>E. coli</i>	5.9×10^5	ND	ND	>3	>3
<i>A. brasiliensis</i>	1.1×10^3	ND	ND	>1	>1
<i>C. albicans</i>	1.3×10^2	1.0×10^4	ND	<1	>1

Table 4.10: Preservative efficacy test results of XG 1.50% w/v with Nipastat® 0.175% w/v and PG 10% w/v (month two). *ND = None detected

Test organism	Total viable cell count (Cfu/ml)				
Two months	Test dates			Log reduction	
	0 hours	14 days	28 days	0h - 14 days	0h - 28 days
5°C					
<i>S. aureus</i>	8.0 x 10 ⁴	ND	ND	>3	>3
<i>P. aeruginosa</i>	4.1 x 10 ⁵	ND	ND	>3	>3
<i>E. coli</i>	3.6 x 10 ⁶	ND	ND	>3	>3
<i>A. brasiliensis</i>	5.0 x 10 ³	ND	ND	>1	>1
<i>C. albicans</i>	2.0 x 10 ²	ND	ND	>1	>1
25°C + 60% RH					
<i>S. aureus</i>	7.0 x 10 ⁵	ND	ND	>3	>3
<i>P. aeruginosa</i>	4.4 x 10 ⁵	ND	ND	>3	>3
<i>E. coli</i>	1.0 x 10 ⁶	ND	ND	>3	>3
<i>A. brasiliensis</i>	7.3 x 10 ³	ND	ND	>1	>1
<i>C. albicans</i>	2.0 x 10 ²	ND	ND	>1	>1
30°C + 65% RH					
<i>S. aureus</i>	6.5 x 10 ⁵	ND	ND	>3	>3
<i>P. aeruginosa</i>	3.7 x 10 ⁵	ND	ND	>3	>3
<i>E. coli</i>	1.8 x 10 ⁶	ND	ND	>3	>3
<i>A. brasiliensis</i>	9.0 x 10 ³	ND	ND	>1	>1
<i>C. albicans</i>	3.4 x 10 ⁴	ND	ND	>1	>1
40°C + 75% RH					
<i>S. aureus</i>	5.0 x 10 ⁵	ND	ND	>3	>3
<i>P. aeruginosa</i>	9.5 x 10 ⁵	ND	ND	>3	>3
<i>E. coli</i>	1.1 x 10 ⁶	ND	ND	>3	>3
<i>A. brasiliensis</i>	5.7 x 10 ³	ND	ND	>1	>1
<i>C. albicans</i>	6.4 x 10 ²	1.0 x 10 ⁴	ND	<1	>1

Table 4.11: Preservative efficacy test results of XG 1.50% w/v with Nipastat® 0.175% w/v and PG 10% w/v (month three). *ND = None detected

Test organism	Total viable cell count (Cfu/ml)				
Three months	Test dates			Log reduction	
	0 hours	14 days	28 days	0h - 14 days	0h - 28 days
5°C					
<i>S. aureus</i>	2.4 x 10 ⁵	ND	ND	>3	>3
<i>P. aeruginosa</i>	6.4 x 10 ⁵	ND	ND	>3	>3
<i>E. coli</i>	7.6 x 10 ⁵	ND	ND	>3	>3
<i>A. brasilliensis</i>	3.3 x 10 ³	ND	ND	>1	>1
<i>C. albicans</i>	2.0 x 10 ³	ND	ND	>1	>1
25°C + 60% RH					
<i>S. aureus</i>	4.6 x 10 ⁵	ND	ND	>3	>3
<i>P. aeruginosa</i>	1.9 x 10 ⁶	ND	ND	>3	>3
<i>E. coli</i>	1.8 x 10 ⁵	ND	ND	>3	>3
<i>A. brasilliensis</i>	3.6 x 10 ³	ND	ND	>1	>1
<i>C. albicans</i>	1 x 10 ³	ND	ND	>1	>1
30°C + 65% RH					
<i>S. aureus</i>	7.6 x 10 ⁵	ND	ND	>3	>3
<i>P. aeruginosa</i>	1.5 x 10 ⁶	ND	ND	>3	>3
<i>E. coli</i>	1.2 x 10 ⁶	ND	ND	>3	>3
<i>A. brasilliensis</i>	4.3 x 10 ³	ND	ND	>1	>1
<i>C. albicans</i>	1.5 x 10 ³	ND	ND	>1	>1
40°C + 75% RH					
<i>S. aureus</i>	2.7 x 10 ⁶	ND	ND	>3	>3
<i>P. aeruginosa</i>	4.0 x 10 ⁶	ND	ND	>3	>3
<i>E. coli</i>	1.6 x 10 ⁶	ND	ND	>3	>3
<i>A. brasilliensis</i>	4.6 x 10 ³	ND	ND	>1	>1
<i>C. albicans</i>	1.5 x 10 ⁴	ND	ND	>1	>1

