

*Leading Article***INVESTIGATION OF FACTORS AFFECTING PHYSICOCHEMICAL PROPERTIES OF PALM-OLEIN CREAMS USING OPTIMISATION TECHNIQUE-QUALITY BY DESIGN**Sheikh KA^a, Saringat B^b, Bukhari NI^c

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ABSTRACT

Semisolid formulations of palm-olein were prepared using design of experiment (DoE) methodology. The influence of formulation aids (emulsifier) and the processing variables (mixing and cooling) were investigated on the physicochemical properties of the formulated systems. The stable semisolid appearance was the response variable. The systems were characterised by microscopy, DSC, Rheology, and XRD. The factorial design generated a matrix of 22 experiments to investigate physicochemical properties of the systems. The systems formed stable semisolids (no syneresis), unstable semisolids showing syneresis or structured fluids, depending on the concentration of stearic acid and the preparation technique. The stable semisolids contained α -crystalline lamellar structure, not present in the unstable structured fluids. In addition, syneretic semisolids showed plate-like crystals, implying pressure sensitivity, associated with polymorphism in the stearic acid. The stable semisolids showed mixture of amorphous and crystalline stearic acid. In contrast, pure amorphous or crystalline stearic acid was present in the unstable semisolids and the structured fluids respectively (confirmed by XRD). Mode of mixing and the concentration of stearic acid appeared to be critical factors ($p < 0.01$). DoE predicted a combination of factors to achieve stable semisolid systems. Confirmatory experiments yielded results within 1% of the predicted responses, demonstrating the reliability of the software.

Key words: Quality by Design (QbD), Design of experiment, Palm-olein, Liquid paraffin, polymorphism, Maltese crosses.

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1. INTRODUCTION

Formulation of novel topical drug delivery systems requires investigation of large number of formulation and processing variables. Generally, the one factor at a time (OFAT) approach is used for the method development and validation, which uses trial and error method and provides information about the effect of individual factor but does not reveal the interactions (if any) between the factors and requires enormous amount of experimentation due to

inclusion of only one factor at a time to investigate its effect on the overall performance. Since the inception of ICH Q8 guidelines for the product development, Quality by Design (QbD) optimisation approach using Design of Experiment (DoE) methodology has become integral part of formulation development and reveals critical factors, their interactions and suggests the best combinations of the factors for optimised outputs with lesser number of experiments (Rowe, 1993; Rowe, 1997; Lewis *et al.* 1999; Anderson and Whitcomb, 2005). Thus, OFAT approach surrenders in favour of QbD.

Several formulation aids and processing variables affect the physicochemical characteristics of the semisolid drug delivery systems (Eccleston, 1986; Sheikh et al., 2011). Formulation aids include emulsifiers and stabilisers while the processes are the modes of heating, mixing, and cooling.

Active pharmaceutical ingredients (API) and excipients may show polymorphism. Conversion from one polymorphic form to another during processing can affect the physicochemical properties of the final formulation (Miller and York, 1985; Ertel and Carstensen, 1988; Rajala and Laine, 1995). Stearic acid, magnesium stearate and oils are the common ingredients for semisolid preparations. Stearic acid exists as polymorphs and may be composed of either pure or mixed homologues. The commonly used stearic acid is not pure but a “triple pressed” homologue mixture of ~60% palmitic acid and 40% stearic acid (Eccleston, 1997). It has been reported that pure stearic acid in liquid paraffin produces unstable semisolid systems (Bozic *et al.*, 1980).

Commercial stearic acid is available as a mixture of monohydrate and dihydrate and its moisture treatment produces different pseudopolymorphic forms affecting its physicochemical properties (Swaminathan and Kildsig, 2001; Bracconi *et al.*, 2005; Okoye and Wu, 2007). Milling of magnesium stearate (MgSt), obtained from stearic acid has also shown to affect its particle size and surface area causing changes in the lubrication properties which may be related to the polymorphic modifications in MgSt (Leinonen *et al.*, 1992).

It is reported that aqueous semisolid systems containing stearic acid are markedly affected by processing variables such as mixing, which produces metastable polymorphic form (Garti *et al.*, 1982; Lin *et al.*, 1994; Eccleston, 1997). Swelling of aqueous stearate creams has been attributed to the existence of α -crystalline lamellar structure (Eccleston, 1997; Mueller-Goymann, 2004). However, swollen lamellar structures appeared to be metastable and under pressure broken down to non-swollen plate like crystalline structures, producing mobile lotions.

Liquid paraffin is synthetic, petroleum-based oil, composed of long chain saturated hydrocarbons which has been used as an oil phase for semisolid formulations for long time. In contrast, palm-olein, natural vegetable-based oil contains saturated and polyunsaturated fatty acids of varying chain lengths and has rarely been used in topical pharmaceutical drug delivery systems (Baie and Sheikh, 2000; Baie *et al.*, 2005).

Therefore, the aim of this work was to investigate the effect of various formulation and processing variables such as type of cooling and mixing in addition to the type and concentration of emulsifier and the polymorphism in stearic acid on the physicochemical properties of liquid paraffin and palm-olein formulations using QbD.

2. MATERIALS AND METHODS

2.1 Materials

Palm–olein was a gift from Lam Soon Edible Sdn Bhd. Liquid paraffin BP was purchased from JM Loveridge Plc, Southampton, England. Span 80, Tween 80 and stearic acid were obtained from Sigma Chemical Co. (St. Louis, USA). Nitrogen and oxygen gases used for DSC were purchased from MOX Gas, Sdn. Bhd. Malaysia. Aluminium crucibles for DSC (40µL capacity) were purchased from Mettler Toledo, Switzerland. Double distilled de–ionised water was used as continuous medium.

