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Article

The Optimization of the Oiling Bath Cosmetic Composition Containing Rapeseed Phospholipids and Grapeseed Oil by the Full Factorial Design

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Abstract: The proper condition of hydrolipid mantle and the stratum corneum intercellular matrix determines effective protection against transepidermal water loss (TEWL). Some chemicals, improper use of cosmetics, poor hygiene, old age and some diseases causes disorder in the mentioned structures and leads to TEWL increase. The aim of this study was to obtain the optimal formulation composition of an oiling bath cosmetic based on rapeseed phospholipids and vegetable oil with high content of polyunsaturated fatty acids. In this work, the composition of oiling bath form was calculated and the degree of oil dispersion after mixing the bath preparation with water was selected as the objective function in the optimizing procedure. The full factorial design 2^3 in the study was used. The concentrations of rapeseed lecithin ethanol soluble fraction (LESF), alcohol (E) and non-ionic emulsifier (P) were optimized. Based on the calculations from our results, the optimal composition of oiling bath cosmetic was: L (LESF) 5.0 g, E (anhydrous ethanol) 20.0 g and P (Polysorbate 85) 1.5 g. The optimization procedure used in the study allowed to obtain the oiling bath cosmetic which gives above 60% higher emulsion dispersion degree 5.001×10^{-5} cm^{-1} compared to the initial formulation composition with the 3.096×10^{-5} cm^{-1}.

Keywords: rapeseed phospholipids; oiling bath cosmetics; emulsion dispersion degree optimization; full factorial design

1. Introduction

One of the basic functions of the stratum corneum (SC)—the most external layer of human skin—beyond the barrier to irritants and microorganisms is the barrier to transepidermal water loss (TEWL). Performing this function is possible because of two factors. One of them is the specialized lipids as the component of the intercellular matrix of SC, and the second is the external SC lipid mantle [1–4]. The major lipid components of the SC intercellular matrix are ceramides, fatty acids and cholesterol while the SC lipid mantle is the mixture of listed lipids and the sebum [1–4]. In the composition of sebum—product of sebaceous glands secretion—are triacylglycerols, wax esters, squalene, cholesterol esters, cholesterol and fatty acids (length of 12–20 carbon atoms with the prevalence of 16–18 carbon atoms) [4]. Components of lipid coat after mixing with sweat makes water in oil emulsion—the hydrolipid mantle [4]. The proper condition of hydrolipid mantle and SC intercellular matrix determines effective protection against TEWL. However, some chemicals, improper use of cosmetics, poor hygiene, old age and some diseases causes disorder in the mentioned structures and leads to TEWL increase [1,5,6].

The maintenance of the proper condition of skin hydrolipid coat and SC intercellular matrix or repairing of their damage is possible with using emollients [7–9]. Emollients are usually the mixture of physiological lipids (e.g., ceramides, free fatty acids, cholesterol) and/or non-physiological lipids (e.g., petrolatum), humectants (e.g., urea, glycerol), antipruritics (e.g., glycine, *Matricaria chamomilla* L. extract), ingredients supporting epidermal differentiation (e.g., dexpanthenol) and surfactants [7]. Respectively to its composition emollients will restore hydrolipid mantle, restore epidermal differentiation, help to break the itching or all of the above [7–9]. The most common presentation forms containing emollients are creams, ointments, topical emulsions, oiling bath preparations, washing emulsions and shampoo. This allows each patient with dry skin for the selection of proper emollient form, considering not only the SC regeneration and reparation but also the hygienic aspects.

Beyond the therapeutic aim, each of emollient form has to provide the defined quality requirements as components and form stability. In case of oiling bath preparations the form stability will refer not only to the storage conditions in the commercial package but also to the stability of emulsion created after mixing the bath preparation with water. The aim of this study was to obtain the optimal formulation composition of an oiling bath preparation based on rapeseed phospholipids and vegetable oil with high content of polyunsaturated fatty acids. Rapeseed lecithin ethanol soluble fraction (LESF) used, contains mainly phosphatidylcholine being biocompatible, native emulsifier. LESF is more resistant to oxidation compared to soybean lecithin and so is a better adjuvant for pharmaceutical technology. Furthermore, rapeseed phospholipids and vegetable oils exhibit skin oiling properties and are a good source of essential fatty acids (EFA), which are a part of the skin hydrolipid mantle. In the study, grapeseed oil was chosen due to high EFA content, above 70% of total. In this work, the composition of oiling bath form was calculated and the degree of oil dispersion after mixing the bath preparation with water was selected as the objective function. The full factorial design 2^3 in the study was used because it is often and effectively applied in the chemical processes and physical unit operations as well as product properties optimization [10–13].

