UNIVERSITY OF MEDICINE AND PHARMACY
"CAROL DAVILA", BUCHAREST
DOCTORAL SCHOOL
PHARMACY DOMAIN

DESIGN AND EVALUATION OF MICROEMULSIONS AS MODERN COLLOIDAL SYSTEMS USEFUL IN THE ACNE TREATMENT

PhD THESIS SUMMARY

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Contents

Introduction

One of the current concerns of scientific research is the study of the passage of therapeutically active molecules to the deep layers of the skin, by finding pharmaceutical formulations based on biocompatible excipients, able to solubilize both hydrophilic and hydrophobic active substances, and at the same time to provide a temporary destructuration of the *stratum corneum*, in support of increasing their bioavailability.

This PhD thesis focuses on the study of microemulsions as ideal vehicles for the transport of active substances from the acne pharmacotherapeutic spectrum and presents the solution of solubility problems of the active substance with beneficial local effects on the affected skin and its integration into a colloidal microemulsion system.

The general hypothesis of the study aims at the possibility of incorporating salicylic acid into microemulsion as transport systems, characterized by clarity and thermodynamic stability, due to the use of a biocompatible mixture of stabilizers with properties of dispersing salicylic acid in a plant lipophilic phase at the nanoscale level.

This paper proposes an investigation of methods for the formulation and preparation of microemulsions using salicylic acid as an active substance. At the same time, the thesis aims to highlight the physical properties of microemulsions by carrying out experimental studies on the internal structure of the systems in relation to the topical mode of administration.

The general-theoretical part of the thesis is structured in three chapters, by providing a perspective on the skin as a functional structure for drug delivery and microemulsions as topical systems that can provide acne treatment to improve the quality of life of acne patients.

Chapter 1 highlights the skin as a structure for drug delivery, by conducting a complex analysis of the anatomical and functional structures that influence the dynamics of drug delivery processes, and how these structures may be influenced by dermatological conditions.

Chapter 2 presents a number of features of acne vulgaris as a dermatological pathology with a multifactorial character and how acne influences patients' quality of life. The chapter concludes with a number of perspectives on common treatments and some natural therapeutic solutions that can be approached to balance the skin.

Chapter 3 is devoted to presenting microemulsions as modern drug delivery vehicles for topical therapy. In addition to general aspects of microemulsion structure, emphasis is placed on the formulation, choice of excipients and the implications of these systems on penetration processes at the stratum corneum. This section provides an opening to the experimental study of microemulsions by presenting characterization methods for the valorisation of microemulsions as topical pharmaceutical systems.

The part for personal contributions is concentrated in **chapters 4-7**. The first two can be seen as a preamble to the experimental studies presented in Chapters 6 and 7.

Chapter 4 comprises the setting of the hypotheses and general objectives of the thesis. The personal contributions part of the thesis revolves around the investigation of microemulsions as biocompatible pharmaceutical systems that can incorporate salicylic acid as an active substance, first through a series of preliminary steps, centred on formulation and excipient screening, followed by a highlighting of some critical quality attributes, analysed in relation to topical administration and the Quality by Design approach.

In accordance with the current level of scientific knowledge and good laboratory practice, the methodology presented both in **Chapter 5** and in the experimental study sections of Chapter 7 was planned and implemented in the Physical and Colloidal Chemistry Discipline of the Faculty of Pharmacy, Carol Davila University of Medicine and Pharmacy, Bucharest, during five distinct phases: (i) the choice of substances and excipients for the formulation and preparation of topical microemulsions; (ii) obtaining oil-in-water microemulsified systems as vehicles for the incorporation of salicylic acid, by applying two distinct preparation methods, namely the room temperature oil phase titration method and the hot aqueous phase titration method, in order to obtain clear and stable systems; (iii) the preliminary physical evaluation of the microemulsions presented in Chapter 6; (iv) the actual evaluation of the microemulsions, focusing on the discovery of their internal structure and the highlighting of critical quality attributes with high relevance for the biopharmaceutical profile of the topical preparation: viscosity, mean droplet size and adhesion work; (v) analysis and interpretation of the obtained data and application of an experimental design program for the experimental run of the selected optimization program.

The novel elements of this thesis are found in the formulation process, as the desired microemulsions were prepared using a biocompatible mixture of excipients, namely Tween 80, propylene glycol and lecithin, a vegetable oil phase, represented by oat oil and pomegranate oil. On the other hand, the solubilisation of salicylic acid in the aforementioned

stabilising mixture and its incorporation in a vegetable lipophilic medium resulted in the formation of a finely dispersed phase in an aqueous hyaluronate medium which can confer an additional moisturising effect.

Therefore, the study of the lipophilic phase droplet dispersion process presented in Chapter 7 was carried out by Dynamic Light Scattering (DLS) spectrometry. This study of the dimensional properties was **the second novel element** of the thesis.

Another innovative element consists in carrying out studies to evaluate the surface properties of microemulsions by determining the surface tension at the liquid/gas interface and the contact angle at a solid surface using the CAM 101 goniometer. The analyses were approached by applying two study models, namely the pendant drop model and the contact angle model. The determinations performed allowed to highlight the behaviour of fluid microemulsified systems in contact with a solid surface, and the flow properties after the initial rheological evaluation of the flow properties. The final point of the goniometric study was the evaluation of the adhesion work of the microemulsions.

Chapter 6 describes the development and evaluation process of oil-in-water microemulsions with salicylic acid starting from a group of systems without active substance as microemulsified vehicles. These vehicles were formulated using a stabilising mixture of Tween 80, propylene glycol and lecithin in a total concentration of 31%. The 4 microemulsions were prepared taking into account the variation of lecithin in four distinct concentrations: 0.5%, 1%, 1.5% and 2%.

Table 1.1 Formulation data for microemulsions prepared in the first step with different lecithin concentrations in the range 0.5 - 2%, maintaining a constant S1/S2/CoS mixture level of 31%.

Form	Lecithin (L) (g%)	Tween 80 (mL%)	PG (mL%)	Oil (mL%)	Water (mL%)	HA (g%)
MEL 1	0,5	20,5	10	1	67	1
MEL 2	1	20	10	1	67	1
MEL 3	1,5	19,5	10	1	67	1
MEL 4	2	19	10	1	67	1

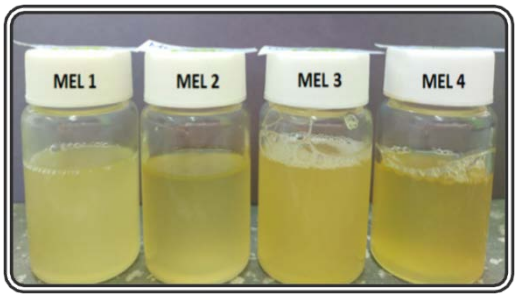

Figure 1.1 Oil-in-water microemulsions (MEL 1 - MEL 4) prepared in the first step with four different concentrations of lecithin, observed after preparation at 25 ± 0.5°C

Of the four systems, the vehicle containing 0.5% lecithin was considered as a model for formulating a separate group of systems. In this case, the lecithin concentration was kept constant at 0.5% and the Tween 80/PG mixture was varied over the concentration range of 30-60%. In both cases, the level of oil phase consisting of oat oil and pomegranate oil (1:1) was maintained at 1% and used as the titration medium. The stabilizer mixture was used for solubilization of 0.5% salicylic acid.