2.2 Methods

2.2.1 Experimental Design

The design of experiment (DoE) approach was used to investigate the potential influence of six formulation– and processing–related factors and their interactions on the physicochemical characteristics of the semisolid formulations as listed in Table 1. The selection of investigated factors was based on literature and previous experience affecting similar formulations and included type of oils (liquid paraffin and Palm–olein), emulsifiers (Span 80 and Tween 80) and their optimum concentrations, stearic acid concentration. The processing variables included mode of cooling and mixing upon cooling.

Table 1: Formulation and process-related factors investigated using quality by design

variables	Designation	Factors	
		Lower level (-)	Higher Level (+)
Oil phase	A.	Palm–olein (A-)	Liquid paraffin (A+)
Emulsifer type	B.	Span 80 (B-)	Tween 80 (B+)
Emulsifier concentrtrion	C.	1% (C-)	10% (C+)
Stearic acid concentration	D.	1% (D-)	5% (D+)
Cooling type	E.	Slow (E-)	Fast (E+)
Mixing upon cooling	F.	“Yes” (F-)	“no” (F+)

After selection of factors (each at two levels), a layout (matrix) for the experimental conditions (Table 2) was generated for 6 factors by the DX[®] 7.1.5, a commercial software for DoE. A factorial design with minimum experimental runs resolution 5 (Minimum Run Res 5) without centre points was selected to investigate the effect of all factors and the interactions between the factors. A matrix of 22 experiments was generated by the software (Table 2).

Based on the generated matrix, the real experiments were carried out using the experimental methodology (*c.f. Preparation of Formulations (Section 2.2.2)*) to capture the effect of various factors on the appearance and stability of the formulations.

The data were visualised by graphical display using Microsoft Excel[®] 2007. To determine the best combination of the factors studied, the data obtained on physical appearance and stability of formulations were entered in the matrix obtained from the DX[®] 7.1.5 and analysed using appropriate DX[®]-suggested model. The data for all responses were randomised and evaluated

by Box Cox plot, which helps to analyse whether any transformation of data is required before the final analysis.

Table 2: Matrix of experimental factors generated by DX[®] 7.1.5 for the formulation of o/w creams

Experiment no.	Oil phase type	Emulsifier type	Emulsifier conc. (%)	Stearic acid (%)	Cooling type	Mixing on cooling
1	Palm-olein	Span 80	10	1	Slow	Yes
2	Palm-olein	Span 80	1	5	Fast	Yes
3	Palm-olein	Tween 80	1	1	Slow	No
4	L. Paraffin	Tween 80	10	1	Slow	No
5	Palm-olein	Tween 80	1	5	Slow	Yes
6	L. Paraffin	Tween 80	1	1	Slow	No
7	Palm-olein	Tween 80	10	5	Fast	No
8	Palm-olein	Span 80	10	5	Fast	No
9	L. Paraffin	Span 80	10	1	Slow	Yes
10	Palm-olein	Tween 80	10	1	Slow	Yes
11	L. Paraffin	Span 80	1	5	Fast	Yes
12	Palm-olein	Span 80	1	5	Fast	No
13	L. Paraffin	Tween 80	1	1	Fast	No
14	Palm-olein	Span 80	10	1	Slow	No
15	L. Paraffin	Tween 80	1	5	Fast	No
16	L. Paraffin	Span 80	10	5	Fast	No
17	L. Paraffin	Tween 80	10	5	Slow	Yes
18	Palm-olein	Tween 80	10	5	Fast	Yes
19	L. Paraffin	Span 80	10	5	Fast	Yes
20	L. Paraffin	Span 80	1	1	Fast	No
21	Palm-olein	Span 80	1	1	Slow	Yes
22	L. Paraffin	Tween 80	1	1	Slow	Yes

The DX[®] suggested a provisional model for the analyses of the results for each factor with a probability (p) value <0.05. Based on the significance level of p value, the factors were included in or excluded from the model. The non-significant factors were excluded from the model using backward elimination with alpha to exit set at 0.1, implemented in DX[®] 7.1.5 and the model was re-fitted with only significant factors and selected based on the goodness-of-fit statistical criteria (Khuri and Cornell, 1987). The optimisation criteria were the visual appearance (semisolid) and the physical stability (no syneresis). The desired level of syneresis was set to be equal or less than 1%.

The DX[®] 7.1.5, on the basis of the contribution of the individual factors in addition to the interactions between the factors predicted experiments with various combinations of the variables producing maximum (100%) desirability to minimum (30%) desirability. The experiment with 100% desirability predicted semisolid creams with no syneresis and the experiment with 30% desirability showed structured fluids or semisolids with syneresis.

The confirmatory experiments were conducted in triplicates to access the accuracy of the prediction of DX[®] 7.1.5 using the maximum desirability conditions (100%). The agreement of predicted and experimental responses was evaluated by observing response values within

prediction at 95% confidence interval. An outcome within this interval validated the model (Weisberg, 1985).

2.2.2 Preparation of Formulations

The oil phase composed of the oil (liquid paraffin/palm-olein), emulsifier and stearic acid and the continuous phase (water and Span 80 or Tween 80) according to the concentrations given in Table 2 were heated separately in a 100mL beaker to 70°C on a water bath. The oil phase was added to the water phase at the same temperature and cooled slowly (the beaker left on the water bath to cool to room temperature (~0.01–0.02°C/min)) or fast (beaker taken out of water bath and left on the bench to cool to room temperature (3–5°C/min)). Upon cooling, the formulations were either continuously mixed using a homogeniser until cold or mixed for a while after initial mixing of two phases at 70°C and then cooling discontinued.