4.2.8 SPECTROPHOTMETRIC AND HPLC ANALYSIS OF PARTITION COEFFICIENTS FOR ESTERS OF PARA-HYDROXYBENZOIC ACID

Owing to their high oil solubility, para-hydroxybenzoates have relatively unfavourable partition coefficients between the oil and water phases of emulsions. Protection of these emulsions requires that lethal levels of the preservative contact the microorganisms in the aqueous phase. In an emulsion containing 50% or more oil, the preservative may be present in the oil phase rather than in the aqueous phase after equilibrium is achieved. Thus a considerable proportion of the preservative can become inactivated by migration into the wrong phase. This migration can occur over a considerable period of time, which is one more reason why prolonged microbiological testing of formulated products is required (Haag and Loncrini, 1984).

To be effective, a preservative agent must be dissolved in sufficient concentration in the aqueous phase of a preparation. Furthermore, only the undissociated fraction or molecular form of a preservative possesses preservative capability, because the ionised portion is incapable of penetrating the microorganism. Microorganisms involved include moulds, yeasts and bacteria, with bacteria generally favouring a slightly alkaline medium and the others an acidic medium. Although few microorganisms can grow at a pH <3 or >9, most aqueous pharmaceutical preparations are withstanding the pH range of 3 to 9. Thus, the preservative selected must be largely undissociated at the pH of the formulation being prepared (Allen, 2008:2110).

4.2.8.1 Materials and methods

The range of para-hydroxybenzoate preservatives used to preserve the Pheroid[®] system varied from single component preservatives (used alone and in combination with PG) to the combination preservatives such as Nipastat[®] and Nipasept[®].

In a simple two-phase system of oil and water a preservative will partition until equation 4.1:

$$C_o/C_w = K_{o/w} \quad \text{(Equation 4.1)}$$

where C_o is the concentration of preservative in oil at equilibrium, C_w is the concentration of preservative in water at equilibrium and $K_{o/w}$ is the oil/water partition coefficient at the given temperature (Parker & Hodges, 2002:665)

Spectrophotometer analysis of single component parabens

For each single paraben, for example methylparaben, the equivalent concentration used in the experiment outline was accurately weighed, combined and heated until dissolved in 15ml of Millipore water. A volume of 15ml of the oil phase (Vit F ethyl ester used in this instance) was measured. The oil and water phases were combined and stirred for 48 hours at 25°C with a magnetic stirrer at 600 rpm. Thereafter the mixture was centrifuged for 20 minutes at 3000 rpm and the supernatant was analysed spectrophotometrically for the paraben concentration at a wavelength of 255 nm. A standard curve was prepared for each individual paraben. The concentration of the paraben preservative accumulated in the water phase was determined by means of linear regression. The concentration of the paraben in the oil phase was determined by means of deduction. Equation 4.1 was used to determine the oil/water partition.

High pressure liquid chromatography (HPLC) analysis of combination parabens

For the experiment, each individual preservative was weighed individually at the concentrations used in the experiment outline. The preservative was dissolved in 15ml of N₂O water phase and in this instance, 15ml of the oil phase (octanol) was accurately measured. The oil and water phases were combined and stirred for 48 hours at 25°C with a magnetic stirrer at 600 rpm. The mixture was centrifuged for 20 minutes at 3000 rpm and the supernatant collected and analysed for the paraben concentrations. For the two combination preparations (Nipastat[®] and Nipasept[®]) a similar procedure was followed, with the addition of PG (10% v/v) added to 13.5ml of the N₂O phase and 15ml of the octanol phase.

The concentration of the individual parabens was determined using an HPLC method. The method of determining the methyl-, ethyl-, propyl-, butyl- and isobutylparaben (depending on the preservative, which was either Nipastat[®] or Nipasept[®]) was developed and validated by Prof Jan du Preez, at the Analytical Technology Laboratory, North-West University, (Potchefstroom Campus).

The sum of the areas of the three peaks (methyl-, ethyl-, and propylparaben) were used in the calculations for Nipasept[®] and the five peaks (methyl-, ethyl-, propyl-, butyl- and isobutylparaben) were used in the calculations for Nipastat[®]. Butylparaben has two isomers, thus producing two peaks. The fact that the two isomers are not baseline-separated does not matter as the areas were summed.