Table 1.2 Formulation data for microemulsions prepared in the second step with 0.5% salicylic acid using lecithin at a constant level of 0.5% as part of the total amount of S1/S2/CoS mixture, having a concentration in the range 30.5% - 60.5%.

Form	Lecithin (L) (g%)	Tween 80 (mL%)	PG (mL%)	Oil (mL%)	Water (mL%)	HA (g%)	SA (g%)
MEAS 1	0.5	20	10	1	67	1	0.5
MEAS 2	0.5	25	12.5	1	59.5	1	0.5
MEAS 3	0.5	30	15	1	52	1	0.5
MEAS 4	0.5	35	17.5	1	44.5	1	0.5
MEAS 5	0.5	40	20	1	37	1	0.5

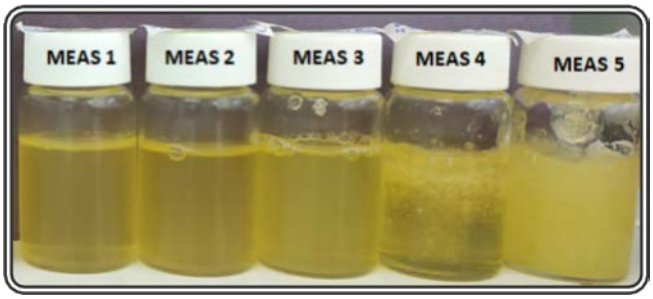

Figure 1.2 Oil-in-water microemulsions (MEAS 1 - MEAS 5) with salicylic acid prepared in the second step, observed after preparation at 25 ± 0.5°C

The quality of the developed systems was analysed by organoleptic examination, describing: appearance, homogeneity, colour and the presence of possible instability phenomena.

The microemulsification capacity of surfactants was assessed by calculating the HLB values for each individual system, using a formula adopted in the study by de Melo Cotrim et al. [1]:

$$HLB_{ME} = \frac{HLB_{Tween\ 80} \cdot F1 + HLB_{Lecithin} \cdot F2}{10 - F_{PG}} \quad (1.1)$$

where F_1 is the fraction of Tween 80, F_2 - the fraction of lecithin, F_{PG} - the fraction of propylene glycol, and the sum of $F_1 + F_2 + F_{PG} = 10$.

For the second group of microemulsions, the stability points were defined by the graphical pseudoternary phase diagram design method. For this purpose, the proportions of three main components were taken into account: the S_1 /S_2 /CoS mixture, the oily phase and the aqueous phase. Triplot 4.1.2. software was used to plot the stability points defining a zone of oil-in-water microemulsions. In a broader sense, the graphical method is useful for screening nanocolloidal systems in order to avoid areas of emulsion formation or areas of instability.

For the MEAS 1-MEAS 5 systems, the pseudoternary phase diagram was constructed and is shown in Figure 1.3 along with its section that is focused on the stability zone, according to each concentration established in the formulation design. The amount of S1, S2 and CoS will influence the physical characteristics of the systems, their flow behaviour and stability over time.

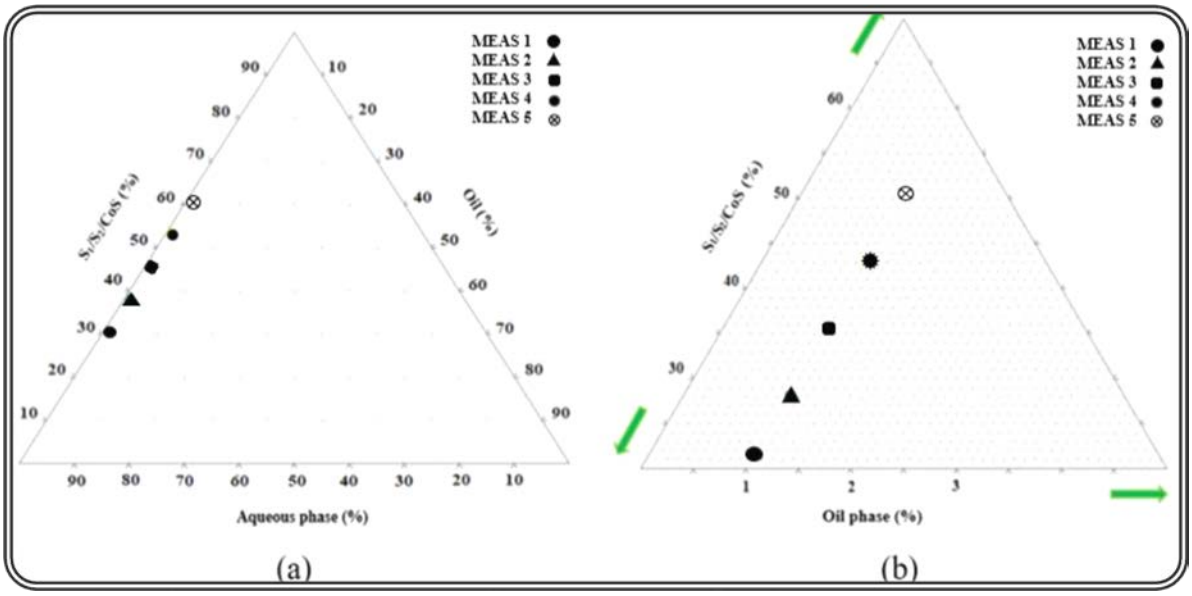

Figure 1.3 Pseudoternary phase diagrams for microemulsions designed in the second formulation phase (MEAS 1 - MEAS 5)

Refractive index analysis demonstrated their isotropy, thus introducing fundamental concepts about colloids (micro- or nanocolloids) and clues about their size, which will be of interest in future studies. The degree of clarity of microemulsions means that the dispersed lipophilic phase chosen to incorporate the active substance is defined by a nanometric size.

The phase diagram for the MEAS 1 - MEAS 5 systems was performed by graphical design, highlighting the influence of formulation factors on the stability and pharmaceutical relevance of the designed microemulsions.

From a rheological point of view, the MEAS 1 - MEAS 3 formulations showed a Newtonian behaviour, with viscosity variations conditioned by their internal structure, i.e. composition.

The study provided important preliminary data on microemulsion formulation, paving the way for an extension of the experimental scope and proposing highly specific stability tests in prospective studies.

Chapter 7 provides a new perspective by continuing the research on salicylic acid microemulsions for dermatological applications highlighted in Chapter 6. The study takes up the MEAS 1 microemulsion model, which is analysed using a new development method combined with complex evaluation methods and experimental design of microemulsions as pharmaceutical systems.

The objective of this study was based on the development of salicylic acid microemulsions as oil-in-water systems using a biocompatible mixture of Tween 80, soy lecithin and propylene glycol in a concentration range of less than 20.1- 40.5 [2]. The excipient mixture, together with the oily phase, represented by oat oil (1-2%) was used to solubilize salicylic acid (0.5%). Aqueous medium enriched with hyaluronic acid (1%) was considered adequate to support a moisturizing effect.

The elements of novelty that define this study are based on the approach of a new way of preparing oil-in-water microemulsions, the broadening of the concentration range of the oily phase and some evaluations that support the study of the internal structure of microemulsions as colloidal systems, together with an application of Quality by Design (QbD) principles throughout the research process, starting from a fractional Box-Behnken factorial matrix [3].