2.2.3 Characterisation of Formulations

2.2.3.1 Visual Inspection

The formulations were inspected for the appearance (structured fluid or semisolid) in addition to any syneresis of the systems. The extent of syneresis was investigated by calculating the amount of oil separated after one week of preparation.

2.2.3.2 Polarised Light Microscopy

A Leica DMLS compound microscope (Histocentre, Malaysia) was used to study the microstructure of the raw materials (liquid paraffin, palm-olein) and all formulations. Slides containing sample were placed between crossed polars and studied at various magnifications (x 5, x 10, x 20 and x 40). Digital photomicrographs were taken using the Image Pro Express software.

2.2.3.3 Hot Stage Microscopy

A TMS 91 hot stage (Linkam Scientific Instruments, UK) attached to the Polyvar microscope was also used to determine the melting point of the various raw materials in addition to investigate the phase changes of all formulations. A thin smear of sample was sandwiched between two 16 mm circular glass cover-slips. The stage was heated slowly from 25°C to 70°C at a rate of 5°C/minute and changes in the microstructure were observed at appropriate magnification (x 20). The melting point was recorded as the temperature between which the sample started to flow and the temperature at which the structure completely disappeared. Thermal changes at various temperatures were recorded and photographed using the Leutron computer software.

2.2.3.4 Differential Scanning Calorimetry

A DSC 822e (Mettler Toledo, Leicester, UK) with the sample robot, was used for the thermal analysis of all samples. Approximately 1.5–10mg of each sample was carefully weighed by difference in 40µL aluminium DSC pans, the pans were sealed and placed in the appropriate position of the sample robot. A standard heating cycle of 30°C–80°C at a rate of 5°C/min was

chosen to observe thermal changes in the samples. The results were plotted together after normalisation to eliminate weight bias due to the weight differences of the samples.

The thermal properties of the formulations were studied by investigating changes in heat transfer (ΔH) during heating cycle.

2.2.3.5 Rheology

A cone and plate Physica MCR 301, air-bearing Pelletier rheometer (Anton Paar, Germany) was used to investigate the flow curves of the formulations. The dimension of the measuring plate was 50 mm and the zero gap was fixed to be 1mm. All experiments were conducted in triplicates at ambient temperature ($\sim 28^\circ\text{C}$).

Flow curves were obtained using shear rate vs shear stress experiments to investigate the zero shear and apparent viscosities of the lipogels. Flow curves were obtained using up and down curves with minimum shear rate of 10^{-3} (s $^{-1}$) and maximum of 10^2 (s $^{-1}$).

The rheological properties were studied by determining apparent viscosity (Pa.s) of each formulation, obtained from the apex of the shear stress vs shear rate curves.

2.2.3.6 X-ray diffraction

A powder X-ray diffractometer (Bruker 8 Advances, Germany) was used for the analysis of the formulations. The method was adopted from Koivisto *et al.*²³ The samples were irradiated with X-rays from a copper target using the following conditions and parameters: Filter Ni, Generator 40kV, voltage 40kV, current 20mA, λ 0.15410nm using a Soller slit. The samples were continuously scanned from 2.3° – 40° at a rate of $2.5^\circ/\text{sec}$ with a step of 0.025° . The XRD spectra were analysed using Diffract Plus software. $\text{CuK}_{\alpha 1}$ values were used for the analysis of each peak.

3. RESULTS

3.1 Characterisation of formulations

Formulation and stability studies were carried out using DoE by conducting 22 experiments (each in triplicate) provided by the matrix generated by DX[®] 7.1.5 as described in the Table 2. The data on visual appearance and stability (no syneresis), microscopy, DSC, rheology and XRD for each formulation generated are listed in Table 3.

3.1.1 Visual appearance

Formulations ranged from structured fluids to semisolid creams with or without syneresis depending on the variables selected (Table 3). Generally, both oils produced semisolid (non-syneretic) creams when Span 80 was used as an emulsifier at 10% concentration, maximum concentration of stearic acid (5%), using slow cooling with no mixing upon cooling. In contrast, structured fluids were obtained with minimum amount of emulsifier (1%), and stearic acid (1%) with fast cooling. Mixing upon cooling had no significant effect on the appearance of structured fluids. However, systems containing maximum amount of stearic acid (5%) with continuous mixing upon cooling produced pressure sensitive semisolid

creams. The systems were semisolid immediately after cooling but changed to structured fluids with gentle stirring. However, these systems changed back to semisolid creams upon storage for a week.

Table 3: Influence of factors on the physical chemical properties of formulations studied by Design of Experiment

Experiment. no	Visual appearance	Syneresis (%)	Polarised Microscopy	Integration (ΔH)	Viscosity (Pa.s)	XRD Data
1	Semisolid	0	MC	-66.4	0.83	AM/CR
2	Str. Fluid	10	AC/PC	-4.5	0.15	Crystalline
3	Str. Fluid	5	AC	-5.2	0.13	Crystalline
4	Semisolid	2	MC	-45.6	0.52	AM/CR
5	Str. Fluid	7	AC/PC	-5.7	0.15	Crystalline
6	Str. Fluid	10	AC	-3.9	0.17	Crystalline
7	Semisolid	1	MC	-40.7	0.50	AM/CR
8	Semisolid	1	MC	-45.1	0.51	AM/CR
9	Semisolid	1	MC	-47.3	0.55	AM/CR
10	Semisolid	1	MC	-56.8	0.82	AM/CR
11	Str. Fluid	10	AC/PC	-3.1	0.11	Crystalline
12	Str. Fluid	6	AC	-6.3	0.13	Crystalline
13	Str. Fluid	8	AC	-6.2	0.12	Crystalline
14	Semisolid	0	MC	-65.7	0.65	AM/CR
15	Str. Fluid	5	AC	-5.4	0.14	Crystalline
16	Semisolid	0	MC	-68.2	0.85	AM/CR
17	Semisolid	5	MC/PC	-25.1	0.25	AM/CR
18	Semisolid	5	MC/PC	-23.4	0.21	AM/CR
19	Semisolid	5	MC/PC	-20.7	0.22	AM/CR
20	Str. Fluid	7	AC	-6.2	0.12	Crystalline
21	Str. Fluid	6	AC	-4.5	0.10	Crystalline
22	Str. Fluid	6	AC	-7.2	0.14	Crystalline