2. Experimental Section

2.1. Chemicals and Materials

LESF was obtained from crude commercial rapeseed lecithin free of erucic acid and glucosinolates (00-type rapeseed) purchased from Oil Factory in Brzeg, Poland purified by the methods described elsewhere [14]. LESF contained 54% phosphatidylcholine (PC), the iodine value 68.5 g I_2/100 g, peroxide value 0.1 mmol O_2/kg and acid value 13.0 mg KOH/g. Grapeseed oil (Ybaara, Dos Hermanas, Spain) was purchased from a local market. Other chemicals obtained from commercial sources were of analytical grade.

2.2. Methods

2.2.1. Choice of Solvent

A quantity of 1 g of Grapeseed oil was mixed with tested alcohol (anhydrous ethanol and 2-propanol) starting from 1 g of alcohol with 1 g intervals until the complete dissolution was observed. Due to dissolution degree 1:20 *w*/*w* in case of 2-propanol comparing to 1:1 *w*/*w* in case of anhydrous ethanol, the 2-propanol was rejected as solvent. Oiling baths prepared were tested on the dispersion degree of emulsions obtained (Section 2.2.4, compositions in Table 1).

Table 1. Composition of oiling bath preparation (SC, stratum corneum; LESF, rapeseed lecithin ethanol soluble fraction).

Ingredient	Quantity (g)	Function
Grapeseed oil	76.0	SC coating
LESF	10.0	SC coating/surfactant
Anhydrous ethanol	10.0	Solvent
Polysorbate [a]	2.0	Surfactant
Ascorbyl palmitate	1.0	Antioxidant
Peppermint oil	1.0	Fragrance

[a] Polysorbate 20, 80 and 85 respectively.

2.2.2. Choice of Nonionic Emulsifier

In the study, we considered non-ionic emulsifiers commonly used in cosmetics such as Polysorbates 20, 80 and 85. The formulations that were prepared for the anhydrous ethanol solution of Grapeseed oil accordingly are stated in Section 2.2.3 and Table 1 and next the dispersion degree of both emulsions obtained were determined (Section 2.2.4).

2.2.3. Oiling Bath Composition and Preparation

The components selected to prepare an oiling bath for optimization based on preliminary experiments (described in Sections 2.2.1 and 2.2.2) are shown in Table 1.

Preparation: LESF, Polysorbate 80 and Ascorbyl palmitate were dissolved in alcohol at 40 °C. Into the clear solution the grapeseed oil was added and homogenized (homogenizer CAT X360, Staufen, Germany; 6000 rpm, 10 min). The preparation obtained was tested as described in Section 2.2.4. Due to emulsion dispersion degree 3.096 × 10^{-5} cm^{-1} in case of using Polysorbate 80 comparing to 2.823 and 2.584 × 10^{-5} cm^{-1} in case of using Polysorbate 20 and 85, the Polysorbate 80 was selected for further experiments.

2.2.4. Dispersion Degree Determination of Bath Emulsions

Exactly 0.4 mL of preparation obtained was mixed with 100 mL water at 40 °C. The dispersion degree was determined using Optiphot-2 microscope (Nikon, Tokyo, Japan) coupled with RGB-2252 camera (Cohu Inc., Poway, CA, USA) and Lucia G Intrigue Pro 4.51 software (Prague, Czech Republic). Samples were observed at magnification 2500 fold. Particles diameter measurements (µm) were leaded in three different fields with 100 objects each. The counted droplets of the dispersion phase were grouped into classes according to the diameter ranges (µm). Total surface area of the oil phase droplets dispersed in water was calculated by the Equation (1):

$$S = \pi \times \sum_{i=1}^{N} f \times d_i^2 \quad (1)$$

where: f_i, the amount of oil droplets in the different classes; d_i, median value of the diameter range of the droplets in different classes.