The method was validated with respect to linearity, accuracy, precision (intra-day and inter-day), ruggedness, sensitivity, specificity and system suitability. The chromatographic conditions were as follows:

Analytical instrument:	HP1100 series HPLC equipped with an HP1100 quaternary gradient pump, HP1100 autosampler, HP1100 diode array detector and Chemstation Rev. A.08.03 data acquisition and analysis software.
Column:	USP 23 (1995) packing L1, p1778 (Luna C18-2 column, 250 x4.6 mm, 5µm, 100 Å pores, 17.8% carbonload, endcapped, Phenomenex, Torrance,CA was used)
Mobile phase:	Acetonitrile/water 60/40, containing 0.1% of orthophosphoric acid.
Flow rate:	1.0 ml/min.
Injection volume:	10 µl.
Detection:	UV at 254 nm.

Use equation 4.1 to determine the oil/water partition coefficient (see Table 4.14 for results obtained).

4.2.8.2 Results & Discussion

In Table 4.12, a summary of the partition coefficients of the individual paraben preservatives are given. According to the literature the expected partition coefficient for the hydroxybenzoates should be the highest for butylparaben and the lowest for methylparaben, indicating the partition coefficient increased with chain length. This could be attributed to the increase in lipophilicity of the preservative with chain length, thereby enhancing the affinity for oil (Wan *et. al.*, 1986:309). The value for the partition coefficient of butylparaben is lower than expected. A repetition of the whole range should be performed to determine the validity of the data retrieved.

Table 4.12: Summary of the o/w partition coefficients for methyl-, ethyl-, propyl- and butylparaben solutions in water and Vit F ethyl ester.

	<i>Methylparaben</i>	<i>Ethylparaben</i>	<i>Propylparaben</i>	<i>Butylparaben</i>
Partition coefficient	12.26	31.20	30.12	24.86
% in Water-phase	7.54%	3.1%	3.21%	3.87%
% in Oil-phase	92.46%	96.9%	96.79%	96.13%

In Table 4.13 a summary of the partition coefficients of the combination preservatives are given. Although the activity of preservative agents, such as parabens, increases with increasing hydrophobicity, i.e., increase in oil/water partition coefficient, their effectiveness in cream formulations is compromised in that a significant percentage of the preservative partitions in the oil phase, thereby decreasing the concentration of the preservatives in the aqueous phase. Studies done by Darwish and Bloomfield (1995:191) suggested the co-solvents (PG in this instance) can increase the activity of paraben preservatives by causing a decrease in the oil/water partition coefficient, thereby increasing the concentration of the free parabens in the aqueous phase of the formulation.

	<i>Methylparaben</i>	<i>Ethylparaben</i>	<i>Propylparaben</i>	<i>Butylparaben</i>
Nipasept®	8.86	20.75	121.41	Below limit of quantification
Nipasept® + PG	4.79	15.21	55.48	Below limit of quantification
Nipastat®	5.38	22.51	113.40	263.90
Nipastat® + PG	4.39	16.27	48.85	341.65

Table 4.13: Summary of the o/w partition coefficients for the combination preservatives (Nipasept® and Nipastat®) solutions in water and octanol with added PG (10% v/v).

4.2.9 CONCLUSION

To summarise the outcome of this accelerated stability test and the effects it had on both the formulas chosen for the final formulation study, we have to look at all the outcomes of the stability tests against which the samples were measured. A choice between two formulas was put up against a pH stability test, viscosity measurements, a PET, visual observation in different packaging materials and under different storage conditions and temperatures and the determination of the oil/water partition coefficient for the Nipastat[®] preservative used in the final formula.

It was clear that the accelerated stability test, especially at the higher temperatures, influenced the results of most of the tests performed. This was especially true of the temperature point of 40°C +75% RH, which proved to be detrimental for both the Carbopol[®] 934P and XG formulations, although the Carbopol[®] 934P exhibited stable pH values over the whole three month test period.

With regard to the preservative efficacy test, Nipastat[®] as a preservative passed the PET for all the formulas containing Carbopol[®] 934P. The conclusion was that stable pH values are very important for both the viscosity values measured for this carbomer and for the partitioning of the paraben preservatives in the water phase of the emulgels. Some of the XG formulations, however, did not pass the PET due to *Candida albicans* growth.

