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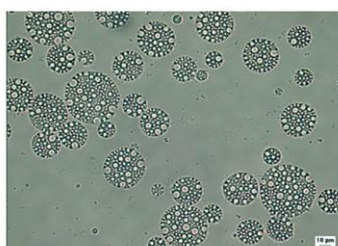
Application of the Fractional Factorial Design in Multiple W/O/W Emulsions

Dragana Vasiljevic¹, Jelena Djuris¹, Sergej Jakimenko¹, Svetlana Ibric¹

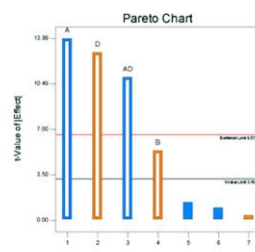
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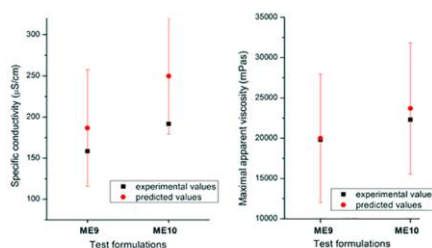
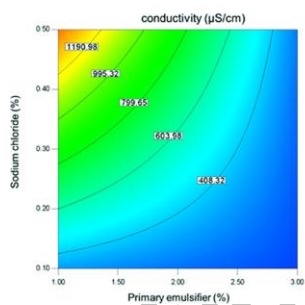
Graphical Abstract



Multiple W/O/W emulsions



Formulation development by using fractional factorial design



INTRODUCTION

Multiple emulsions (W/O/W or O/W/O) are complex dispersion systems, also known as “emulsions of emulsions”. These emulsion systems, at least in theory, have significant potential in the fields of cosmetics, pharmaceuticals and food industry to provide prolonged release of active substances, the possibility of combining incompatible substances in one product and/or protection of sensitive substances. In practice, however,

significant problems may arise because of their thermodynamical instability and strong tendency for coalescence, flocculation and creaming ^[1-5].

In the formulation development of multiple W/O/W emulsions, the following variables should be considered: (i) primary W/O emulsifier (usually a low HLB number polymer or surfactant), (ii) secondary O/W emulsifier (usually a high HLB number polymer or surfactant), (iii) secondary volume fraction, that is between 0.4 and 0.8 depending on the required viscosity (iv) nature of electrolyte, (v) thickener or additives, and (vi) processing of primary and secondary emulsions at high shear or low shear mixing rates, respectively. ^[6,7] As mentioned, successful development of a multiple emulsion formulation requires selection of the appropriate amount of several excipients, including emulsifiers and electrolytes. In order to avoid the traditional trial-and-error approach, which could potentially lead to preparation of numerous emulsion samples without necessarily reaching the optimal one, experimental design should be used for formulation development and optimization. Various experimental design techniques have been used for formulation development and/or optimization of W/O or O/W emulsions ^[8-11], whereas the application of experimental design for development of the more complex multiple emulsions is seldom. There are several examples of the application of experimental design in formulation development and/or optimization of multiple emulsions: stable water-in-oil-water cosmetic emulsions were developed according to the quality by design concept ^[12]; orthogonal experimental design was used to optimize formulation of water-in-oil-water emulsion for intestinal insulin delivery ^[13]; central

composite design was used for development of water-in-oil-water emulsion formulation with diclofenac sodium^[14], etc.

The aim of this work was to develop semi-solid multiple W/O/W emulsions for potential cosmetic or dermatopharmaceutical application by using fractional factorial design and evaluate the influence of the primary and secondary polymeric emulsifier concentrations (PEG 30-dipolyhydroxystearate and poloxamer 407, namely), as well as the type and electrolytes concentration (magnesium sulfate heptahydrate and sodium chloride) on the rheological properties, conductivity and physical stability after centrifugation of the multiple W/O/W emulsions.