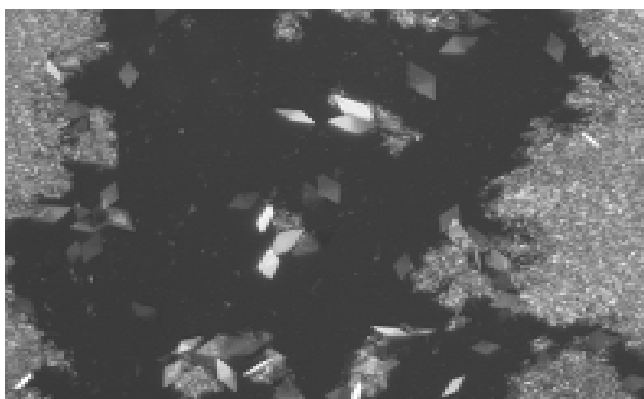
Note: MC- Maltese Crosses; PC- Plate-like crystals; MC/PC- Maltese crosses and plate-like crystals; AC- Anisotropic crystals; AC/PC- Anisotropic crystals and plate-like crystals; AM/CR- Mixture of amorphous and crystalline materials

3.1.2 Polarised Microscopy

Figure 1 shows the photomicrographs of the selected formulations. The semisolid (non-synergetic) creams showed presence of numerous “Maltese crosses” in addition to the anisotropic crystals (Figure 1a). The number of Maltese crosses increased with an increase in the amount of emulsifier and the stearic acid. Contrarily, structured fluids showed only anisotropic crystals (not shown). No “Maltese crosses” were observed in these systems. The pressure sensitive semisolid creams showed presence of few Maltese crosses in addition to the plate-like crystals (Figure 1b).



(a)



(b)

Figure 1: Photomicrographs of the selected formulations (a) Stable (non-synergetic) semisolid creams and (b) pressure sensitive synergetic semisolid creams

3.1.3 Hot Stage Microscopy

All formulations showed melting of anisotropic crystals between ~ 40 and $\sim 50^{\circ}\text{C}$. In contrast, Maltese crosses in the stable semisolid creams appeared to melt at $\sim 55^{\circ}\text{C}$. The plate-like crystals in the pressure sensitive semisolid creams melted at $\sim 42^{\circ}\text{C}$.

3.1.4 Differential Scanning Calorimetry

Figure 2 shows DSC data for the selected samples. All formulations showed different DSC data each showing one to two endotherms. The stable (non-synergetic) semisolid creams showed two endotherms peaking between $\sim 48^{\circ}\text{C}$ and $\sim 55^{\circ}\text{C}$. In contrast, the structured fluids showed only one endotherm peaking at $\sim 45^{\circ}\text{C}$. The pressure sensitive semisolids showed two endotherms peaking between ~ 43 and $\sim 50^{\circ}\text{C}$. The stable semisolid formulations showed significantly higher integration (ΔH) ($P < 0.05$) compared to structured fluids and the pressure sensitive semisolid formulations (Table 3).

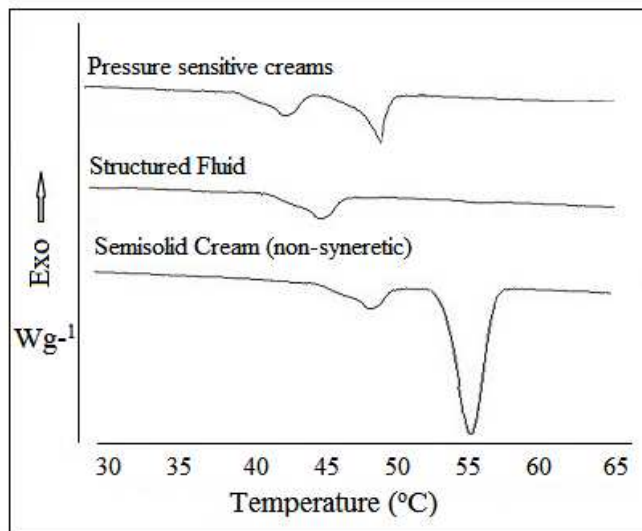


Figure 2: DSC spectra of selected formulations. A standard heating cycle of 30°C–80°C at a rate of 5°C/min was used

3.1.5 Rheology

All formulations showed non-Newtonian behaviour with flow curves in the form of anti-clock-wise hysteresis loops. Rheograms of all samples were different from each other showing varying hysteresis loops *i.e.* stable semisolid formulations showing the broadest loop (not shown).

All formulations showed different apparent viscosities, which were calculated from the apex of the loop (100s^{-1}). The semisolid (non-syneretic) formulations showed highest viscosities (Table 3). In contrast, the structured fluids showed lowest apparent viscosities.

3.1.6 X-ray Diffraction

Figure 3 shows the XRD data for the selected samples. The structured fluids demonstrated sharp peaks at 2θ between 25° and 30°. The pressure sensitive syneretic semisolid creams showed a broad peak at $2\theta=20^\circ$. No sharp peaks were seen in these systems. In contrast, all stable semisolid creams showed two sharp peaks at 2θ between 25° and 30° in addition to the broad peak at $2\theta=20^\circ$ (Figure 3).

