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Formulation and Optimisation of Cream Loaded by Kojic Acid Dipalmitate Using Design-Expert® Software

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ABSTRACT

The cosmetic industry has proved to be one of the most potential economic sources. Whitening cream is one of the decorative cosmetic products that has a unique market all over the world. The quality of creams depends on the ingredients used in the formulation. The final characteristics of the cream formulation depend on the ratio of the oil phase, emulsifier/coemulsifier and water, which are considered the main variables. The Statistical Mixture Design has been proven to be a powerful tool for determining the relationship between all the variables in the formulation. This study used a ternary phase diagram with contour graphics to assess the effect of variable changes. Kojic acid dipalmitate (KDP), Virgin Coconut Oil (VCO) and Emulium Kappa® (EK) were used to prepare a whitening cream formulation. The systems were designed using the Scheffé model. The effects of the components on the apparent viscosity, yield value, and spreadability (the main criteria) were assessed. Ten formulations were prepared using Design-Expert® software. The experimental error was reduced by repeating four formulations that critically affected the formulation properties. The final formulation's apparent viscosity, yield value and spreadability were determined based on the properties of two commercial whitening creams. The results indicate that the Mixture Design, with the aid of Design-Expert® software, can be used successfully and efficiently to optimise cream formulation composed of Kojic acid dipalmitate (KDP), Virgin Coconut Oil (VCO) and Emulium Kappa® (EK).

Keywords: Ternary phase diagram, Design-Expert® software, Kojic Acid Dipalmitate, Scheffé model.

I. INTRODUCTION

The Design of Experiment (DOE) or Statistical Experimental Design (SED) is a concept for the planning of informatics experiments which can be used in many formulations [1]. In pharmaceutical technology, DOE is recommended greatly [2]. DOE requires prior knowledge of the procedure used so that a robust and valid statistical model for the examined factors can be achieved [3] with a minimum number of experiments, i.e. minimum time, resources, and effort [4-6]. For stable and effective dosage forms development, careful selections of integral components are essential, which can be achieved through pre-formulation studies [7]. In cosmetic cream formulations, DOE plays a vital role in product development because it is not easy to predict the optimum values of the formulation properties, such as spreadability and viscosity [8].

Screening is usually carried out at the first stage to reduce the number of factors and to determine the important outcomes under which both squared and interaction terms in the model are of interest. In contrast, optimisation is mostly carried out after the screening. Thus, the best setting for the important variables is determined. These two elements (i.e., screening and optimisation) are considered the main elements of the DOE concept [1, 9-11]. Because of the involvement of multivariable process parameters, the optimisation process is considered a tedious process [10]. Furthermore, the optimisation process involves three major steps: (i) performing the

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statistical design experiments, (ii) estimating the coefficient in a mathematical model, and (iii) predicting the response and checking the adequacy of the model [3]. There are several techniques of DOE used for formulation development, such as Cross Technique, Factorial Design, and Mixture Design [6]. Factorial design is the most popular experimental design used to study systems having independent factors [2, 4, 6] and to determine the relationship between two or more components [8]. In the mixture components, the ratios of the components are dependent on one another, where the sum has to be equal to 1 or 100% [2, 6, 7, 9]. A mathematical model has to be used, too. Furthermore, Mixture Design has been used to explore how much change in mixture composition will affect the properties of the mixture [1, 2, 7], and it has been adopted to optimise the composition of the systems to describe the response as a function of the mixture composition utilising a mathematical model [2, 6, 9, 12, 13]. For a three-component system, Mixture Design can be represented by an equilateral triangle of two-dimension space [4, 12, 13]. The relationship between the formulation variables was investigated effectively by Statistical Mixture Design [6].

It is important to obtain knowledge about potential physical and chemical interaction between mixture components to accelerate drug development rapidly [7], and this can be achieved by using Response Surface Methodology (RSM) [1, 10] to visualise and select optimal conditions immediately [1, 9-11]. In the 1950s, Response Surface Methodology (RSM) was developed by Box and Wilson [14]. Response surface contour can be depicted only after discovering an acceptable statistical model function [2]. Response surface plots clearly show the influence of two factors on recovery value in the investigated area and are presented in three-dimensional spaces [11]. Examining these three-dimensional graphs may help to determine a region with acceptable values of responses [2] because RSM combines experimental design and statistical techniques for model optimisation and building [14]. Moreover, the linear or quadratic effect of the experimental variables and the response can be mapped onto the surface contour plot [10, 14].

In the pharmaceutical industry, computer software can be used with DOE [1, 15]. In reality, this software can analyse multi-responses simultaneously, efficiently, and very accurately [14]. An example of such software is Design-Expert® software, which has been described by many authors [3, 5, 6]. Therefore, Design-Expert® software was used in this study.

Finally, it is important to point out that most of the three components of the design mixture employ a ternary phase diagram in the pre-formulation study. This ternary phase diagram was described by many researchers [13, 16-20]. A phase diagram can capture the relationship between a mixture of phase behaviour and its composition. Constructing a ternary phase diagram is usually time-consuming, particularly when delineating a phase boundary. The pseudo-ternary phase diagram is useful for the identification of the region of interest, e.g. o/w emulsion region [18].

Kojic acid (5-hydroxy-2-(hydroxymethyl)-4-pyrone) is a skin-whitening agent and a natural product [21]. Kojic acid has antibacterial action and inhibits the tyrosinase enzyme. Mushrooms and food fermentation by bacteria and fungi such as *Penicillium* and *Aspergillus* are considered the source of kojic acid [22-25]. The storage properties and the inhibitory effect in cosmetics are non-stable due to heat sensitivity, which could deteriorate faster by the effect of heat and light [26]. As a result, semisynthetic derivatives of kojic acid have been synthesised [22]. The modification was carried out at the C-7 hydroxyl group to produce hydroxyphenyl ether or ester or form peptide derivatives or glycosides [22]. Dipalmatic ester was added to kojic acid to be used in cosmetics as kojic acid dipalmitate. This ester is hydrolysed in the skin by the effect of esterase to produce kojic acid [26].