The total volume of the droplets was calculated from the Equation (2):

$$V = \frac{\pi}{6} \times \sum_{i=1}^{N} f \times d_i^3 \quad (2)$$

The dispersion degree was next calculated according to the Equation (3):

$$P = \frac{S}{V} \times 10^{-5} \quad (3)$$

2.2.5. Optimization of the Oiling Bath Composition

It is not clear from the results of preliminary experiments whether the optimum composition would be within the tested concentrations. For that reason, to determine the optimal oiling bath composition the mathematical model based on full factorial experimental design 2^3 was applied (design 2^3, a combination of three variables on two levels) with the dispersion degree as the response function. Real variables (the contents of LESF, anhydrous ethanol and Polysorbate 80) were selected according to preliminary experiments. Real variables, its values and symbols are showed in Table 2. Based on a preliminary study on the effect of oiling bath compositions on the emulsion dispersion degree, we defined a range of variables necessary in the design of an experimental matrix.

Table 2. Real values and code symbols of the variables used in the optimization procedure. L, rapeseed lecithin ethanol soluble fraction; E, anhydrous ethanol; P, Polysorbate 80.

Variables (z_j)	**L (g)**	**E (g)**	**P (g)**
Code symbols	x_1	x_2	x_3
Basic level (z_j^0)	10	10	2
Interval of variation (Δz_j)	2	5	1
Higher level (+)	12	15	3
Lower level (−)	8	5	1

Calculations and statistical analysis were based on Statistica PL v 6.0 for Microsoft Windows.

3. Results and Discussion

Based on data showed in Table 3, eight optimization experiments and three experiments on basic level (Table 2) were carried out.

Table 3. Ingredients concentration used in optimization experiments of oiling bath preparation. L, rapeseed lecithin ethanol soluble fraction; E, anhydrous ethanol; P, Polysorbate 80.

Experiment Number	**L (g)**	**E (g)**	**P (g)**
1	12	15	3
2	12	15	1
3	12	5	3
4	12	5	1
5	8	15	3
6	8	15	1
7	8	5	3
8	8	5	1
9 [a]	10	10	2

[a] three experiments on the basic level were carried out.

To simplify the calculation of regression coefficients, the real values of the variables (z_j) used in the experiments were standardized to become dimensionless values (x_j): +1 for the higher level, −1 for the lower level and 0 for the basic one (z_j^0) from the Equation (4):

$$x_j = \frac{z_j - z_j^0}{\Delta z_j} \quad (4)$$

The design matrix and the results of optimization experiments (dispersion degree values) are shown in Table 4.

The results (dispersion degree values) obtained in optimization experiments were used to designate the regression Equation (5) in a polynominal form and to calculate the coefficients (b_j) of the regression equation by the least square method (Equation (6)). The regression coefficients were calculated from the standard equation, which in a matrix notation has a form:

$$X^T X B = X^T Y \quad (5)$$

where X is the matrix of independent variables (Table 4); Y is the column vector of results (dispersion degree values, Table 4); B is the column vector of regression coefficients and X^T is transposition of an X-matrix. Incorporation into the regression equation of a dummy variable $x_0 = +1$ gives a set of standard equations with as many equations as the number of unknown coefficients (number of experiments):

$$\hat{y} = b_0x_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{123}x_1x_2x_3 \quad (6)$$

$$b_j = \frac{1}{N}\sum_{i-1}^{N} x_i y_i \quad (7)$$

Table 4. The full factorial experiments design matrix (+1—high level, −1—low level) and results of optimization experiments.

Experiment Number	Standardized Variables Level								Dispersion Degree DD × 10^{-5} (cm^{-1})
	x_0	x_1	x_2	x_3	x_1x_2	x_1x_3	x_2x_3	$x_1x_2x_3$	
1	+1	+1	+1	+1	+1	+1	+1	+1	2.722
2	+1	+1	+1	−1	+1	−1	−1	−1	2.905
3	+1	+1	−1	+1	−1	+1	−1	−1	3.760
4	+1	+1	−1	−1	−1	−1	+1	+1	3.281
5	+1	−1	+1	+1	−1	−1	+1	−1	4.323
6	+1	−1	+1	−1	−1	+1	−1	+1	3.868
7	+1	−1	−1	+1	+1	−1	−1	+1	2.843
8	+1	−1	−1	−1	+1	+1	+1	−1	3.489

The variance of the response function (dispersion degree values of bath emulsion) (s^2) was determined based on the additional three complementary experiments carried out for the basic level of ingredients concentration:

$$s^2 = \frac{\sum_{u=1}^{N}(y_u^0 - \bar{y}^0)}{f} \quad (8)$$

where $f = 2$ is a degree of freedom. The resulting variance was 0.0094.