The first parameter, viscosity, was optimized and revealed by rheological evaluation, in accordance with the definition of the Newtonian character of the systems. Next, the study of the internal structure of the microemulsions was carried out by applying photon correlation spectroscopy techniques and the determination of the electrokinetic zeta potential. Finally, the goniometric study provided a broad insight into the surface phenomena and behaviour of microemulsions in contact with a solid surface, as well as their quantification by analysing the surface tension at the liquid/gas interface, the contact angle and the adhesion work, respectively, considering Young's and Dupré's equations as mathematical models for goniometric analysis.

The application of QbD principles in the development of microemulsions aimed at optimizing the composition of the systems, highlighting three critical quality attributes: viscosity, droplet size and adhesion work. The three attributes were analysed as dependent variables of Tween 80 and propylene glycol (PG) mixture concentration (2:1), lecithin and oat oil concentrations. The application of response surface methodology combined with prediction models of optimal points was considered a valuable analysis technique to obtain an optimal preparation characterized by high viscosity, low dispersed phase particle size and low adhesion work, suitable for the application and topical action of the designed and analyzed systems.

Tabel 1.3 Factor design for microemulsion development with three independent variables (X_1, X_2, X_3) and three levels of variation - lower, middle, upper - coded for each factor, which will accompany the dependent variables during the optimization process

Factor	Variable	Lower (-1)	Medium (0)	Superior (+1)
X_1	Tween 80/PG (%)	20	30	40
X_2	Lecithin (%)	0,1	0,3	0,5
X_3	Oat oil (%)	1	1,5	2

Fractional factorial design modeling with 3 factors and 3 levels was performed using Minitab software (Trial version, Minitab, LLC, State College, PA, USA) [409]. Each independent variable X_1, X_2, X_3, was set 3 levels of variation: "-1", "0" and "+1".

Table 1.4 lists the factor design for $N = 3^3$ experiences (3 factors with 3 levels of variation), in which the variables are rendered according to the coding scheme explained above. Thirteen variants of MELSA 1-MELSA 13 coded formulas were proposed.

Table 1.4 Experimental matrix for MELSA 1 - MELSA 13 microemulsions developed by applying a Box-Behnken model

Form	Experiment	Tween 80/PG (%)	Lecithin (%)	Oat oil (%)
		X_1	X_2	X_3
MELSA 1	1	-1	-1	0
MELSA 2	2	+1	-1	0
MELSA 3	3	-1	+1	0
MELSA 4	4	+1	+1	0
MELSA 5	5	-1	0	-1
MELSA 6	6	+1	0	-1
MELSA 7	7	-1	0	+1
MELSA 8	8	+1	0	+1
MELSA 9	9	0	-1	-1
MELSA 10	10	0	+1	-1
MELSA 11	11	0	-1	+1
MELSA 12	12	0	+1	+1
MELSA 13	13	0	0	0

The composition of the salicylic acid microemulsions developed according to the 3^3 Box-Behnken fractional experimental matrix, denoted MELSA 1 - MELSA 13, is shown in Table 1.5 and corresponds to the final formulations analysed in the following steps. At the same time, the appearance - identified by clarity and correspondingly coded HLB values determined for each system have been rendered.

Table 1.5. Composition of oil-in-water microemulsions with salicylic acid, formulated according to the experimental fractional matrix type 3^3 Box-Behnken, presented according to the clarity parameter and HLB values

Form	**Clarity**	**Tween 80 /[1] PG (2:1)** (%)	**Lecithin (%)**	**Oil** (%)	**[2] HA** (%)	**[3] SA** (%)	**Water** (%)	**HLB ME**
		(X_1)	**(X_2)**	**(X_3)**				
MELSA 1	++	20	0,1	1,5	1	0,5	76,9	14,90
MELSA 2	+++	40	0,1	1,5	1	0,5	56,9	14,46
MELSA 3	++	20	0,5	1,5	1	0,5	76,5	14,62
MELSA 4	+++	40	0,5	1,5	1	0,5	56,5	14,80
MELSA 5	+++	20	0,3	1	1	0,5	77,2	14,75
MELSA 6	+++	40	0,3	1	1	0,5	57,2	14,87
MELSA 7	+	20	0,3	2	1	0,5	76,2	14,75
MELSA 8	+++	40	0,3	2	1	0,5	56,2	14,87
MELSA 9	+++	30	0,1	1	1	0,5	67,4	14,93
MELSA 10	+++	30	0,5	1	1	0,5	67	14,72
MELSA 11	+	30	0,1	2	1	0,5	66,4	14,93
MELSA 12	+	30	0,5	2	1	0,5	66	14,72
MELSA 13	+++	30	0,3	1,5	1	0,5	66,7	14,85

Note: The values given in the table for each component are expressed as a percentage (%) and have been calculated for 20 mL final microemulsion; [1]PG - propylene glycol; [2]HA - hyaluronic acid; [3]SA - salicylic acid

Figure 1.4 shows the steps in the preparation of microemulsions, starting from the preparation of the lipophilic phase, solubilisation of salicylic acid and obtaining a high clarity microemulsion by the hot aqueous phase titration process.