In this study, Mixture Design was used to optimise the composition of a three-component system composed of Kojic acid dipalmitate (KDP), Virgin Coconut Oil (VCO) and Emulium Kappa® (EK). The main objective of this research is to quickly and efficiently investigate the response over the entire factor space. Another objective is to locate the region of interest where the response has reached its optimum or near optimum value. The selected responses in this study were viscosity, yield value, and spreadability. The Response Surface Methodology was employed to determine the best visual combination.

II. METHODS AND MATERIAL

Kojic acid dipalmitate (KDP) (the whitening agent) was purchased from Beijing Brilliance Biochemical Co., Ltd. (Hou Modern, China). Emulium Kappa® (EK) was purchased from Gattefossé, France. Propylene glycol (PG) was purchased from Sigma. Virgin coconut oil

(VCO) was purchased from Adirondack Co., Ltd (Selangor, Malaysia) and was filtered through 0.45 µm methyl cellulose filter paper before use. Methyl paraben and propyl paraben (the preservatives) were purchased from Sigma. The commercial references used in the current study, NIVEA® whitening cream (NIVEA, Malaysia) and Hazeline® white and Natural Lightening Cream (Unilever, Malaysia) were purchased from Tesco store (Penang, Malaysia).

Cream preparation

Creams were prepared on a laboratory scale (batch size 10 g). EK was solubilised in the oil phase at 65 °C, and PG was solubilised in the aqueous phase and was brought to the same temperature. An oil phase was then added to the aqueous phase at a temperature of 60 °C and mixed at a speed of 2000 rpm for 10 minutes. During the cooling phase, the cream was continuously stirred (290 rpm) with two homogenisation steps of 3 minutes each (Ultra-Turrax, TP 18/10, Ika-Werk, Janke and Kunkle, Staufen, Germany) until the temperature of the cream dropped to 30 °C. Mixing should be done at a low speed because a higher speed will hinder the solidifying of the waxes. All batches were prepared by using the same manufacturing procedure.

Construction of ternary phase diagram

The ternary phase diagram was constructed to investigate the relationship between the phase behaviour of a mixture and its composition. Each of the three apexes of the triangle represents 100% of the weight of one component. Each line joining the two apexes (at the periphery) represents two-component mixtures. The area inside the triangle represents all the possible combinations to give a system of three components. The three components were EK/PG (surfactant/cosurfactant mixture), the VCO (oil phase), and deionised water (aqueous phase). A straight line from the middle (50%) of each line to the opposite apexes was drawn, and nine points on each line were formulated (total point=27) to do a screening for the ternary diagram [27]. It is hard to think that each of the three particular components will be allowed to vary from 0% to 100%; at the same time, it is tedious and pointless to try and study all the possible combinations of the proportions of the components when it is already known that many of these are probably out of interest or are excluded [28].

The resulting formulations were analysed using a light microscope (Nikon, USA) to determine their types (o/w or w/o). A drop of the emulsion was placed on a slide and mixed with a drop of methylene blue (a water-soluble dye) using a spatula. The slide was then covered with a cover slide before testing under the microscope. From these 27 points, only o/w emulsion points were selected for further scanning, whereas other points were neglected. Further scanning was performed to determine the precise border of the area of interest. Thus, the area with the required points was scanned by formulating other points surrounding the points of interest and the border points that had been connected to construct the final area.

Mixture Design

The factors that affect the combination were counted. The software selected the design according to several factors, which fitted the Scheffé model. The variables (EK/PG, VCO and water) were the factors. The parameters were the temperature, the time (period of mixing), and the stirring speed. The parameters used in the current study were those recommended by the manufacturer of EK (temperature=65°C, time=10 min, mixing speed=2000 rpm). Moreover, the active ingredient and the preservatives were also excluded. Therefore, only three variables were considered as the factors and were termed as the components in the design. The lower and upper limits should be determined carefully for each single factor.

The upper limit of the emulsifier/co-emulsifier system could be determined only after determining the area of interest. In contrast, the lower limit was determined according to the manufacturer's (Gattefossé) specifications. Practically, zero percent was not used because it would abandon one of the three components of the combination; therefore, a higher per cent would be used. A percentage between 4-6% (w/w) was suggested by the manufacturer but with the use of a polymer. Since polymer was not used in the formulation in this study, 6% would be selected as the lower limit for using EK.

Subsequently, the lower and higher limits for the oil phase were 10 and 30, respectively. A percentage lower than 10% of the oil phase would not produce a cream formulation. A percentage higher than 30% of the oil phase would obtain a high-greasiness preparation, which is not recommended in cosmetic formulations. This was why the oil phase was selected beyond the lower and upper limits.

Optimization using Design-Expert® software

The model formulation was treated by Design-Expert® software (version 6.0.10, State-Easy Inc., Minneapolis, USA). The study type was a mixture study. The design model used was the Scheffé model. The mixture order was linear and quadratic. The initial design was D-optimal. The selected portion was termed Domain. The experimental responses, apparent viscosity (Y_1), yield value (Y_2), and spreadability (Y_3), were recorded and fed to the software.