The regression equation coefficients and their statistical significance (Student's t-test) were calculated as stated in Table 5. All regression coefficients obtained for full factorial experiments are calculated with the same accuracy ($s_{bj} = 0.034$):

$$s_{b_j} = \frac{\sqrt{s^2}}{\sqrt{N}} \quad (9)$$

where $s = 0.0969$ is a standard deviation of the response function and $N = 8$ is a number of optimizing experiments.

When the error estimation value (t_{bj}) obtained from the Equation:

$$t_{b_j} = \frac{|b_j|}{s_{b_j}} \quad (10)$$

(b_j is a coefficient value) is lower than the critical value $t_{p(f)} = 4.3$ from a table of Student's distribution for significance level $P = 0.05$ and degree of freedom $f = 2$, the respective regression coefficient is statistically insignificant.

Table 5. Coefficients of the regression equation of the full factorial experiment, statistically significant coefficients ([a]) error estimation value for s_{bj} = 0.034 and regression equation coefficients statistically significant ([a]).

Symbol of Regression Equation Coefficient	Regression Coefficient Value	Error Estimation Value
b_0	3.399 [a]	99.970
b_1	−0.232 [a]	6.824
b_2	0.056	1.635
b_3	0.013	0.382
b_{12}	−0.409 [a]	12.029
b_{13}	0.061	1.794
b_{23}	0.055	1.618
b_{123}	−0.220 [a]	6.471

Taking the significant coefficients of the full factorial experiment into consideration, the regression equation takes the form:

$$\hat{y} = 3.399 + 0.232x_1 - 0.409x_1x_2 - 0.220x_1x_2x_3 \quad (11)$$

Statistical significance of Equation (11) was estimated by *F*-Fisher test (*F*) used the formula:

$$F = \frac{s_{res}^2}{s^2} \quad (12)$$

where:
s^2—variance of the results of experiments on the basic level;
s^2_{res}—residual variance:

$$s_{res}^2 = \frac{\sum_{i=1}^{N}(y_i - \hat{y}_i)^2}{N - l} \quad (13)$$

$F_{cal.}$ =1.702 < $F_{0.05(2,3)}$ = 19.2. So that $F_{0.05(2,3)}$ is higher than $F_{cal.}$ The Equation (11) is statistically significant.

To confirm the statistical significance of the Equation (11), the correlation coefficient was calculated according to the Equation:

$$R^2 = \frac{\sum_{i=1}^{N}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{N}(y_i - \bar{y}_i)^2} \quad (14)$$

The calculated correlation coefficient has a value R = 0.986.

The evaluations of Equation (11) by *F*-Fisher test show the statistical significance of that equation. The high value of the correlation coefficient confirms the proper description of the examined composition and the influence of selected oiling bath preparation ingredients on the bath emulsion dispersion degree. In Equation (11) one of the first-degree monomial (main effect) and some of the second-degree values (interdepended effect of variables) are high significant. Thus, it could be suggested that the response function (emulsion dispersion degree) describes the optimal composition examined in the quasi-stationary surface (near the extremum). Based on this, next experiments are not necessary and the regression Equation (11) in the normalized form were transformed into standard form (real value) for the computation. It was transformed into real variables using the Equation (4) where: z_1—L (LESF), z_2—E (anhydrous ethanol), z_3—P (Polysorbate 80). After transforming the Equation (11) obtained the form:

$$y = 4.869 - 0.147L + 0.0031LE - 0.031E - 0.022ELP + 0.22LP + 0.22EP - 2.2P \quad (15)$$

Subsequently, the regression equation was optimized for the maximum value of the response function by an electronic data processing method. The computation was performed used standard computer program Statistica PL v 6.0 to search for the maximum value of the response function (emulsion dispersion degree) with the restrictive values: emulsion dispersion degree 3.0×10^{-5}–8.0×10^{-5} cm^{-1}, L 5–20 g, E 5–20 g, P 0.5–1.5 g. Based on calculations results, the optimal composition of oiling bath was L (LESF) 5.0 g, E (anhydrous ethanol) 20.0 g and P (Polysorbate 85) 1.5 g. For that composition the maximum emulsion dispersion degree calculated from Equation (15) was 5.474×10^{-5} cm^{-1}. In control experiment carried out for optimal composition (L, E, P) as described in Sections 2.2.3 and 2.2.4, the real emulsion dispersion degree 5.001×10^{-5} cm^{-1} was obtained. Calculated function value for 5 g L, 20 g E and 1.5 g P was 5.474. The final results of optimization procedure are shown in Table 6.