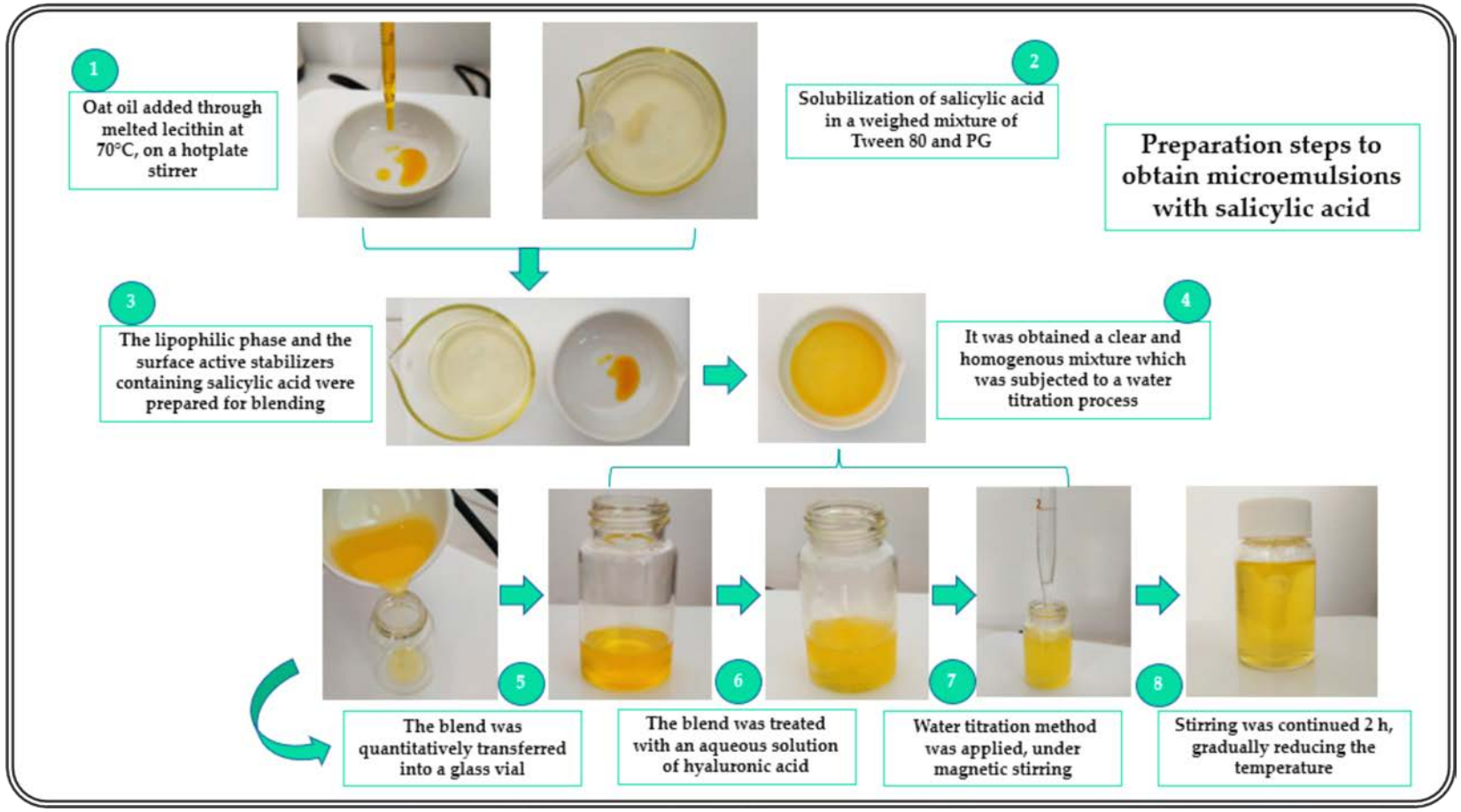

Figure 1.4 Main steps in the preparation of oil-in-water microemulsions with salicylic acid

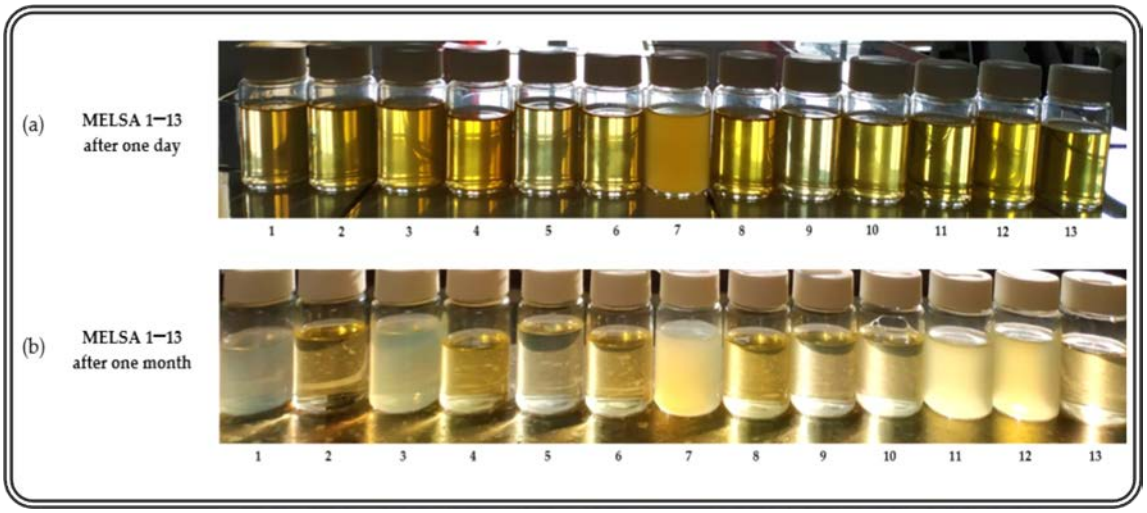

Figure 1.5 Visualisation of MELSA 1 - MELSA 13 microemulsions after one day of preparation - case (a), respectively after one month - case (b), at room temperature, 25 ± 0.5°C

Selecting a maximum Tween 80/PG level of 40%, combined with 0.1%, 0.3% and 0.5% lecithin, was favourable to ensure complete dispersion of the oily phase in the 1-2% concentration range, resulting in clear systems.

Physical evaluations of the prepared microemulsions were carried out, including pH analysis, conductivity study, refractive index determination, rheological evaluation, droplet size analysis, and goniometric analysis of oil-in-water microemulsions with salicylic acid. Some examples of these determinations are given below.

In agreement with the **conductometry determinations**, the microemulsions were defined as oil- in-water type systems with high conductivity values, as can be seen in Table 1.6. These were placed in the range 506.0 ± 3.0 - 1088 ± 1.7 μS/cm, and were correlated with the presence of the Tween 80/PG mixture and water. The influence on conductivity of lecithin and oat oil was insignificant ($p > 0.05$).

Table 1.6 Conductivity values for microemulsions MELSA 1-MELSA 13, determined at 25± 0,5°C

Form	**Tween 80 /[1]PG (2:1) (%)**	**Water (%)**	**Conductivity (μS/cm)**
MELSA 1	20	76,9	1042 ± 1,2
MELSA 2	40	56,9	506,0 ± 3,0
MELSA 3	20	76,5	1088,0 ± 1,7
MELSA 4	40	56,5	621,3 ± 0,6
MELSA 5	20	77,2	1065,0 ± 1,0
MELSA 6	40	57,2	524,3 ± 0,6
MELSA 7	20	76,2	986,3 ± 1,5
MELSA 8	40	56,2	542,3 ± 0,6
MELSA 9	30	67,4	772,0 ± 1,0
MELSA 10	30	67	728,7 ± 1,5
MELSA 11	30	66,4	717,3 ± 1,5
MELSA 12	30	66	786,0 ± 0
MELSA 13	30	66,7	661,3 ± 1,5

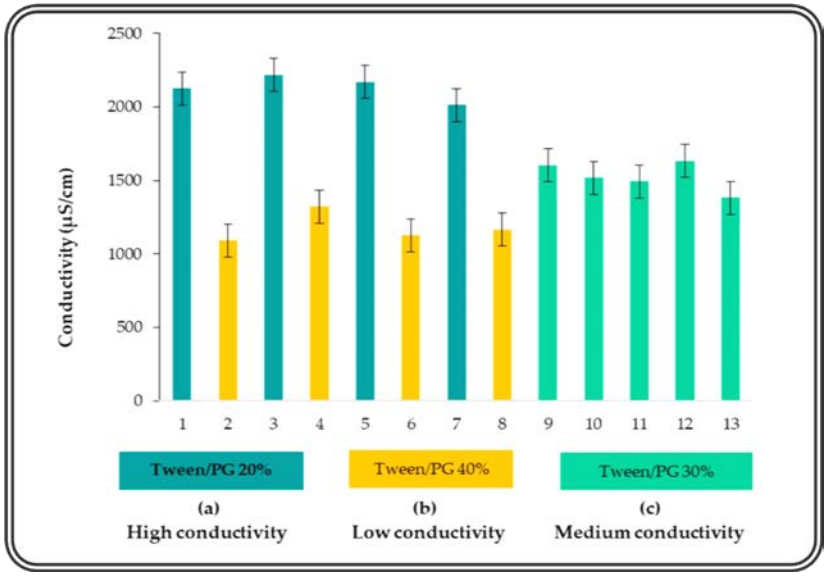

Figure 1.6 Graphical representation of conductivity values, divided into three groups, according to Tween 80/PG concentration as systems with (a) high conductivity, (b) low conductivity and (c) intermediate conductivity.