The criteria for optimisation were taken from the reference creams (NIVEA® and Hazeline®). From these criteria, the minimum and the maximum limits for each of the experimental responses were selected. The results were fed into the software to select the best combination solution. Subsequently, Design-Expert® software had analysed the variance of the experiment and determined the errors. The Mixture design mostly follows first order canonical equation:

$$Y = \alpha_0 + \alpha_1 X_1 + \alpha_2 X_2 + \alpha_3 X_3 + \varepsilon \quad \text{Eq. (1)}$$

Because the sum of all components is in unity, $\sum X_j=1$, α_0 could be replaced by $\alpha_0(X_1+X_2+X_3)$:

$$Y = (\alpha_0 + \alpha_1) X_1 + (\alpha_0 + \alpha_2) X_2 + (\alpha_0 + \alpha_3) X_3 + \varepsilon$$

Given $\beta_j = \alpha_0 + \alpha_j$, equation 1 can be written as follows

$$Y = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \varepsilon \quad \text{Eq. (2)}$$

Y_j is the predicted response

β_j is the estimated coefficient for factor X_j

X_1 is the surfactant/cosurfactant concentration (%), with $6 < X_1 < 18$

X_2 is the VCO concentration (%), with $10 < X_2 < 30$

X_3 is the water concentration (%), with $52 < X_3 < 84$

The main effects (X_1, X_2, and X_3) represent the average results of changing one factor at a time from its low to high value, while the interactions (X_1X_2, X_1X_3, and X_2X_3) represent how the response changes when two factors are changed simultaneously [1, 13, 14].

The point in the factor space that might be defined in terms of the constituent of the mixture, or the components (X_1, X_2, and X_3) can be defined in the same way, in terms of the pseudo components (X'_1, X'_2, and X'_3). They can be defined as responses in terms of the L-transformation pseudo component.

$$Y = \beta'_1 X'_1 + \beta'_2 X'_2 + \beta'_3 X'_3 + \varepsilon \quad \text{Eq. (3)}$$

The second order or quadratic equation can be derived by using the above argument:

$$Y = \beta'_1 X'_1 + \beta'_2 X'_2 + \beta'_3 X'_3 + \beta'_{12} X'_1X'_2 + \beta'_{13} X'_1X'_3 + \beta'_{23} X'_2X'_3 + \varepsilon, \quad \text{Eq. (4)}$$

There are no squared terms, βjjXj2, in the canonical equation because X1 (1- X2 - X3) can replace Xj2 and so on, and the resulting expression can then be included in the first-order and rectangular term.

Apparent viscosity (Y_1)

The viscosity follows the first-order equation according to the software analysis. Therefore, the equation that fits this response is:

$$Y_1 = \beta'_1 X'_1 + \beta'_2 X'_2 + \beta'_3 X'_3 + \varepsilon \quad \text{Eq. (5)}$$

A multistep flow curve measurement was run using a controlled–stress rheometer (StressTech, ReoLogica Instruments AB, Lund, Sweden, STRESS RHEOLOGIC Basic software, version 3.6) with a cone and plate sensor system of 40 mm diameter on smooth surfaces. The stress increased from 0.1 to 100 Pa at 50 linear steps to obtain the ascending curve.

Yield value (Y_2)

Yield value fits the second-order equation. The equation that fits this response is:

$$Y_2 = \beta'_1 X'_1 + \beta'_2 X'_2 + \beta'_3 X'_3 + \beta'_{12} X'_1X'_2 + \beta'_{13} X'_1X'_3 + \beta'_{23} X'_2X'_3 + \varepsilon \quad \text{Eq. (6)}$$

The yield value measures the lowest shear stress needed to break the structure and start the flow. This study measured it with the same equipment as described in section 2.2.4.1. The stress was increased from 0.1-100 Pa at 50 linear steps. The stress that caused the cream to flow and the cone to move would represent the yield value.

Statistical analysis

Analysis of variance (ANOVA) was used to compare the results of these experiments. The significance of the interactions between the factors could be determined by using ANOVA. The t-test, using a 95% ($\propto$=0.05) significance level, was performed to test the statistical significance of the regression model. The overall regression relationship between the response and the entire set of variables was determined using the F-test at a 95% ($\propto$=0.05) significance level. Finally, the regression model validity was assessed using lack-of-fit tests and statistical assumptions. Design-Expert® and SPSS software for Windows were used to perform the statistical analysis. All models of optimisations were solved and plotted by using Design-Expert® software.

III. RESULTS AND DISCUSSION

Ternary phase diagram

The formulation of o/w cream was based on constructing the ternary phase diagram, as explained in section 2.2.2. Fig 1a shows the resulting points from the screening process. Thus, the A-points represent o/w cream, the B-points represent w/o cream, and the C-points represent the gel. Then, scanning was performed for the points forming o/w emulsion (i.e., A-points) to precisely determine the area's border. Next, the border points were connected, and the final area of interest (o/w emulsion) was constructed, as shown in Fig 1.

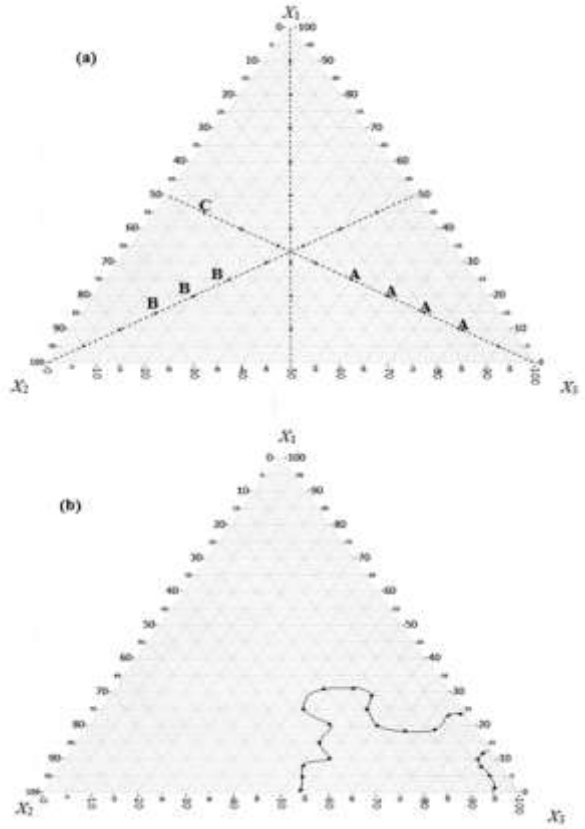

Fig 1: Ternary phase diagram: (a) Screening A-point=o/w emulsion, B-point= w/o emulsion, and C-point= gel; (b) Area of interest (o/w emulsion). X_1= EK/PG; X_2 = VCO; X_3= water.