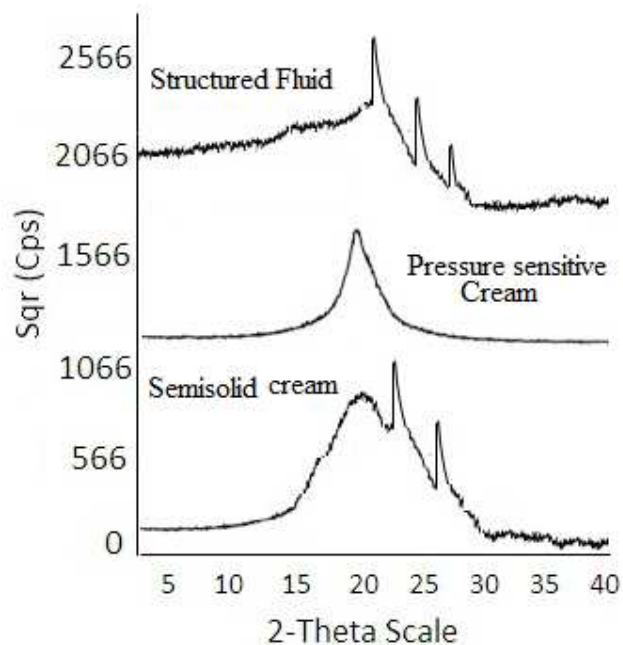


Figure 3: XRD spectra of selected formulations showing sharp to broad peaks.

The data from Table 3 was analysed on DX[®] 7.1.5 using factorial design model. The software randomised and evaluated the need for data transformation for all responses by Box Cox plot and no data transformation was required, as the lambda was equal to 1 (best lambda= 1.48). The data was analysed by ANOVA implemented in DX[®] 7.1.5, which shows influence of the factors, depending on the p values (Table 4).

Table 4: ANOVA showing contribution of various factors on the physicochemical properties of formulations

Parameters	Source	Sum of Squares	Mean Square	F Value	p-value Prob > F
Visual appearance and Syneresis	Model	55.50	6.17	19.53	< 0.0001
	A–Oil phase	11.83	11.83	37.47	< 0.0002
	B–Emulsifier type	4.08	4.08	12.92	0.0670
	C–Emulsifier conc.	7.31	7.31	23.16	0.0004
	D–Stearic acid conc.	2.34	2.34	7.41	0.0185
	E–Type of cooling	5.22	5.22	40.55	0.0001
	F–Type of mixing	4.95	4.81	13.73	0.0015
	AB	5.60	5.60	14.74	0.0012
	AD	2.91	2.91	9.23	0.0103
	DE	8.54	8.54	23.04	0.0005

3.2 Influence of Factors

The Pareto chart demonstrates influence of various experimental variables and interactions between them on the overall outcome and categorises the factors into significant, critical and non-significant. The factors showing t -value more than the lower limit (2.77645) were considered as significant whereas the factors with t -value above Bonferroni limit (6.84714) were the critical factors (Figure 4).

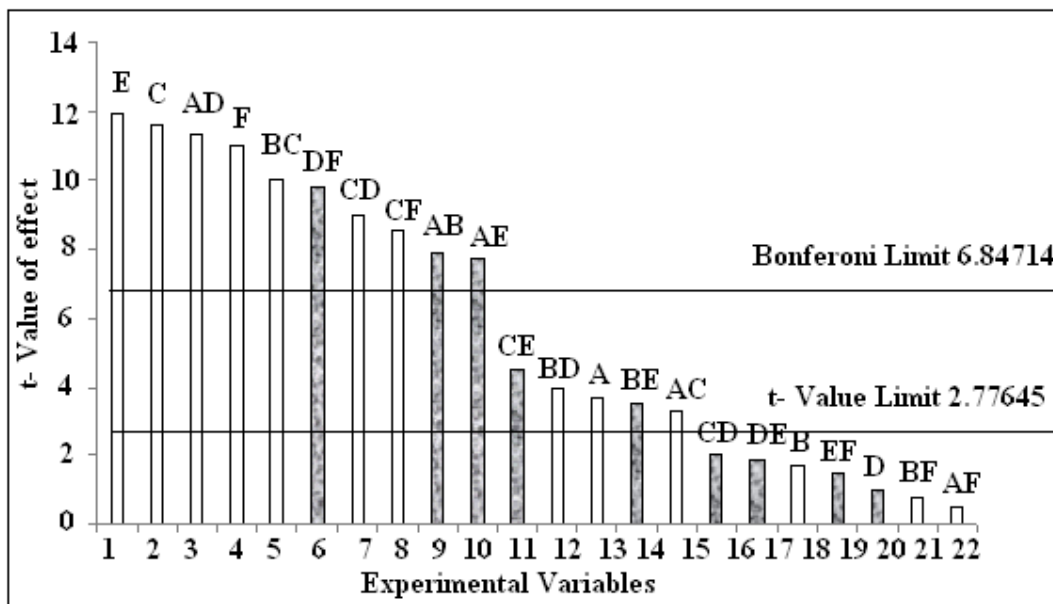


Figure 4: Pareto chart showing significance of the factors and their combinations on visual appearance and synerisasis of studied formulations.