For further product development, the importance of the packaging materials used to store the Pheroid[®] emulgel as well as the challenges the formulator has to face to mask the fish oil smell of the product, can be a daunting task. Although the Carbopol[®] 934P formulation passed the accelerated stability test it is advisable that a minimum of two years stability testing must be performed on the formula to determine the possible instabilities or shortcomings of this promising preparation, the emulgel.

With regard to packaging it is evident that foil packaging was superior to amber glass bottles for maintaining the physical state of the Pheroid[®] emulgel formulations. This was probably due to the fact the foil packaging was more effective in limiting the influence of oxygen on the Pheroid emulgel formulations.

SUMMARY AND FUTURE PROSPECTS

SUMMARY

Emulsion formulations are well known as a potential carrier system for the delivery or targeting of API's to specific sites in the body. The biotechnological approach of the Pheroid® drug delivery system, which forms a part of submicron emulsion type formulations, are based on the ability to entrap drugs with high efficiency and deliver these with remarkable speed to target sites in the body. In this study the possibility of developing emugel formulations for Pheroid® technology was investigated.

In a preformulation study, three different viscosity-increasing agents were used in combination with the Pheroid® emulsion system to form an emugel dosage form. The agents used were XG (1.00% w/v; 1.50% w/v; 2.00% w/v), Carbopol® 934P (0.1% w/v; 0.2% w/v; 0.3% w/v) and HPMC (2.00% w/v and 3.00% w/v). The effect of PG as a co-solvent was investigated, as well as two different methods of manufacturing employed.

The influence of these gelling agents on the physical properties of the emugel formulations were monitored at time intervals of 24 hours, 72 hours, seven days and 28 days after manufacturing. The pH values measured for the XG formulation stayed stable while the HPMC formulations showed instabilities at the 28 day stability interval. Carbopol® 934P was stable around a neutral pH value of 7, which was needed for total neutralisation of the carbomer.

The influence of the gelling agents on the viscosity measurements showed a shear thinning behaviour, with a definite yield point value, for the XG formulations. Formula F11A with 1.50% w/v XG and 10% PG prepared according to method A had the most stable viscosity measured over 28 days. Carbopol® 934P exhibited shear-thinning flow behaviour since the viscosity decreased with an increase in shear rate. The HPMC gels showed instabilities as the viscosity values on day-28 could not be measured any longer.

Visual assessment of the sample showed the instabilities of the HPMC formulations. Microscopy images taken of these formulas confirmed the disintegration of the system into separate oil and water phases.

As most oral liquids, especially aqueous preparations, offer suitable conditions for growth of microorganisms. A range of paraben-ester was investigated as preservatives. The theory of the advantage of PG to increase the efficacy of paraben

preservatives was also tested. The preservative of choice for the accelerated stability study was Nipastat[®] which comprises a combination of methylparaben, ethylparaben, propylparaben, butylparaben and isobutylparaben in various concentrations. The basic Pheroid[®] formulation showed the possibility of self-preservation. The oil-in-water partition coefficient of the parabens was determined with spectrophotometer analysis for single component parabens and HPLC for combination parabens.

The best possible formulations obtained during the early formulation tests were manufactured in bulk for storage and testing purposes. A validated stability programme for three months was followed at three storage temperatures. The two formulas chosen were Carbopol[®] 934P (0.2% w/v) with PG (10% w/v) and XG (1.50% w/v) with PG (10% w/v). The type of storage materials used for the accelerated stability study were amber glass bottles and foil sachets. The foil sachets proved efficient to protect the Pheroid[®] emulgels against oxidation which was evident with the amber bottles.

The formula that passed the preservative efficacy test was Carbopol[®] 934P. This formula also exhibited stable pH values and acceptable viscosity values were attained and the formula stayed stable at most temperatures except during high temperature storage (40°C + 75% RH).

FUTURE PROSPECTS

Based on the results and conclusions of the study, the following aspects should be addressed in future studies:

1. Attention must be given to optimise the Pheroid[®] formulary for patient acceptance. Suitable colourants and flavourants must be found to mask any discolouring or smell variations in the Pheroid[®] formula. Suitable sweeteners should be incorporated when products for children are manufactured to prevent tooth decay. Olfactory tests must be performed to determine compliance.
2. The product Pheroid[®] emulgel formulations should be exposed to long-term stability testing to determine the long-term stability of the formulation.
3. The preservation system for the Pheroid[®] emulgel formulations should be optimised, especially with regard to the growth of yeasts such as *Candida albicans*.

4. The influence of the incorporation of active pharmaceutical ingredients on the physical properties and stability of the Pheroid® emulgel formulations should be investigated.