Within the area of interest, a pseudo-ternary phase diagram (L-transformation) was constructed based on the upper and lower limits of the components (Table 1).

In general, where the mixture composition was optimised, the experimental range lay between 0 and 97.79% since creams were prepared with constant drug and preservative contents of 2 and 0.21%, respectively (Table 1). The upper range for the emulsifier/coemulsifier system is 18%, as shown in Fig 2.

TABLE I: FACTORS INCLUDED IN THE FORMULATION.

Factor (Component in mixture)	Associated variable	Lower limit
EK:PG (9:1) (%)	X1	6
VCO (%)	X2	10
Water (%)	X3	q.s. 97.79%
KDP (%)	Fixed	2%
Preservatives (%)	Fixed	0.21%
Heat (°C)	Fixed	65
Time (minute)	Fixed	10
Mixing rate (rpm)	Fixed	2000

A parallelogram shape (Domain) was prepared for cream optimisation using Design-Expert® software in this area. This parallelogram can form a triangle, and the apexes of this triangle are termed pseudo components (Fig 2).

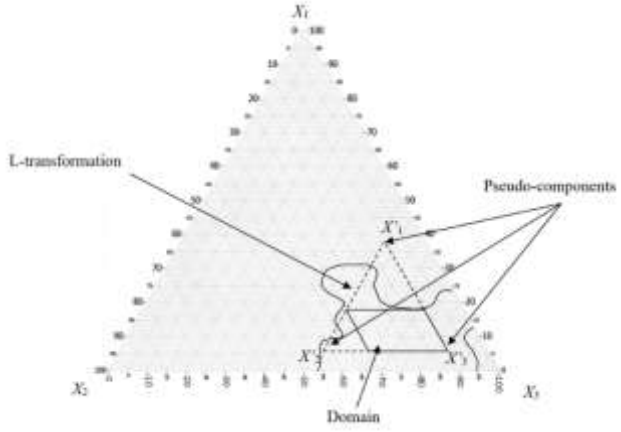

Fig 2: Factor and design area for formulation. X_1=X'_1= EK/PG; X_2=X'_2= VCO; X_3=X'_3= deionized water.

Optimization using Design-Expert® software Since the area of interest still consisted of hundreds of possible combinations, Design-Expert® was used to help minimise the cost and to save the time required to reach the best combinations.

Mathematically, the expression for the Scheffé model, in terms of pseudo components, is equivalent to the model expressed in the components. The Domain was studied using 10 points; thus, it was possible to observe the effect of the emulsifier percentage and VCO percentage on the apparent viscosity, yield value, and spread ability, as shown in Fig 2. One possible weakness was that the centre points were considered for each component. Any error in the experiment would lead to serious problems; therefore, the centre point was replicated (e.g., Run 1). On the other hand, the data points of pure diluents were less useful than those of the mixtures.

The Design-Expert® software optimised the formulation according to the criteria previously decided upon. The criterion was obtained from a list of reference items. These references should have the required properties like apparent viscosity, yield value, and spread ability. Two reference creams were selected to determine the lower and the upper criteria under which the formulation had been optimised. These creams were lightening creams, and they were o/w base cream. These were NIVEA® and Hazeline® cream. Apparent viscosity, yield value, and spread ability for these reference creams were measured and tabulated, as shown in Table 2. Next, the software fed the results to select the best combination solution.

TABLE II CRITERIA OF THE REFERENCE CREAMS

The criteria	NIVEA® cream	Hazeline® cream
Spreadability (cm2 g-1/2)	0.294	0.296
Viscosity (Pa.s)	4.6	3.5
Yield value (Pa.)	47.5	3.5

Diagnostic plots of residual effects

The particular models that illustrated a specific number of effects could be concluded from the ANOVA Tables (Table 5-7) for each of the three responses. The distribution of the residual values should also be examined [4]These distributions represent the differences between predicted and observed values. The normal probability plot of the residuals for each response is presented in Fig 5 separately. The normal probability was assessed by examining how close the observed values were to the theoretical ones. Since all fall on a straight line, as shown in Fig 3, it can be concluded that they follow a normal distribution.

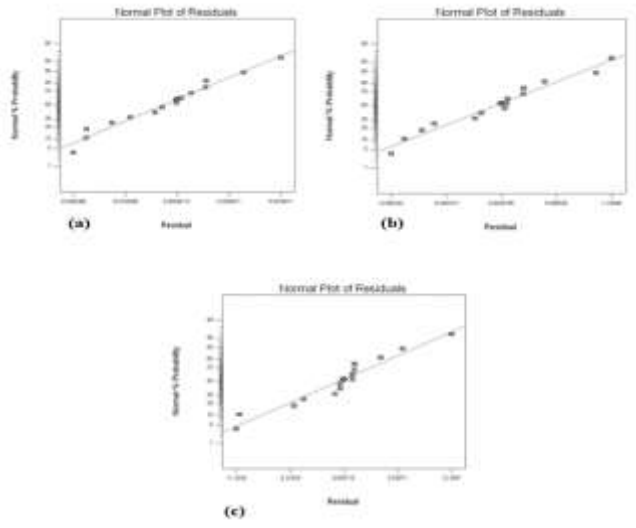

Fig 3: Normal probability plots of residuals for (a) apparent viscosity, (b) yield value, and (c) spread ability.

Furthermore, Fig 4 shows the distributions of the predicted values versus the actual values listed in Table 8, which shows how close they are. Since they all fall on a straight line, it could be concluded that there were no significant differences between them.