MATERIALS AND METHODS

Materials

The oil phase consisted of caprylic/capric triglycerides (Myritol[®] 318, Fina, Belgium). The polymeric surfactants used were triblock copolymer of polyhydroxystearic acid / poly(ethylene oxide) / polyhydroxystearic acid (PEG 30-dipolyhydroxystearate, Arlacel[®] P135, ICI (now Croda), Kortenberg, Belgium), as primary lipophilic emulsifier and ethoxylated propylene oxide copolymer (Poloxamer 407, Lutrol[®] PE/F127, BASF, Ludwigshafen, Germany), as secondary hydrophilic emulsifier. The other substances used were magnesium sulfate heptahydrate (Centrohem, Belgrade, Serbia), sodium chloride (Sigma-Aldrich, Steinheim, Germany) and purified water.

Methods

Experimental Design

Multiple emulsions formulations were prepared according to a fractional factorial design set-up (software Design Expert, version 7.0.0; Stat-Ease, Inc., Minneapolis, MN, U.S.A.). A 2^{4-1} fractional factorial design was performed by varying the following variables on the two levels (-1 and +1): primary emulsifier (PEG 30-dipolyhydroxystearate) concentration (1% and 3% in primary, i.e. 0.8% and 2.4% in final W/O/W emulsions), secondary emulsifier (Poloxamer 407) concentration (0.8% and 1.2%), electrolyte magnesium sulfate heptahydrate (0.1% and 0.5% in primary, i.e. 0.08% and 0.4% in final W/O/W emulsions) and electrolyte sodium chloride (0.1% and 0.5% in primary, i.e. 0.08% and 0.4% in final W/O/W emulsions), as represented in Tables 1 and 2.

Preparation Of The Samples

The preparation method was a two-step procedure first proposed by Matsumoto et al. ^[15] The stirring was performed by laboratory mixer (Heidolph RZR 2020, Heidolph Elektro GmbH & Co., KG, Kelheim, Germany). For preparation of the primary emulsion (first step), oil phase consisted of the lipophilic emulsifier and caprylic/capric triglycerides while water phase contained purified water with dissolved electrolyte (magnesium sulfate heptahydrate or sodium chloride). Both oily and water phases were heated up to 80 ± 2 °C. Water phase was then added in small increments to the oily phase at 1000 rpm for 5 min. The mixing was continued at 1500 rpm until the emulsion temperature was approximately 25 °C. In the second step, the primary W/O emulsion was added slowly to the water phase (containing the hydrophilic emulsifier) while the system was stirred at

500 rpm at room temperature. After complete introduction of the primary emulsion the stirring was continued for 30 min.

Centrifugation Test

Centrifugation test was performed with 5 g of the samples at $1500 \times g$ using laboratory centrifuge MPW 56 (MPW, Warsaw, Poland). Samples were inspected for eventual phase separation after 30 min of centrifugation.

Conductivity Measurements

Conductivity was measured directly on the undiluted emulsions with Conductivity Meter CDM 230 (Radiometer, Copenhagen, Denmark) at 22 ± 1 °C, to examine the release of the electrolyte initially entrapped in the internal water phase.

Rheological Measurements

Rheological measurements were performed on rheometer Rheolab MC 120 (Paar Physica, Stuttgart, Germany), coupled with cone and plate measuring device MK 22 (diameter 50 mm, 1° angle, gap 50 μm), at 20 ± 0.2 °C. Values of maximal (at the shear rate 4 s^{-1}) and minimal (at the shear rate 200 s^{-1}) apparent viscosities and hysteresis area were used for the samples flow analysis.

All measurements were performed in triplicate, 72 h after preparation of the samples.

RESULTS AND DISCUSSION

Factorial analysis was performed to study the influence of the input parameters (Table 1) on emulsions properties.