Figure 1.7 shows the experimental DLS path for the MELSA 8 system, starting from the laser action on the sample, followed by data acquisition and determination of the droplet size, according to the Cumulant model, expressed as intensity (a.u.) versus diameter (nm).

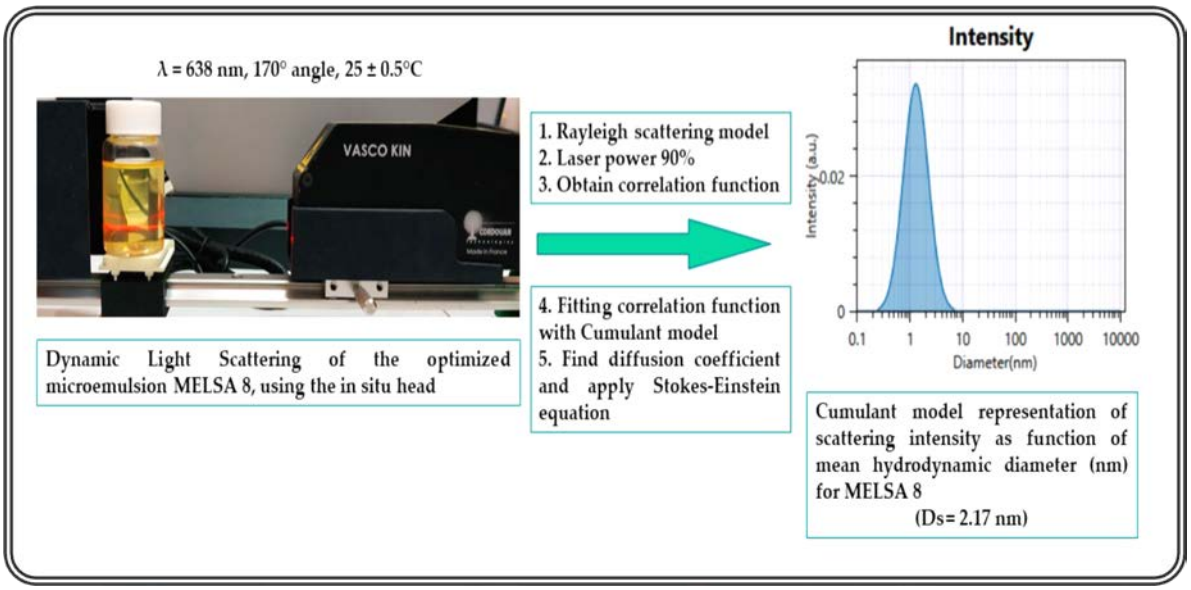

Figure 1.7 Representation of the DLS method for MELSA 8 microemulsion using the in-situ analysis accessory of the VascoKin apparatus in five successive steps to obtain a response, according to the Cumulant algorithm

Table 1.7 shows the cumulative data specific to DLS and electrokinetic zeta potential determinations for MELSA 1 - MELSA 13 systems at 25 ± 0.5°C.

Table 1.7 Cumulative presentation of the results obtained from droplet size analysis and determination of the electrokinetic zeta potential in relation to the three formulation factors for MELSA 1 - MELSA 13 microemulsions at 25 ± 0.5°C

Form	Tween 80/PG (%)	Lecithin (%)	Oil (%)	Diameter (nm)	PDI	Zeta potential (mV)
MELSA 1	20	0,1	1,5	11,35 ± 1,05	0,268 ± 0,011	-1,98 ± 0,02
MELSA 2	40	0,1	1,5	3,32 ± 1,85	0,091 ± 0,010	-2,78 ± 0,05
MELSA 3	20	0,5	1,5	21,88 ± 1,25	0,039 ± 0,017	-3,78 ± 0,05
MELSA 4	40	0,5	1,5	5,88 ± 1,16	0,070 ± 0,010	-2,14 ± 0,05
MELSA 5	20	0,3	1	3,43 ± 1,73	0,297 ± 0,075	-3,83 ± 0,01
MELSA 6	40	0,3	1	1,58 ± 0,20	0,119 ± 0,023	-3,16 ± 0,06
MELSA 7	20	0,3	2	37,72 ± 4,55	0,250 ± 0,025	-2,80 ± 0,02
MELSA 8	40	0,3	2	2,17 ± 1,54	0,352 ± 0,027	-1,72 ± 0,03
MELSA 9	30	0,1	1	5,14 ± 2,15	0,134 ± 0,018	-1,43 ± 0,03
MELSA 10	30	0,5	1	5,30 ± 2,53	0,218 ± 0,037	-2,33 ± 0,05
MELSA 11	30	0,1	2	6,45 ± 2,55	0,202 ± 0,011	-2,38 ± 0,03

MELSA 12	30	0,5	2	26,02 ± 3,78	0,557 ± 0,038	-1,38 ± 0,05
MELSA 13	30	0,3	1,5	4,11 ± 1,65	0,240 ± 0,021	-2,38 ± 0,02

The discovery of the surface properties of microemulsions was based on the measurement of free surface energy and contact angle using **the CAM-101 goniometer** equipped with a Hamilton syringe fitted with a C209-30 needle and a digital camera (KSV Instruments, Espoo, Finland), as reported in previous studies [5, 6].

Figure 1.8 shows the captured images by the CAM-101 digital camera for each drop of MELSA 1 - MELSA 13 samples released from the Hamilton syringe and measured.

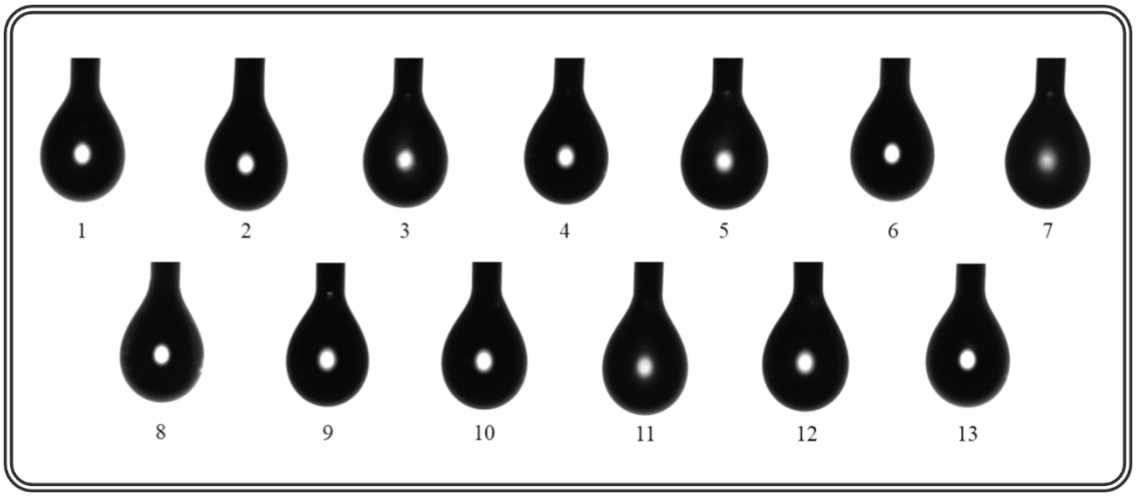

Figure 1.8 Captured images for microemulsion droplets, coded (1 - 13), evaluated by the pendant drop model, for measuring γ_{LG}, at 25 ± 0.5°C

Figure 1.9 shows captures for each microemulsion sample (1-13) and studied by applying the *contact angle (CA)* model. Their behaviour can be observed by considering wettability and droplet adhesion on contact with a solid surface.