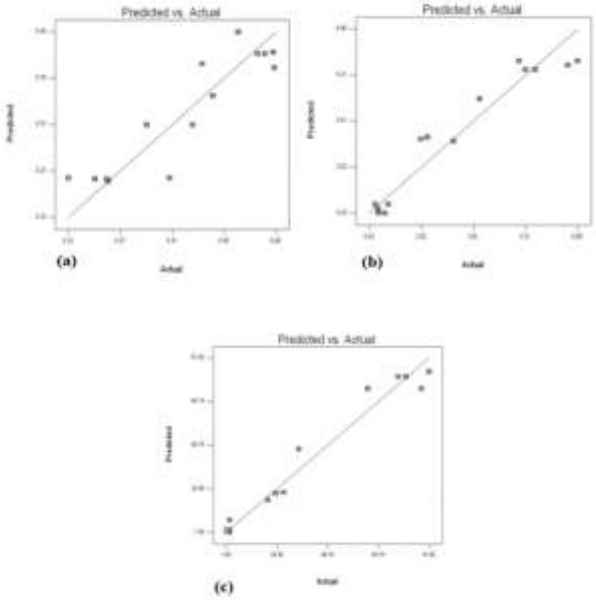

Fig 4: Predicted versus actual values for (a) apparent viscosity, (b) yield value, and (c) spread ability.

Assessing factor effects with the trace plot

The effect of each factor could be assessed graphically using the trace plot (Fig 5). When EK/PG was increased, the apparent viscosity increased, too, which means that this factor has a significant effect on apparent viscosity. In contrast, VCO and water decreased the apparent viscosity, as shown in Fig 5a. Similarly, yield value response follows similar behaviour as viscosity but with a curve since it follows a quadratic equation model, as shown in Fig 5b. Furthermore, an interesting behaviour for spread ability was observed. The spread ability decreased dramatically with the increased emulsifier concentration. However, the spread ability also increased with the increase in VCO and water content to a specific limit, after which, when it was near zero reference blends, spread ability started to decrease again in a curve shape since it follows a quadratic equation, as shown in Fig 5c. The deviation from a reference blend for the spread ability is shown in Fig 5d, which presents the distance of the optimum combination from the variables [3, 10].

Fortunately, one solution was proposed by the Design-Expert® software, which is the optimum combination with the required properties (Table 5 and Fig 8). The suggested optimum combination was formulated (n=3)

and studied, and the results were compared with the predicted values given by the Design-Expert® software using a student t-test. Apparent viscosity and spread ability showed no significant differences between the predicted and the experimental results (p= 0.604 and 0.127, respectively). On the other hand, there was a substantial difference between the expected and experimental yield values, which was p= 0.017, which might be due to the antagonistic effect of the ingredient on the yield value.

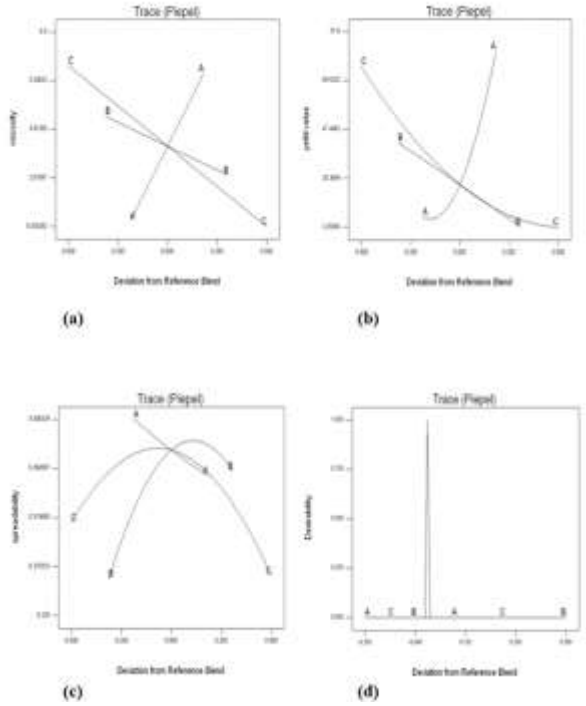

Fig 5: The contour traces for (a) apparent viscosity, (b) yield value, (c) spreadability, and (d) desirability. A is Emulium Kappa: Propylene glycol (9:1); B is VCO; C is De-ionized water.

Overall, HLB value is an essential criterion for selecting a surfactant/cosurfactant system. To form an o/w emulsion, the HLB value should be greater than 10. For this reason, EK and PG should have HLB values of 11 and 4.3, respectively, combined at a ratio of 9:1 to give a more stable o/w emulsion with an HLB value of 10.35. PG decreases the bending stress of the interface, which increases the flexibility of the interfacial film [18].

A high content of VCO may result in high greasiness. Therefore, this study chose 30% of the VCO content as the highest. Furthermore, no cream could be formed at a lower VCO percentage (< 10%); thus, 10% was considered the lowest level of VCO. On the other hand, the upper level of EK/PG (18%) was selected because it was within the upper internal margins of the interested area, as shown in Fig 1 and 2. The pseudo-ternary phase diagram (L-transformation), a set of possible experiments known as candidate points, covered the experimental domain and represented a good design for each model. The viscosity, yield value, and spreadability were taken as responses. L-transformation was used to precede the study because no additional experiments are needed to fit a model of a higher degree, which makes this transformation advantageous over an entire factor space. Furthermore, more calculus would provide better results [2]. The minimum and maximum values of the components of the mixture system are shown in Table 1, while Table 2 shows the experimental value for fourteen runs.

Based on acceptance criteria for apparent viscosity, the yield value and spreadability were selected from two reference commercialised creams, NIVEA® and Hazeline® (Table 3). The selected promising formulation was produced and analysed [1, 12]. The better fit was received for the first-order or second-order mathematical model by using Design-Expert software [5]. Eventually, the interactions between the examined factors and the main effects were concluded. Thus, the complicated interactions were clarified through graphical presentations [4].