3.2.1 Oil phase

The Palm-olein (A-) and liquid paraffin (A+) were used as oil phases. The influence of type of oil phase and its interactions with other factors is explained by the Pareto chart (Figure 4) as well as ANOVA statistics (Table 4). Type of oil phase showed a significant effect on the overall outcome of the experiment ($P < 0.0002$) showing positive effect (represented by the colourless bars) as the t -value was above the lower line (Figure 4) but it was below the Bonferroni limit (a measure of critical effect). However, its interactions with stearic acid concentration (AD) and the type of emulsifier (AB) appeared as critical factors (above Bonferroni limit). The interaction of factors AD showed positive effect. In contrast, interaction of factors AB showed negative influence.

3.2.2 Type of emulsifier

The emulsifiers used were Span 80 (B-) or Tween 80 (B+). Eulsifer type appeared to be insignificant variable (P value 0.067) as shown in Table 4 and the t -value was below the lower line in the Pareto chart (Figure 4). However, it showed significant interactions with other experimental factors to produce critical effect especially with the concentration of emulsifier (BC) producing positive effect (Figure 4).

3.2.3 Emulsifier concentration

The two emulsifiers, each at two concentrations 1% (C-) and 10% (C+) were used in this study. The emulsifier concentration alone (C) and its interactions with other factors such as Span 80 (BC), stearic acid (CD) and mode of mixing (CF) showed critical positive effect on overall results as all of these factors were above the Bonferroni limit (Figure 4).

3.2.4 Stearic acid concentration

Two stearic acid concentrations 1% (D-) and 5% (D+) were selected. Stearic acid concentration alone had a critical (Bonferroni value 8.94) but negative influence (Blue colour) on the overall results. However, it showed significant interactions with other factors such as oil phase (AD) and concentration of emulsifier (CD) to produce positive effect (colourless bar) (Figure 4).

3.2.5 Mode of cooling

The formulations were cooled either slowly (E-) or fast (E+). The type of cooling appeared to be the most critical factor (Bonferroni value 11.92) showing positive effect when taken into consideration in isolation (slow cooling) producing non-synergetic semisolid creams. However, its interaction with type of oil phase produced critically negative effect (AE-coloured bar). Other interactions such as emulsifier type and mode of cooling (BE) and emulsifier concentration and mode of cooling (CE) also showed significantly negative influence (Figure 4). Fast cooling produced synergetic semisolids.

3.2.6 Mode of mixing upon cooling

To find out the effect of mixing on properties under study, the formulations were either mixed continuously until cold (F-) or mixing stopped after initial amalgamation of phases at 70°C (F+). It showed critical effect on the final appearance of the formulation (Bonferroni value 10.5) (Figure 4). In addition, its interaction with stearic acid concentration (DF) also showed a significant (negative) effect on the overall results.

The equation 1 quantifies the overall influence of all factors and the interactions between the factors on the appearance and stability of the formulations.

$$\text{Semisolid creams} = 4.28 + 0.21A + 0.09B + 0.71C - 0.34D + 0.89E + 0.61F + 0.65AD + 0.39BC - 0.17BE \quad (\text{Equation 1})$$

Order of effect of factors and interactions between the factors was as follows:

$$E > C > AD > F > BC > D > A > BE > B$$

The interactions between various factors for each formulation provided significant amount of data and it is not possible to show all data here therefore typical interaction plots showing effect of various factors as a function of appearance and stability of the formulations are shown in Figures 5 and 6.

Figures 5 and 6 show the influence of factor interactions on the appearance of formulations. The investigation of the interactions of various factors showed that when palm-olein was used as an oil phase (A-) with slow cooling (E+) and high stearic acid concentration (D+), changing cooling mode (F- or F+) have insignificant effect on the appearance of formulations (Figure 5) as it produced semisolids in each case. However, liquid paraffin (A+) with same variables showed significant contribution of the change in the type of mixing upon cooling from (F-) to (F+) producing non-synergetic stable semisolids and synergetic structured fluids respectively (Figure 5).

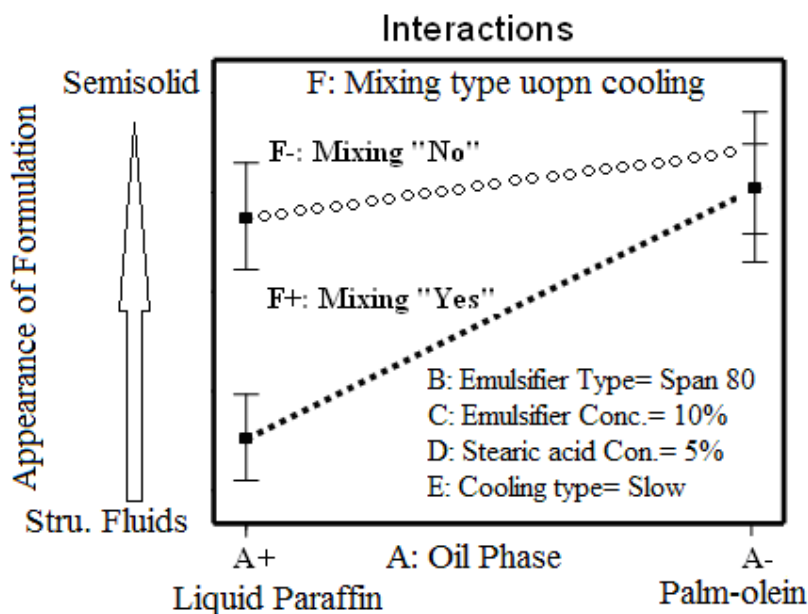


Figure 5: Influence of mode of mixing upon cooling and its interaction with other factors on the physicochemical properties of formulations.