Table 6. Final results of optimization procedure.

Optimization Result	Value
Calculated response function from Equation (10) (emulsion dispersion degree DD × 10^{-5} cm^{-1})	5.474
Emulsion dispersion degree from control experiment (emulsion dispersion degree DD × 10^{-5} cm^{-1})	5.001
Difference between calculated and control values (%)	8.64
Maximum value obtained in the primary experiments (emulsion dispersion degree DD × 10^{-5} cm^{-1})	3.096
Increase in emulsion dispersion degree after optimization (%)	61.53

4. Conclusions

The optimization procedure used in the study allowed us to obtain the oiling bath preparation which gives above 60% higher emulsion dispersion degree 5.001×10^{-5} cm^{-1} compared to the initial formulation composition with the 3.096×10^{-5} cm^{-1}.

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Author Contributions

All authors contributed equally to the experimental and theoretical parts of this work. The first three authors were also contributed equally to writing and editing this manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Harding, C.R. The stratum corneum: Structure and function in health and disease. *Dermatol. Ther.* **2004**, *17*, 6–15.
2. Proksch, E.; Brander, J.M.; Jensen, J.M. The skin: An indispensable barrier. *Exp. Dermatol.* **2008**, *17*, 1063–1072.
3. Rawlings, A.V.; Harding, C.R. Moisturization and skin barrier function. *Dermatol. Ther.* **2004**, *17*, 43–48.
4. Wójcik, A.; Budzisz, E.; Rotsztejn, H. Skin surface lipids and their measurements. *Postepy Dermatol. Alergol.* **2011**, *6*, 498–505.
5. Sator, P.-G.; Schmidt, J.B.; Hönigsmann, H. Comparison of epidermal hydration and skin surface lipids in healthy individuals and in patients with atopic dermatitis. *J. Am. Acad. Dermatol.* **2003**, *48*, 352–358.
6. Seyfarth, F.; Schliemann, S.; Antonov, D.; Elsner, P. Dry skin, barrier function, and irritant contact dermatitis in the elderly. *Clin. Dermatol.* **2011**, *29*, 31–36.
7. Proksch, E.; Lachapelle J.-M. The management of dry skin with topical emollients—Recent perspectives. *J. Dtsch. Dermatol. Ges.* **2005**, *3*, 768–774.
8. Proksch, E. The role of emollients in the management of diseases with chronic dry skin. *Skin Pharmacol. Physiol.* **2008**, *21*, 75–80.
9. Lodén, M. Role of topical emollients and moisturizers in the treatment of dry skin barrier disorders. *Am. J. Clin. Dermatol.* **2003**, *4*, 771–788.
10. Smilkov, K.; Petreska Ivanovska, T.; Petrushevska Tozi, L.; Petkovska, R.; Hadjieva, J.; Popovski, E.; Stafilov, T.; Grozdanov, A.; Mladenovska, K. Optimization of the formulation for preparing *Lactobacillus casei* loaded whey protein-Ca-alginate microparticles using full-factorial design. *J. Microencapsul.* **2014**, *31*, 166–175.
11. Severino, P.; Santana, M.H.A.; Souto, E.B. Optimizing SLN and NLC by 2^2 full factorial design: Effect of homogenization technique. *Mater. Sci. Eng. C* **2012**, *32*, 1375–1379.
12. Gómez-Gaetel, C.; Bustos, G.L.; Godoy1, R.R.; Saez, C.K.; Novoa, G.P.; Fernández, E.M.; Tsapis, N.; Fattal, E. Successful factorial design for the optimization of methylprednisolone encapsulation in biodegradable nanoparticles. *Drug Dev. Ind. Pharm.* **2013**, *39*, 310–320.
13. Mahdi, S.; Shahabadi, S.; Reyhani, A. Optimization of operating conditions in ultrafiltration process for produced water treatment via the full factorial design methodology. *Sep. Purif. Technol.* **2014**, *132*, 50–61.
14. Górecki, M.; Sosada, M.; Boryczka, M.; Fraś, P.; Pasker, B. Rapeseed lecithin hydroxylation by chlorine replacing with hydroxyl groups in chlorinated phospholipids. *Acta Poloniae Pharm.* **2012**, *69*, 927–931.