The three-dimensional response surface plots for apparent viscosity, yield value, and spread ability, as a function of mixture composition, were constructed using Design-Expert® software to quickly choose an actual optimum condition and better visualisation, as shown in Fig 3 [11]. Furthermore, the interactions and responses of the variables could be noticed. Moreover, such interactions that were of statistical significance should not be neglected. The emulsifier system (EK/PG concentration) seemed to be the most critical factor influencing all responses, and this finding corresponds to the previous findings [33]. However, o/w emulsion could be formed only in the presence of an emulsifier. Increasing EK/PG concentration seems to increase the apparent viscosity and yield value, while spread ability decreases with the increase in EK/PG concentration. Fig 3b shows no significant curvature in the yield value response when the EK/PG changed approximately 15%.

In contrast, Fig 3c shows an exciting and significant curvature when the emulsifier concentration was compared, thus indicating that these factors significantly influence the resultant response. This study shows that VCO and water concentration have a critical effect on the yield value and spread ability but little impact on the

viscosity compared to EK/PG concentration. This study also shows that the yield value continuously decreases with the increased VCO and water concentration. It can be concluded that the yield value reaches a minimum point when the water reaches a maximum, as shown in this model. Spreadability seems to show biphasic behaviour. This study increased in the first phase with the rising VCO and water concentration. Still, after a limited concentration, it decreased, representing the second phase. This behaviour could be attributed to the internal structure of the cream.

The accuracy and usefulness of this statistical model could be proved by conducting a verification test under the optimised experimental condition. To simplify the computations, the transformation of the actual concentration of EK/PG, VCO, and water-based should be made on the Scheffé model so that a minimum concentration corresponds to zero and a maximum concentration corresponds to one. This finding corresponds with the finding reported before [13].

Apparent viscosity fits the first-order model with an F value=68.08 ($P < 0.001$) (Table 4). The goodness of fit (R^2) and ANOVA represented efficient tests for the models and were applied to verify the adequacy of the regression model in terms of a lack-of-fit test. [1] (Table 5). The yield value and spread ability fit the second-order model (Tables 6 and 7), i.e., the linear model, which was insignificant with F values of 48.01 and 7.59, respectively. For viscosity, yield value, and spread ability, the alternative hypothesis, "lack-of-fit", is rejected (p-value < 0.05). The residual response sum of squares (SS) implied in this test is separated into the components of model error and pure error. Furthermore, the differences were obtained by counting an F test. One degree of freedom (DF) was included in each ANOVA main effect since the factors in this study had two levels only, and this finding corresponds with those of other reports [4]. A higher correlation coefficient (R^2=0.9252) for apparent viscosity and yield value (R^2=0.9677) indicate a good fitness, i.e., the dependant variables and the responses have a good agreement, and this finding corresponds with that as reported before [7, 12]. Although spread ability variation may not be well predicted due to the lower correlation coefficient value (R2=0.8259) compared to the apparent viscosity and yield value, it could be considered acceptable. The model's lack of fit is highly insignificant, corresponding with the other statistics, indicating that it is a good model. A good reason for the lack of fit is that the estimation of the experimental error was too big, and these results correspond with the work that had been reported before [2].

The measurement of the closeness using residual analysis of the mixture surface as predicted by the observed response values at the design points could be acceptable. It could correct the proposed response model, i.e., apparent viscosity, yield value, and spread ability. These findings correspond with those of other reports [7].

There could be undesirable results, meaning the demands are too high for this set of components or pseudo components. Therefore, another set of components must be chosen, and the experiment must be repeated to obtain the optimisation from the start. A different set of components were only permitted to be compared when the responses were compared at optimal composition. It was observed in the present study that the responses varied significantly with the variation in the three factors: EK/PG, VCO, and water. These results correspond with the work reported before [2, 3].

Contour plot graphics of the responses are shown in Fig 4. The optimised formulation could be chosen quickly by superimposing the contour plots depending on the demands. Thus, the responses over the total area of interest can easily be located, and this finding corresponds with those of other reports [2, 3, 9, 13]. Furthermore, the model equation was illustrated using the response contour diagram, which shows the effect of components on the cream characteristics and interprets the mixture area. This finding corresponds with what had been reported before [1].

The average probability plots of each response residual are presented in Fig 5, and the closeness of actual values to the theoretical distribution is assessed. The distribution is normal since all values fall on a straight line. Next, the distribution of the residual values should be examined. The differences between the predicted and actual values are listed in Table 8 and shown in Fig 6. The random noise and the actual effects could be separated efficiently using standard distribution plots. According to the outliers, each response was investigated, and it was found that all points were placed in a normal distribution. This finding corresponds with those of other reports [1, 4].

Finally, the effect of each factor was also assessed graphically using the trace plot. Fig 5 shows the trace plots for the responses. The trace plots seem to correlate with the response surface and contour plots. Thus, the effect of the factors on the apparent viscosity is linear since it follows the first-order equation. Fig 5a presents the impact of the factor variations on the apparent

viscosity. Therefore, the increase in EK/PG also increased the viscosity dramatically, while VCO and water decreased the viscosity. Concerning yield value, the initial rise in EK/PG showed no effect on the yield value, but after a 10-15% increase, the yield value increased in a significant way (Fig 5b). Finally, the exciting effects of VCO and water on spread ability were identified. Thus, spread ability increases as water and VCO increase but to a limited extent, after which the reverse effect is achieved, unlike EK/PG, which decreased the spread ability dramatically (Fig 5c).

IV. CONCLUSION

The physicochemical properties of whitening cream, demonstrated by this study, can be manipulated by varying the content concentration. Mixture experimental design and surface response methodology were used to study and optimise the influence of ingredient concentration using only a truncated set of empirical experiments. By checking the assumption, the statistical modelling of this empirical data has proved to be appropriate. Due to instrumental and mathematical analysis, the emulsifier and the VCO concentrations seem to have a crucial effect on the required demands or consumer acceptance of the cream prepared. The usefulness of the experimental design methodology in optimisation was shown clearly. Therefore, this research can be the groundwork for understanding the characteristics of the whitening cream prepared by EK/PG and VCO.