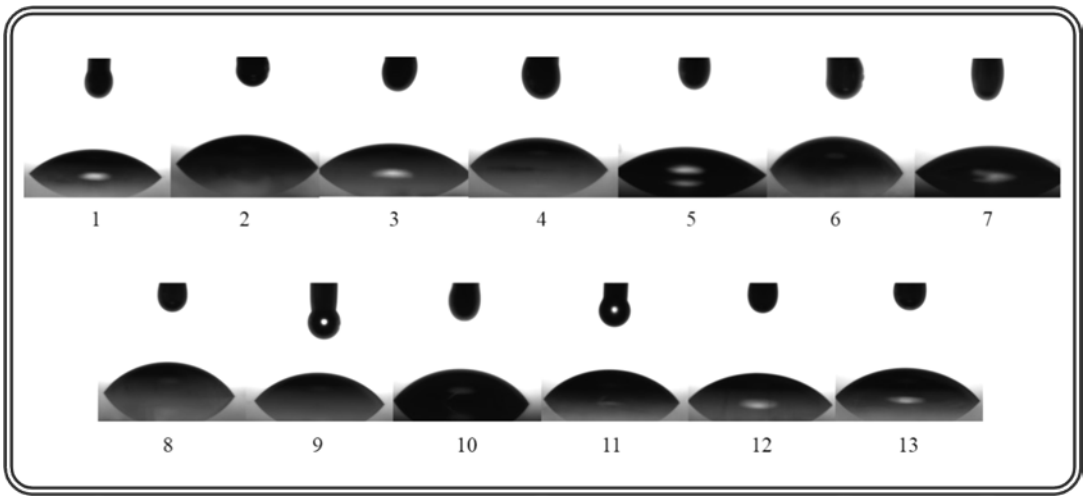

Figure 1.9 Captured images of microemulsion samples (1-13) applied to glass slides during the γ_{LG} and contact angle evaluation study at 25 ± 0.5°C.

The formulation composition **optimization process**, in terms of critical quality attributes, was based on the development of a fractional factorial design type 3^3, characterized by 13 experiments designed using Minitab software. Analysis of the responses namely Y_1: viscosity (cP), Y_2: mean droplet size (MPS) - Ds (nm), and Y_3: work of adhesion - W (mN/m), as a function of formulation factors defined as X_1: Tween 80/PG (%), X_2 : lecithin (%), and X_3 : oat oil (%), was performed by applying the response surface methodology (RSM).

Optimisations of the three dependent variables: viscosity (Y_1), mean droplet size (Y_2) and work of adhesion (Y_3), were carried out according to the independent variables (X_1 - X_3), and the example of the optimisation of the viscosity parameter (Y_1) according to the factors X_1: Tween 80/PG (%) and X_2: Lecithin (%) is detailed below.

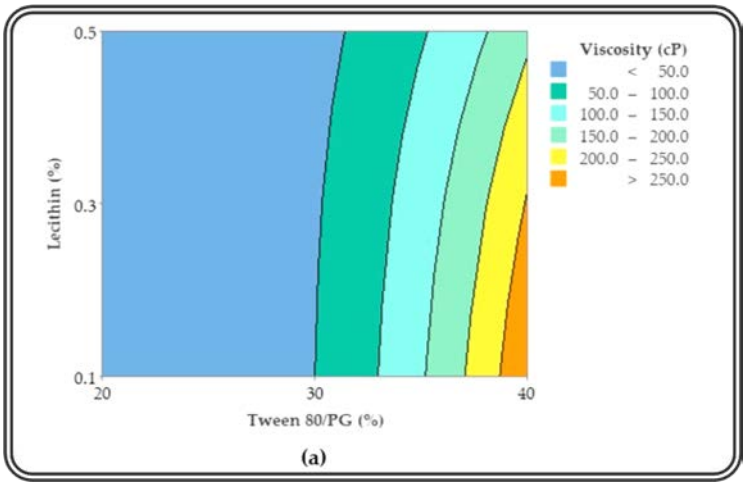

Figure 1.10 Contour plot for viscosity parameter (Y_1) as a function of factors X_1 : Tween 80/PG (%) and X_2 : Lecithin (%)

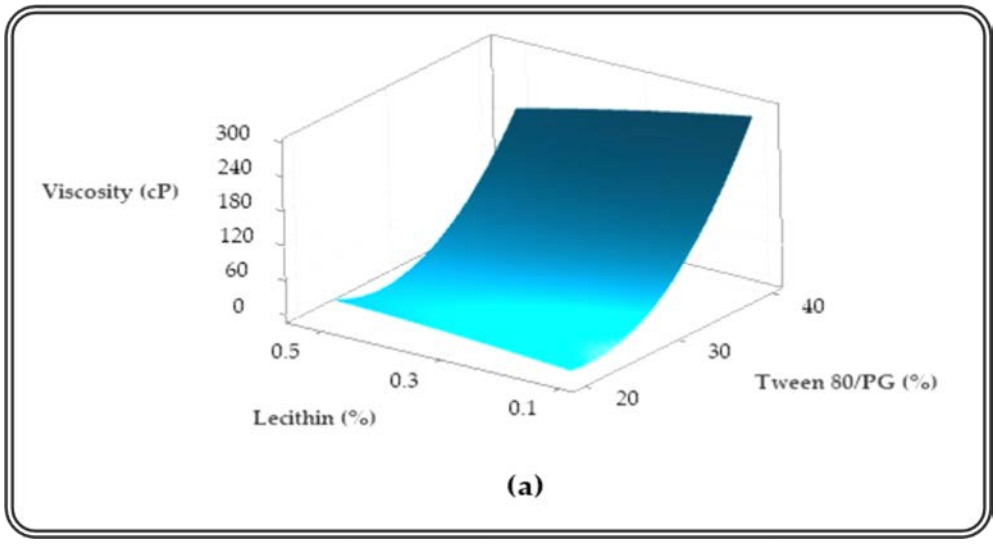

Figure 1.11 Gradient response area for viscosity (Y_1) as a function of Tween 80/PG and lecithin concentration

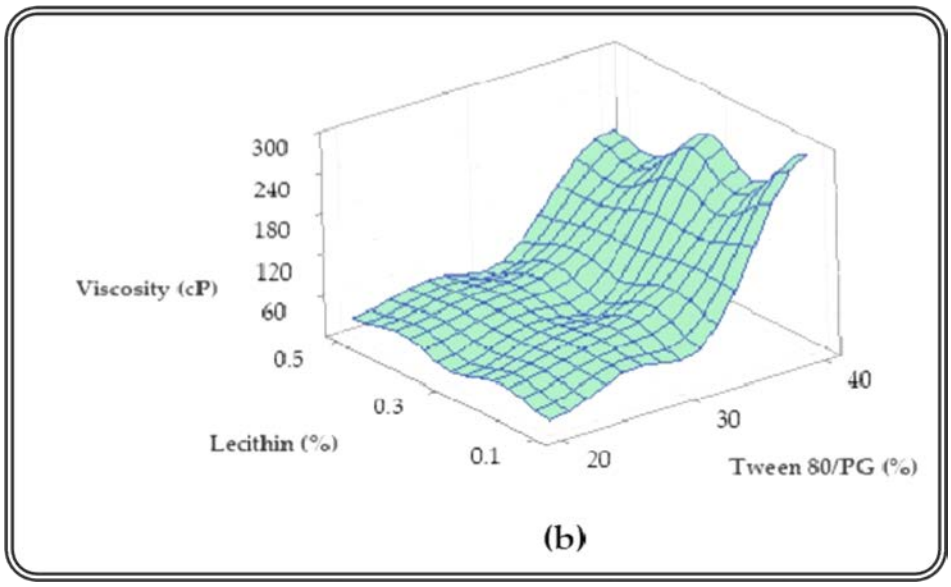

Figure 1.12 Relief plot for viscosity (Y_1) as a function of Tween 80/PG and lecithin concentration