Changes in emulsifier type showed significant effect on the physicochemical properties of the palm-olein formulations. In contrast, the liquid paraffin formulations showed no change in the appearance. When Span 80 (B⁻) was used as an emulsifier both oils produced semisolid creams. However, with Tween 80 (B⁺), the palm-olein produced structured fluids (Figure 6). DoE suggested following combination of formulation-aids and processing variables to achieve the desired output *i.e.* non-synergetic semisolid creams:

Oil: Palm-olein; Emulsifier: Tween 80; Emulsifier concentration: 10%; Stearic acid concentration: 5%; Mode of cooling: slow; Mixing upon cooling: yes.

Confirmatory experiments using the above formulation-aids and the processing variables produced non-synergetic semisolids of similar microscopical, thermal and rheological properties.

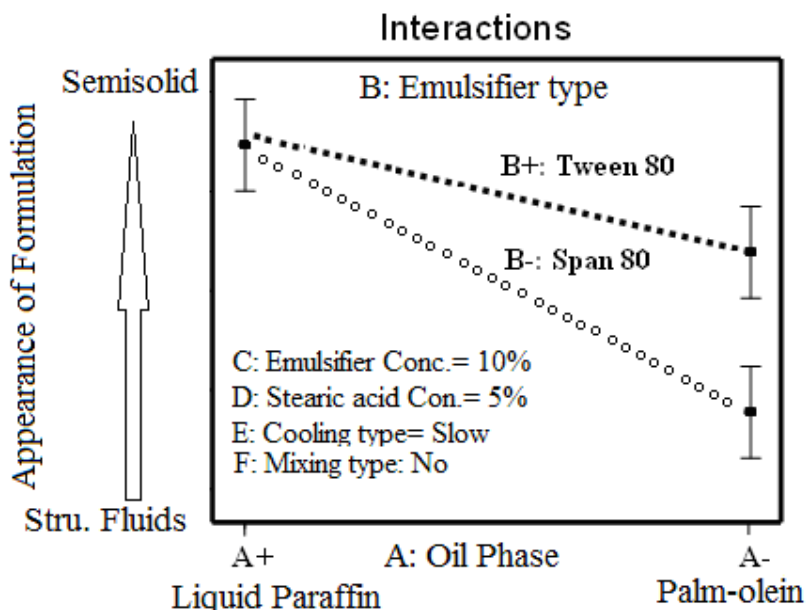


Figure 6: Influence of emulsifier type and its interaction with other factors on the physicochemical properties of formulations.

4. DISCUSSION

Formulations containing either liquid paraffin or palm-olein as an oil phase were prepared by mixing the oil and water phases at 70°C using Span 80 or Tween 80 as emulsifiers and stearic acid as stabiliser. The systems were cooled either slowly or fast with or without continuous mixing. The influence of the variables was investigated on the physicochemical properties of the systems using optimisation technique Quality by Design.

The systems obtained were either stable (non-synergetic) semisolid creams, synergetic semisolid creams or mobile structured fluids showing very different microscopic, rheological, thermal and polymorphic properties.

Rheological properties of topical drug delivery systems such as semisolid or mobile nature are associated with the presence of three-dimensional α -crystalline lamellar gel network phase (Eccleston *et al.*, 2000; Sheikh *et al.*, 2010). The literature is vague since the α -crystalline lamellar phase and the lamellar liquid crystals could not be discriminated. Stability of colloidal dispersions has been associated with the formation of mesomorphic lamellar structures such as micelles, vesicles, liquid crystals, hexagonal or nanoparticle (Rao *et al.*, 1992; Kriwet and Mueller-Goymann, 1993; Mueller-Goymann, 2004). Various liquid crystalline phases (hexagonal, cubic and lamellar) that are produced in concentrated surfactant solutions are known. However, lamellar liquid crystals are fundamentally different that they do not swell significantly and convert to micelles instead forming liquids. The lamellar liquid crystals may simply extend in the aqueous phase producing only two-dimensional systems showing little swelling and entrap the oil droplets forming liquid emulsions (Tadros and Vincent, 1983; Muller-Goymann, 2004; Tadros *et al.*, 2005).

The present work describes existence of α -crystalline lamellar structures in the stable semisolid creams. This view was supported by the polarised microscopy where stable semisolids showed existence of “Maltese crosses” between cross-polars (Figure 1a), which are indicative of α -crystalline lamellar phases Eccleston, 1986; Eccleston *et al.*, 2000). In contrast, unstable semisolids and structured fluids did not show “Maltese crosses”, but rather clusters of crystals, suggesting absence of α -crystalline lamellar structures. The unstable semisolid contained both plate-like crystals (Figure 1b) and lamellar structures (not seen); the latter only disappeared on stirring producing liquids. Various researchers have reported significant effect of process variables such as stirring, temperature and solvent on the appearance and stability of the aqueous formulations containing stearic acid (Garti *et al.*, 1980; Timmins *et al.*, 1990; Lin *et al.*, 1994; Eccleston, 1997). Eccleston (1997) showed that aqueous stearate creams are markedly affected by the mode of mixing and described the effect of stress on the swollen crystalline structures. It was shown that in some systems, the swollen lamellar structures appeared to be metastable and changed to non-swollen structures under pressure showing plate-like crystals and attributed these differences to the marked polymorphism in the stearate creams. Therefore, pressure sensitivity of synergetic semisolid systems can be attributed to the plate-like crystals. The presence of plate-like crystals is associated with the amorphous state of the stearic acid, confirmed by the XRD data (Figure 3).