V. REFERENCES

1) Martinello, T., Kaneko, T. M., Velasco, M. V., Taqueda, M. E., & Consiglieri, V. O. (2006). Optimization of poorly compactable drug tablets manufactured by direct compression using the mixture experimental design. *International journal of pharmaceutics*, *322*(1-2), 87–95. https://doi.org/10.1016/j.ijpharm.2006.05.034.
2) Huisman, R., Van Kamp, H. V., Weyland, J. W., Doornbos, D. A., Bolhuis, G. K., & Lerk, C. F. (1984). Development and optimization of pharmaceutical formulations using a simplex lattice design. *Pharmaceutisch weekblad. Scientific edition*, *6*(5), 185–194. https://doi.org/10.1007/BF01999941.
3) Rossi, M. J., & Oliveira, V. L. (2011). Growth of the Ectomycorrhizal Fungus Pisolithus Microcarpus in different nutritional conditions. *Brazilian journal of microbiology : [publication of the Brazilian Society for Microbiology]*, *42*(2), 624–632. https://doi.org/10.1590/S1517-83822011000200027.
4) Loukas Y. L. (1998). A computer-based expert system designs and analyzes a 2(k - p) fractional factorial design for the formulation optimization of novel multicomponent liposomes. *Journal of pharmaceutical and biomedical analysis*, *17*(1), 133–140. https://doi.org/10.1016/s0731-7085(97)00173-8.
5) Petrovic, A., Cvetkoviae, N., Trajkovic, S., Ibric, S., Popadic, D., & Djuric, Z. (2006). Mixture design evaluation of drug release from matrix tablets containing carbomer and HPMC. *Journal of controlled release : official journal of the Controlled Release Society*, *116*(2), e104–e106. https://doi.org/10.1016/j.jconrel.2006.09.073.
6) He, H., Li, A., Li, S., Tang, J., Li, L., & Xiong, L. (2021). Natural components in sunscreens: Topical formulations with sun protection factor (SPF). *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie*, *134*, 111161. https://doi.org/10.1016/j.biopha.2020.111161.
7) Mura, P., Furlanetto, S., Cirri, M., Maestrelli, F., Marras, A. M., & Pinzauti, S. (2005). Optimization of glibenclamide tablet composition through the combined use of differential scanning calorimetry and D-optimal mixture experimental design. *Journal of pharmaceutical and biomedical analysis*, *37*(1), 65–71. https://doi.org/10.1016/j.jpba.2004.09.047.
8) Contreras, M. D., & Sanchez, R. (2002). Application of a factorial design to the study of the flow behavior, spreadability and transparency of a Carbopol ETD 2020 gel. Part II. *International journal of pharmaceutics*, *234*(1-2), 149–157. https://doi.org/10.1016/s0378-5173(01)00954-1.
9) Rispoli, F. J., & Shah, V. (2007). Mixture design as a first step for optimization of fermentation medium for cutinase production from Colletotrichum lindemuthianum. *Journal of industrial microbiology & biotechnology*, *34*(5), 349–355. https://doi.org/10.1007/s10295-007-0203-y.
10) Sayyad, S. A., Panda, B. P., Javed, S., & Ali, M. (2007). Optimization of nutrient parameters for lovastatin production by Monascus purpureus MTCC 369 under submerged fermentation using response surface methodology. *Applied microbiology and biotechnology*, *73*(5), 1054–1058. https://doi.org/10.1007/s00253-006-0577-1.
11) Zivanović, L., Licanski, A., Zecević, M., Jocić, B., & Kostić, M. (2008). Application of experimental design in optimization of solid phase extraction of mycophenolic acid and mycophenolic acid glucuronide from human urine and plasma and SPE-RP-HPLC method validation. *Journal of pharmaceutical and biomedical analysis*, *47*(3), 575–585. https://doi.org/10.1016/j.jpba.2008.01.046.
12) Patel, D. M., Patel, N. M., Pandya, N. N., & Jogani, P. D. (2007). Gastroretentive drug delivery system of carbamazepine: formulation optimization using simplex lattice design: a technical note. *AAPS PharmSciTech*, *8*(1), 11. https://doi.org/10.1208/pt0801011.
13) Zhu, W., Yu, A., Wang, W., Dong, R., Wu, J., & Zhai, G. (2008). Formulation design of microemulsion for dermal delivery of penciclovir. *International journal of pharmaceutics*, *360*(1-2), 184–190. https://doi.org/10.1016/j.ijpharm.2008.04.008.

14) Slyper, M., Porter, C. B. M., Ashenberg, O., Waldman, J., Drokhlyansky, E., Wakiro, I., Smillie, C., Smith-Rosario, G., Wu, J., Dionne, D., Vigneau, S., Jané-Valbuena, J., Tickle, T. L., Napolitano, S., Su, M. J., Patel, A. G., Karlstrom, A., Gritsch, S., Nomura, M., Waghray, A., … Regev, A. (2020). A single-cell and single-nucleus RNA-Seq toolbox for fresh and frozen human tumors. *Nature medicine*, *26*(5), 792–802. https://doi.org/10.1038/s41591-020-0844-1.

15) Ismail, N. S., El Dine, R. S., Hattori, M., Takahashi, K., & Ihara, M. (2008). Computer based design, synthesis and biological evaluation of novel indole derivatives as HCV NS3-4A serine protease inhibitors. *Bioorganic & medicinal chemistry*, *16*(17), 7877–7887. https://doi.org/10.1016/j.bmc.2008.07.084.