The solution obtained from the analysis showed a model system with the following composition: Tween 80/PG (X_1): 40%, lecithin (X_2): 0.1% and oat oil (X_3): 2%. The predicted viscosity was 263.83 cP, with a coefficient value for the desired response of 0.8953. The result of this analysis can be corroborated with the data obtained in the experimental course, by highlighting two characteristic systems with viscosities around the predicted value, namely MELSA 6 with a viscosity of 287.77 cP and MELSA 8 with a viscosity value of 217.73 cP. By considering the proposed limitation, referred to maximizing the oat oil concentration to 2%, it can be deduced that MELSA 8 is eligible to be the optimal system.

CONCLUSIONS

The PhD thesis entitled "Design and evaluation of microemulsions as modern colloidal systems useful in anti-acne treatment" pursued and achieved all the proposed objectives: the choice of active substance and excipients for obtaining oil-in-water microemulsions; obtaining oil-in-water microemulsions as vehicles for the incorporation of salicylic acid by applying the oil-phase titration method at room temperature (selected for the first study) and the hot aqueous phase titration method (selected in the second study), respectively; preliminary physical evaluation of the microemulsions, in accordance with the data presented in the first study in Chapter 6; physical evaluation of microemulsions to discover the internal structure and highlight critical quality attributes relevant to the biopharmaceutical profile of topical preparations, in line with the results of the study presented in Chapter 7; analysis and interpretation of the data obtained by applying an experimental design; discovery of optimal model systems that fulfil a number of critical quality attributes suitable for topical microemulsions.

Thus, in the **first experimental study** a procedure for the **development and evaluation of oil-in-water microemulsions with salicylic acid** is outlined, starting from a group of blank microemulsified vehicles. The vehicles were formulated using a stabilizer mixture, consisting of Tween 80, propylene glycol and lecithin, in a total concentration of 31%. In this group, four concentrations of lecithin were selected, 0.5%, 1%, 1.5% and 2%. A single vehicle formulated with 0.5% lecithin was considered as a model for the development of a different group of microemulsions, characterized by a variation of Tween 80/PG in the range 30-60%.

For these types of microemulsions, the level of **oil phase** (*oat oil and pomegranate oil) (1:1)* was set at **1%**. In the second group, the stabilizer mixture was used for solubilization of 0.5% salicylic acid.

Preliminary physical evaluations that focused on *pH, conductivity and refractive index studies were* useful for a preliminary characterisation of the systems and a definition of some quality elements of the preparations. Thus, the pH was suitable for the topical formulations and was between 4.26 and 4.37 for the first group of microemulsions (MEL 1- MEL 4) and between 3.57 and 4.13 for the second group (MEAS 1 - MEAS 5). It was also considered that a low pH can be favourable for an acne formula preparation.

Further, the *conductivity* showed high composition-dependent values only for the second group of systems, confirming the type of oil-in-water microemulsions. At the same time, the *refractive index* study demonstrated the isotropy of the prepared systems. In the second group, a proportional increase of the refractive index was observed with increasing the concentration of Tween 80/PG to 60%, from 1.3749 to 1.4081.

The pseudoternary phase diagram was designed for systems prepared in the second stage and the influence of formulation factors on the stability and pharmaceutical relevance of the developed microemulsions was revealed.

The study culminated in the identification of *rheological profiles* for three representative microemulsions belonging to the second group. The viscosity variation between 31.5 - 1317.3 cP was specific for fluid microemulsions with a **Newtonian flow**. At the same time, it was concluded that the viscosity of the three systems is dependent on the concentration of Tween 80/PG.

This preliminary study has provided a series of data on the formulation of microemulsions and at the same time an opening to discover the key features of microemulsions as topical nanocolloids by approaching complex experimental techniques.

In the **second study**, the microemulsion development process was put in a new light through a different approach and was directed towards the application of *Quality by Design* principles. A model system discovered in the previous study was taken and formulated with a **mixture of Tween 80/PG (2:1) 30%, lecithin 0.5%, a mixture of oat oil and pomegranate oil (1:1) 1%, salicylic acid 0.5%, hyaluronic acid 1% and water 67%**.

The microemulsion development process was carried out by applying *Quality by Design* concepts and modelling a **three-factor, three-level fractional factor design (3^3) of the Box-Behnken type**. Thus, 13 oil-in-water microemulsion formulations were defined by applying the hot aqueous phase titration method. The 13 experiments defined by the experimental matrix 3^3 were based on the variation of three independent variables (formulation factors), on three levels of minimum, medium and maximum, noted -1, 0 and +1, and coded X_1: Tween 80/PG (20-40%), X_2: Lecithin (0.1-0.5%), X_3: Oat oil (1-2%). The designed systems, equivalent to the 13 experiments, named MELSA 1 - MELSA 13, were physically analysed, with a particular focus on three critical quality

attributes, coded as Y_1: viscosity (cP), Y_2: mean droplet size (nm) and Y_3: work of adhesion (mN/m). Based on this model, an extensive optimization process was performed by applying the Response Surface Methodology (RSM). Clarity was considered as an exclusion criterion for opalescent or low clarity systems.

In terms of preliminary physical parameters, *pH* placed between 3.49 and 3.71 was considered suitable for an acne formula preparation that may be involved in restoring skin pH affected by acne pathology. *The conductivity study* confirmed the type of oil-in-water microemulsions, while *the refractive index* was characteristic of isotropic systems and was significantly influenced by the presence of Tween 80/PG.

In the *rheological analysis*, the viscosity ranged from 16.94 - 292.69 cP, and the flow profiles of the microemulsions were typical of Newtonian systems. The systems were described as fast flowing fluids with low viscosity values (16.94-51.23 cP) when Tween 80/PG was selected at a minimum and medium level, while more viscous microemulsions (188.17-292.69 cP), were formed when using a maximum concentration of Tween 80/PG (X_1). X_1 was thus the only parameter that influenced the viscosity response, as observed in the contour plots and response areas of Y_1 as a function of X_1 and X_2. The two variables X_2 and X_3 showed no significant influence on Y_1.

The predictive analysis revealed a model system with a viscosity of 263.83 cP, leading to the choice of two optimal systems, MELSA 6 (Y_1: 287.77 cP) and MELSA 8 (Y_1: 217.73 cP). By pursuing clarity as an exclusion criterion, MELSA 8 was considered a high viscosity microemulsion model suitable for topical application.

To complete the microemulsion quality profile, as a **novel element, DLS analysis** provided information on the internal structure of microemulsions by observing intensity (a.u.) profiles as a function of droplet diameter (nm), in agreement with the Cumulant model, specific to droplets with average diameter below 100 nm.