The prepared W/O/W emulsions were white and homogenous systems, but the consistency varied from liquid lotion (ME3) to hard cream (ME6). Multiple characteristics of prepared emulsions were confirmed by an optical microscope (Figure 1). After centrifugation test, phase separation did not occur only in the samples ME2, ME6 and ME8, indicating better physical stability of these W/O/W emulsions (Table 3). Factorial analysis revealed that the separated phase volume is under the linear influence of concentrations of the primary emulsifier (PEG 30-dipolyhydroxystearate, parameter **A**) and magnesium sulfate heptahydrate (parameter **C**) used as electrolyte. The predominant factor influencing the phase separation is the concentration of the primary emulsifier. In numerical terms, the equation quantifying these effects stands as follows:

$$\begin{aligned} \text{Separated phase volume} &= 0.68 - 0.17 \times \mathbf{A} - 0.41 \times \mathbf{C} \\ R^2 &= 0.8778 \end{aligned} \tag{1}$$

Concentration of the primary emulsifier influences phase separation negatively (Eq. 1), meaning that the increase in the primary emulsifier concentration leads to the smaller volume of the separated phase. This observation is in agreement with the experimental findings that the samples prepared with the higher concentration of the primary emulsifier (2.4 % w/w) were the more stable (i.e. smaller volume of the separated phase was observed). The influence of magnesium sulfate heptahydrate concentration is also negative, meaning that the more stable emulsions were prepared using higher concentrations of this electrolyte.

Conductivity values of all primary emulsions (PE1 - PE8) were lower than 0.01 $\mu\text{S}/\text{cm}$. Conductivity values of investigated multiple emulsions ranged from 113.3 $\mu\text{S}/\text{cm}$ to 1563.3 $\mu\text{S}/\text{cm}$ (Table 3), which indicated that the change of emulsion type occurred in the second emulsification step. Differences in the conductivity of the multiple emulsions occurred as a consequence of electrolyte (magnesium sulfate heptahydrate or sodium chloride) release from the inner water phase in which it was initially incorporated. Rupture of some multiple droplets, which could occur in the second phase of the emulsification process, could lead to mixing of the outer water phase with some amount of the inner water phase ^[16]. It may be assumed that this phenomenon is more likely to occur in samples ME3 and ME5, indicating lower stability of these emulsions. Analysis of the significance of the influence of factors varied through experimental design (using ANOVA test) revealed that changes in conductivity are mainly affected by the changes in the concentration of the primary emulsifier (PEG 30-dipolyhydroxystearate, parameter **A**), sodium chloride (parameter **D**) and secondary emulsifier (Poloxamer 407, parameter **B**). Statistically significant ($p < 0.05$) interaction between the primary emulsifier and sodium chloride concentrations (parameters **A** and **D**) was also observed. Ranking of the significance of influences is represented in Figure 2. Conductivity is predominantly affected by concentrations of the primary emulsifier and sodium chloride used as electrolyte.

Quantitatively, the dependence of the conductivity on the formulation factors can be represented with the following equation:

$$\begin{aligned} \text{Conductivity} &= -559.04 + 55.06 \times \mathbf{A} + 585.62 \times \mathbf{B} + 3810.62 \times \mathbf{D} - 1200.62 \times \mathbf{A} \times \mathbf{D} \\ \mathbf{R}^2 &= 0.9942 \end{aligned} \quad (2)$$

The influence of parameters A, B and D on emulsions conductivity is linear and positive, meaning that the conductivity increases with the increase in their respective concentrations.

Figure 3. represents the influence of the primary emulsifier and sodium chloride concentration on the conductivity of the studied multiple emulsions. Negative interaction between the factors **A** and **D** can be interpreted according to the Eq. 2, implying that changes in the sodium chloride concentration greatly affect the conductivity only if the primary emulsifier concentration is at its lower value (0.8 % w/w). If the primary emulsifier concentration is at its higher value (2.4 % w/w) changes in sodium chloride concentration do not have influence on the conductivity. This conclusion is in agreement with the experimental findings for less stable samples ME3 and ME5. Therefore, concentration of the primary emulsifier needs to be carefully selected if sodium chloride is used as electrolyte, since conductivity might be greatly affected. This phenomenon was not observed for electrolyte magnesium sulfate heptahydrate. It could be assumed that magnesium sulfate heptahydrate stabilized investigated W/O/W emulsions prepared with 0.8 % primary emulsifier PEG-30 dipolyhydroxystearate more efficiently than sodium chloride. The other authors ^[17] concluded that sodium chloride may destabilize W/O/W emulsions.