16) Wu, H., Ramachandran, C., Weiner, N. D., & Roessler, B. J. (2001). Topical transport of hydrophilic compounds using water-in-oil nanoemulsions. *International journal of pharmaceutics*, *220*(1-2), 63–75. https://doi.org/10.1016/s0378-5173(01)00671-8.

17) Kepczyński, M., Lewandowska, J., Romek, M., Zapotoczny, S., Ganachaud, F., & Nowakowska, M. (2007). Silicone nanocapsules templated inside the membranes of catanionic vesicles. *Langmuir : the ACS journal of surfaces and colloids*, *23*(13), 7314–7320. https://doi.org/10.1021/la063442i.

18) Shafiq, S., Shakeel, F., Talegaonkar, S., Ahmad, F. J., Khar, R. K., & Ali, M. (2007). Development and bioavailability assessment of ramipril nanoemulsion formulation. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V*, *66*(2), 227–243. https://doi.org/10.1016/j.ejpb.2006.10.014.

19) Shah, R. B., Zidan, A. S., Funck, T., Tawakkul, M. A., Nguyenpho, A., & Khan, M. A. (2007). Quality by design: characterization of self-nano-emulsified drug delivery systems (SNEDDs) using ultrasonic resonator technology. *International journal of pharmaceutics*, *341*(1-2), 189–194. https://doi.org/10.1016/j.ijpharm.2007.04.009.

20) Dixit, R. P., & Nagarsenker, M. S. (2008). Self-nanoemulsifying granules of ezetimibe: design, optimization and evaluation. *European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences*, *35*(3), 183–192. https://doi.org/10.1016/j.ejps.2008.06.013.

21) Zhu, X., & Li, S. (2023). Nanomaterials in tumor immunotherapy: new strategies and challenges. *Molecular cancer*, *22*(1), 94. https://doi.org/10.1186/s12943-023-01797-9.

22) Kang, S. S., Kim, H. J., Jin, C., & Lee, Y. S. (2009). Synthesis of tyrosinase inhibitory (4-oxo-4H-pyran-2-yl)acrylic acid ester derivatives. *Bioorganic & medicinal chemistry letters*, *19*(1), 188–191. https://doi.org/10.1016/j.bmcl.2008.10.119.

23) Momtaz, S., Lall, N., Hussein, A., Ostad, S. N., & Abdollahi, M. (2010). Investigation of the possible biological activities of a poisonous South African plant; Hyaenanche globosa (Euphorbiaceae). *Pharmacognosy magazine*, *6*(21), 34–41. https://doi.org/10.4103/0973-1296.59964.

24) Liu, J., Zhou, D., Liu, X., Wu, K., & Wan, C. (2009). Determination of kojic acid based on the interface enhancement effects of carbon nanotube/alizarin red S modified electrode. *Colloids and surfaces. B, Biointerfaces*, *70*(1), 20–24. https://doi.org/10.1016/j.colsurfb.2008.12.003.

25) Kalasho, B. D., Minokadeh, A., Zhang-Nunes, S., Zoumalan, R. A., Shemirani, N. L., Waldman, A. R., Pletzer, V., & Zoumalan, C. I. (2020). Evaluating the Safety and Efficacy of a Topical Formulation Containing Epidermal Growth Factor, Tranexamic Acid, Vitamin C, Arbutin, Niacinamide and Other Ingredients as Hydroquinone 4% Alternatives to Improve Hyperpigmentation: A Prospective, Randomized, Controlled Split Face Study. *Journal of cosmetic science*, *71*(5), 263–290.

26) Balaguer, A., Salvador, A., & Chisvert, A. (2008). A rapid and reliable size-exclusion chromatographic method for determination of kojic dipalmitate in skin-whitening cosmetic products. *Talanta*, *75*(2), 407–411. https://doi.org/10.1016/j.talanta.2007.11.021.

27) Feng, F., Jiang, Y., Jia, Y., Lian, X., Shang, C., & Zhao, M. (2023). Exogenous-organic-matter-driven mobilization of groundwater arsenic. *Environmental science and ecotechnology*, *15*, 100243. https://doi.org/10.1016/j.ese.2023.100243.

28) Ip, S. H., Johnson, M. L., & Ackers, G. K. (1976). Kinetics of deoxyhemoglobin subunit dissociation determined by haptoglobin binding: estimation of the equilibrium constant from forward and reverse rates. *Biochemistry*, *15*(3), 654–660. https://doi.org/10.1021/bi00648a032.

29) Bonacucina, G., & Palmieri, G. F. (2006). Acrylic polymers as thickening agents for tetraglycol cosolvent. *Journal of pharmaceutical sciences*, *95*(4), 726–736. https://doi.org/10.1002/jps.20567.

30) Loew, L. M., Brosseau, L., Tugwell, P., Wells, G. A., Welch, V., Shea, B., Poitras, S., De Angelis, G., & Rahman, P. (2014). Deep transverse friction massage for treating lateral elbow or lateral knee tendinitis. *The Cochrane database of systematic reviews*, *2014*(11), CD003528. https://doi.org/10.1002/14651858.CD003528.pub2.

31) Yilmaz, E., & Borchert, H. H. (2006). Effect of lipid-containing, positively charged nanoemulsions on skin hydration, elasticity and erythema--an in vivo study. *International journal of pharmaceutics*, *307*(2), 232–238. https://doi.org/10.1016/j.ijpharm.2005.10.002.

32) Ruiz Martinez, M. A., López-Viota Gallardo, J., de Benavides, M. M., de Dios García López-Duran, J., & Gallardo Lara, V. (2007). Rheological behavior of gels and meloxicam release. *International journal of pharmaceutics*, *333*(1-2), 17–23. https://doi.org/10.1016/j.ijpharm.2006.09.045.

33) Zhang, J., Fan, Y., & Smith, E. (2009). Experimental design for the optimization of lipid nanoparticles. *Journal of pharmaceutical sciences*, *98*(5), 1813–1819. https://doi.org/10.1002/jps.21549.