The presence of a minimal amount of oil phase resulted in very finely dispersed droplets with a hydrodynamic diameter variation between 1.58-37.72 nm. The mean droplet size was significantly influenced by the presence of oat oil (X_3) and Tween 80/PG (X_1). The two factors acted in opposite ways on the generation of oil-in-water microemulsions: X_1 caused a reduction of the mean particle size towards the minimum value and X_3 influenced an increase of the particle size towards the maximum value.

In the optimization process, predictive analysis was conducted to obtain a

microemulsion model with an average droplet size below 10 nm, designed with a maximum X_3-factor of 2%. This criterion was met by two systems MELSA 8 (Y_2: 2.17 nm) and MELSA 11 (Y_2: 6.45 nm). By applying the exclusion criterion, MELSA 8 was again considered a model system.

DLS analysis was accompanied by **polydispersity index (PDI)** measurement and microemulsion characterization in terms of **electrokinetic zeta potential**. The PDI values ranged from 0.039-0.557 and defined two groups of systems: monodisperse, with values between 0.039-0.119 and a group of polydisperse systems with PDI values reaching the maximum value of 0.557. In this case, it was suggested that further studies are needed to relate PDI to microemulsion composition by applying more elaborate analysis models.

On the other hand, the zeta potential was found with negative values close to 0 which were justified by the presence of non-ionic surfactants. It has been proposed that the stabilization process of microemulsions can be achieved by other types of repulsive forces than electrostatic ones, such as steric repulsions.

Finally, the study of the surface properties of topical oil-in-water microemulsions was carried out using the **CAM 101 goniometer**. This step brought to the fore new elements in the field of knowledge of the internal behaviour of microemulsions in relation to solid surfaces, with particular relevance to the study of colloids.

As the goniometric study is timidly approached in the case of microemulsions, this analysis proposal supports in an original way the discovery of surface properties, defined by *testing the surface tension at the liquid-gas interface (γLG)* and the *contact angle (CA)*, by applying the two goniometric analysis models in accordance with the Young equation: the pendant drop model and the contact angle model.

The parameters γLG and CA were considered two relevant factors for the **study of the adhesion work** as a third response Y_3, involved in the definition of topical microemulsions. In the pendant drop model, γLG ranged from 32.57-37.36 mN/m, with values typical of stable systems with low surface tensions, favoured by the presence of the Tween 80/PG stabilising mixture, which further implies the presence of very low interfacial tensions at the oil/water interface.

On the other hand, in agreement with the CA model, γLG was studied as a parameter involved in the adhesion process. It was observed that the factor X_1 had a significant influence in generating contact angles between 40.74-57.91°, below 90°,

which defined a good wettability at the solid surface.

At the end of this study, the involvement of **oat oil** for solubilization of the active substance and promotion of strong adhesion processes was appreciated and can be analyzed by studying the surface properties. The study of adhesion properties made a connection between surface parameters and between physical parameters selected as critical quality attributes by highlighting their relevance for topical administration.

In conclusion, **the MELSA 8 system was close to the optimal systems generated from the predictive analyses, and met the critical quality attributes investigated. The system was formulated with Tween 80/PG 40%, lecithin 0.3%, oat oil 2%, salicylic acid 0.5%, hyaluronic acid 1% and water 56.2%**. This type of microemulsion was considered an interesting system to pursue, with potential for development as a preparation for topical delivery of a dermatological active substance such as salicylic acid.

This type of microemulsion can be further adapted to integrate both hydrophilic and lipophilic active substances with respect and attention to the solubilising potential of the components. Their study is all the more challenging as research advances towards the use of the microemulsion technique to obtain nanoparticle systems with potential in the dermatological sphere.

Through the subject of study and the results obtained from the experimental pathway, the PhD thesis "Design and evaluation of microemulsions as modern colloidal drug delivery and delivery systems for anti-acne treatment" offers an original perspective on the study of microemulsions as biocompatible nanocolloidal systems for topical delivery of acne drugs.

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Dissemination of research results

The results presented in this PhD thesis were materialized in 2 articles published in ISI-listed international journals, 1 article published in Proceedings volumes of ISI-indexed scientific events and 1 article published in Proceedings volumes of BDI-indexed scientific events. Part of the data accumulated during the years of doctoral study were presented at national scientific events, which will be presented below.

Published works on the topic of the PhD thesis

M.C. Anicescu, C.E. Dinu-Pîrvu, M.T. Talianu, M.V. Ghica, V. Anuța, R.M. Prisada, A.C. Nicoară, L. Popa, Insights from a Box–Behnken optimization study of microemulsions with salicylic acid for acne therapy, *Pharmaceutics*, 2022, 14(1), 174, https://doi.org/10.3390/pharmaceutics14010174, ISSN 1999-4923, FI - 6,321/2020, https://www.mdpi.com/1999-4923/14/1/174

C.M. Anicescu, C.E. Dinu-Pîrvu, M.V. Ghica, M.T. Talianu, L. Popa, Preliminary study regarding the formulation and physical evaluation of some biocompatible, oil-in-water microemulsions with salicylic acid for dermatologic use, *Farmacia*, 2021, 69(3), 434-445, https://doi.org/10.31925/farmacia.2021.3.6, ISSN 0014-8237, FI - 1,433/2020. https://farmaciajournal.com/wp-content/uploads/art-06-Anicescu_Ghica_Popa_434-445.pdf

M.T. Talianu, L. Popa, M.V. Ghica, C.E. Dinu-Pîrvu, **C. Anicescu**, Design, formulation and evaluation studies of dermatocosmetic microemulsions based on oat oil, pomegranate oil and hyaluronic acid, *Proceedings of The Romanian National Congress of Pharmacy*, 17th Edition, Bucharest, Romania, 26-29 septembrie 2018, p. 218-222, ISBN 978-88-85813-28-1.http://apps.webofknowledge.com.ezproxy.medgrid.eu/ Diploma of Excellence - Presentation Award, Pharmaceutical Technology Section.

M.C. Anicescu, C.E. Dinu-Pîrvu, M.V. Ghica, V. Anuța, R.M. Prisada, M.T. Talianu, L. Popa, Preliminary analysis of emulsion based formulations containing pumpkin seed oil and hemp seed oil for internal use, *Proceedings of the 8th International Conference on Advanced Materials and Systems (ICAMS)*, Bucureşti, 1-3 octombrie 2020, p. 115-120, https://doi.org/10.24264/icams-2020.II.1, ISSN 2068-0783. http://icams.ro/icamsresurse/2020/files/lucrari/II_biomaterials_biotechnologies_01.pdf

Papers presented at national scientific events

M.C. Anicescu, L. Popa, M.V. Ghica, C.E. Dinu-Pîrvu, M.T. Talianu, Design and evaluation of lecithin/Tween 80 O/W microemulsions for a topical anti-acne drug delivery system: insights from a preliminary study, lucrare poster (ID 145) prezentată la *Congresul Universității de Medicină și Farmacie „Carol Davila”, ediția a 7-a,* Bucureşti, 10-12 octombrie 2019, volum de rezumate: *Supliment Maedica - a Journal of Clinical Medicine*, 2019, Vol.14, p. 34, ISSN 2501-6903.https://www.congresumf.ro/editia-2019/