The results of the steady-state rheological measurements have shown that all the investigated samples exhibited non-Newtonian thixotropic behavior, as demonstrated by the shear stress-shear rate curves given in Figures 4 and 5. The download curve is below

the upward curve, indicating thixotropy in the system. In such a case, the shear stress induces structural changes, i.e., a break of multiple droplets – the maximum shear produces a decrease in the volume fraction, which results in a decrease in the viscosity [18].

Values of the maximal and minimal apparent viscosities and hysteresis area of the investigated W/O/W multiple emulsions are given in Table 4.

Maximal apparent viscosity (at the shear rate of 4 s^{-1}) of multiple emulsions varied from 421 mPas (ME3) to 45433 mPas (ME6) (Table 4). The rheological characteristics of the investigated W/O/W emulsions were markedly influenced by the concentration of lipophilic and hydrophilic emulsifiers as well as by the type and concentration of the electrolyte.

Factorial analysis of the formulation factors influence on the maximal apparent viscosity of the studied multiple emulsions revealed that the maximal apparent viscosity is predominantly affected by changes in the primary emulsifier and magnesium sulfate heptahydrate concentration. Influence of the primary emulsifier and magnesium sulfate heptahydrate concentration on the maximal apparent viscosity of the investigated W/O/W emulsions is given in Figure 6. As represented in Eq. 3, interaction between these two parameters was also observed.

$$\begin{aligned} \text{Maximal apparent viscosity} &= 2520.41 - 1555.41 \times \mathbf{A} - 30244.14 \times \mathbf{C} + 35467.49 \times \mathbf{A} \times \mathbf{C} \\ R^2 &= 0.8805 \end{aligned} \quad (3)$$

Positive interaction between the primary emulsifier and magnesium sulfate heptahydrate concentration can be interpreted in the following manner: greatest changes in the maximal apparent viscosity can be expected when the primary emulsifier concentration is at its higher value (2.4 % w/w) and the concentration of magnesium sulfate heptahydrate is varied.

Identified interactions between the effects of primary emulsifier and electrolytes are important since this highlights that concentrations of these ingredients in multiple emulsions cannot be independently varied. Furthermore, these interactions are also important for development of multiple emulsions of optimal properties.

Two test formulations were prepared (ME9 and ME10) in order to evaluate the model equations (Eqs. 1 – 3) obtained through experimental design. The goal was to obtain semi-solid multiple emulsions, of the desired maximal viscosity ranging from 20000 to 35000 mPas, with the minimal values of the conductivity and the separated phase volume. The composition of the test formulations were proposed by the software used for analysis of experimental design data, and are represented in Table 5.

Prepared formulations (ME9 and ME10) were semi-solid emulsions. Low values of conductivity (158.4 $\mu\text{S}/\text{cm}$ and 191.7 $\mu\text{S}/\text{cm}$; Table 6) and no phase separation after centrifugation were observed, which is in agreement with the predefined conditions. Experimentally obtained and predicted responses (for separated phase volume, conductivity and maximal apparent viscosity) are compared in Table 6 and Figure 7.

Table 6 and Figure 7 demonstrate good agreement of the experimentally obtained results and multiple emulsions properties predicted by the models generated through experimental design. These results support further application of the experimental design in the development of complex systems such as multiple emulsions.

CONCLUSIONS

The concentration of the primary emulsifier had the greatest impact on the investigated parameters of W/O/W emulsions. Obtained results indicated that magnesium sulfate heptahydrate was more efficient as electrolyte stabilizing these systems, compared to sodium chloride. The applied factorial design method enabled determination of the optimal concentrations of the primary lipophilic emulsifier (PEG-30 Dipolyhydroxystearate) and secondary hydrophilic emulsifier (Poloxamer 407), as well as the concentration of electrolytes (magnesium sulfate heptahydrate and sodium chloride), in order to obtain semi-solid W/O /W emulsions for potential use in cosmetics or dermatopharmacy. Optimized formulations of multiple emulsions had desired maximal apparent viscosities and low values of conductivity. After centrifugation test, phase separation does not occur in these samples. These results support further application of the experimental design in the development of complex systems such as multiple emulsions.