This view was further supported by the present DSC data as all stable semisolid formulations showed similar thermal properties suggesting similar microstructures (Table 3). The high temperature endotherm (55°C) in stable semisolid systems was related to the melting of “Maltese crosses”, which was confirmed by hot stage microscopy, confirming the existence of α -crystalline lamellar structure. In contrast, unstable semisolids or liquids did not show this endotherm (55°C), which established lack of α -crystalline lamellar structures in these systems. In addition, pressure sensitive semisolids showed broad endotherm peaking at 42°C, which was related to the melting of plate-like crystals as confirmed by hot stage microscopy, confirming weakening of α -crystalline lamellar structure.

Viscosity values measure the ability of any system to resist the structural breakdown during a shearing process (Eccleston, 1977; Realdon *et al.*, 2001; Ribeiro *et al.*, 2004; Tadros, 2004). Furthermore, viscosity determinations provide information about the structural organisation of the formulations (Mueller-Goymann, 2004). There is an increased organisation of the α -crystalline lamellar structure with an increase in the viscosity (Tadros, 2004). It can be postulated that an increase in viscosity causes structural organisation mainly due to formation of α -crystalline lamellar structure. This postulation was confirmed by the rheology data as the stable semisolid systems showed significantly higher apparent viscosities than the synergetic semisolid or liquid systems (Table 3).

The XRD data showed that stearic acid is present either mainly in amorphous or crystalline state. The broad peaks in the XRD spectra are generally related to the amorphous state and sharp peaks to the crystalline state (Gunstone, 1967). The stable semisolid creams showed existence of two sharp peaks at $2\theta=24-30^\circ$ in addition to a broad peak at $2\theta=20^\circ$, suggesting that stearic acid is present essentially in the mixture of crystalline and amorphous states producing α -crystalline lamellar structures giving rheological strength to the systems. In contrast, the synergetic semisolid nature of unstable systems can be attributed to the broad peak at $2\theta=20^\circ$, suggesting existence of stearic acid mainly in the amorphous state. The

structured fluids on the other hand showed three sharp peaks in the same region suggesting the presence of essentially crystalline material. Amorphous materials are easy to disintegrate and penetration of the vehicle is rather easier due to lack of any ordered structure compared to the mixture of amorphous and crystalline materials (Garti *et al.*, 1982; Leinonen *et al.*, 1992). In the present study the synergetic semisolids were brittle, fragile and crumbled to touch. Due to lack of structural organisation, lamellar structure was not fully developed and the systems showed syneresis as a consequence.

In the present study, the effect of six (6) factors (Table 1) was investigated on the properties of formulations. In this context, using OFAT approach, 64 experiments are needed to be conducted to obtain a complete set of data for the investigation of the effect of 6 factors ($2K^6$). However, with the use of DoE, the same information was obtained with just 22 experiments. This observation was in agreement to the finding of Nielloud *et al.*, (2003) who also reported significant reduction in number of experiments with the use of quality by design approach in the formulation of submicron emulsions. In addition, a set of parameters and their combination were predicted to optimise the formulation, the objective of the present study. The DoE revealed that concentration of emulsifier, type of cooling and the type of mixing upon cooling were the most critical factors for the overall appearance and stability of the formulations. Although, other factors also contributed in obtaining the optimised formulations but statistically they appeared to be non-critical.

The type of cooling had a significant effect on the formulation properties. Slow cooling produced stable systems whereas fast cooling resulted in syneresis, a characteristics of unstable systems. The continued mixing upon cooling adversely (negatively) effected the stability of formulations of liquid paraffin when stearic acid concentration was at maximum-(AD) and (DF) interactions (Figure 5) producing synergetic semisolids. This observation is believed to be due to the polymorphic modification in stearic acid from crystalline to amorphous state upon continued mixing during cooling as confirmed by the XRD data (Figure 3) and is attributed to the pressure sensitivity of the stearic acid (Sheikh *et al.*, 2010). However, systems were stable semisolids when mixing was discontinued immediately after initial mixing of two phases at 70°C. In contrast, palm-olein formulations were not affected by the change in mixing type, suggesting that the α -crystalline lamellar structures in these systems were essentially protected by the palm-olein resisting change in the crystal state of the stearic acid upon stirring.

The emulsifier type also showed a significant effect on the appearance of systems. The palm-olein produced stable semisolids only in the presence of Tween 80 as an emulsifier (AB interaction). Structured fluids were obtained with the Span 80. In contrast, liquid paraffin formulations had indifferent effect of the type of emulsifier as all formulations were stable semisolids (Figure 4).

Therefore, DoE by investigating influence of various factors and their interactions provided various combinations to obtain optimised stable semisolid formulations using both oils separately and in combination, proving to be a cost effective methodology.

5. CONCLUSIONS

Quality by design “QbD” optimisation technique was used to formulate palm-olein based semisolid systems. It was possible to investigate the critical factors and their interactions on

overall outcome *i.e.* physicochemical properties of the semisolid systems, in a significantly fewer number of experiments (22) compared to OFAT approach (66).

The formation of stable semisolid systems with both oils depends on the type and concentration of emulsifier used and preparation technique. Generally, slow cooling and discontinued mixing upon cooling produced stable semisolids. Fast cooling and continued mixing upon cooling containing stearic acid (5%) produced unstable pressure sensitive semisolids showing syneresis of oil in liquid paraffin systems. In contrast, low amount of emulsifier (1%) generally produced fluids.

The stable semisolid systems showed presence of “Maltese crosses”, suggesting existence of α -crystalline lamellar structures. The unstable semisolids and fluids showed to lack α -crystalline lamellar structures and the unstable semisolids showed presence of plate-like crystals, implying pressure sensitivity.

Stearic acid was essentially in the mixture of crystalline and amorphous state in stable semisolid systems producing α -crystalline lamellar phases. In contrast, it was mainly in the amorphous and crystalline states in unstable semisolids and structured fluids respectively.

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