ACKNOWLEDGEMENT

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Table 1.

Parameter	Low level (-1)	High level (+1)
A – Primary emulsifier (PEG 30-dipolyhydroxystearate), % w/w	0.8	2.4
B – Secondary emulsifier (Poloxamer 407), % w/w	0.8	1.2
C – Magnesium sulfate heptahydrate, % w/w	0.08	0.4
D – Sodium chloride, % w/w	0.08	0.4

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Table 2.

Primary W/O emulsion	PE1	PE2	PE3	PE4	PE5	PE6	PE7	PE8
Caprylic/Capric Triglycerides	19.0	17.0	19.0	17.0	19.0	17.0	19.0	17.0
PEG 30-dipolyhydroxystearate	1.0	3.0	1.0	3.0	1.0	3.0	1.0	3.0
Magnesium sulfate heptahydrate	0.1	0.1	0.1	0.1	0.5	0.5	0.5	0.5
Sodium chloride	0.1	0.5	0.5	0.1	0.5	0.1	0.1	0.5
Purified water	79.8	79.4	79.4	79.8	79.0	79.4	79.4	79.0
Multiple W/O/W emulsion	ME1	ME2	ME3	ME4	ME5	ME6	ME7	ME8
Primary W/O emulsion	80.0	80.0	80.0	80.0	80.0	80.0	80.0	80.0
Poloxamer 407	0.8	0.8	1.2	1.2	0.8	0.8	1.2	1.2
Purified water	19.2	19.2	18.8	18.8	19.2	19.2	18.8	18.8

Table 3.

	ME1	ME2	ME3	ME4	ME5	ME6	ME7	ME8
Separated phase volume (ml)	0.5	0	0.5	0.2	0.2	0	0.35	0
Conductivity ($\mu\text{S}/\text{cm}$)	224.3	222.0	1563.3	312.0	1210.0	113.3	461.0	370.3

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Table 4.

	ME1	ME2	ME3	ME4	ME5	ME6	ME7	ME8
Maximal apparent viscosity (mPas)	2553	9540	421	1400	3937	45433	3216	26433
Minimal apparent viscosity (mPas)	272	1193	139	234	309	2257	355	1753
Hysteresis area (Pa/s)	1414	1188	690	69	358	10831	2458	6759

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Table 5.

Formulation factors	ME9	ME10
A – Primary emulsifier (PEG 30-dipolyhydroxystearate), % w/w	1.84	2.19
B – Secondary emulsifier (Poloxamer 407), % w/w	0.86	0.99
C – Magnesium sulfate heptahydrate, % w/w	0.33	0.30
D – Sodium chloride, % w/w	0.09	0.12

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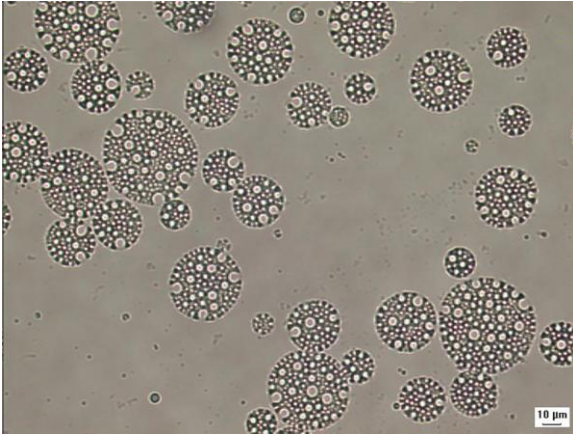
Table 6.

Output parameters (responses)	ME9		ME10	
	Experimental values	Predicted values	Experimental values	Predicted values
Separated phase volume (ml)	0	0.12 ± 0.10	0	0.06 ± 0.10
Conductivity ($\mu\text{S}/\text{cm}$)	158.4	$186.6 \pm$ 70.88	191.7	$249.7 \pm$ 70.40
Maximal apparent viscosity (mPas)	19800	$19989 \pm$ 7976.9	22300	$23695 \pm$ 8134.3

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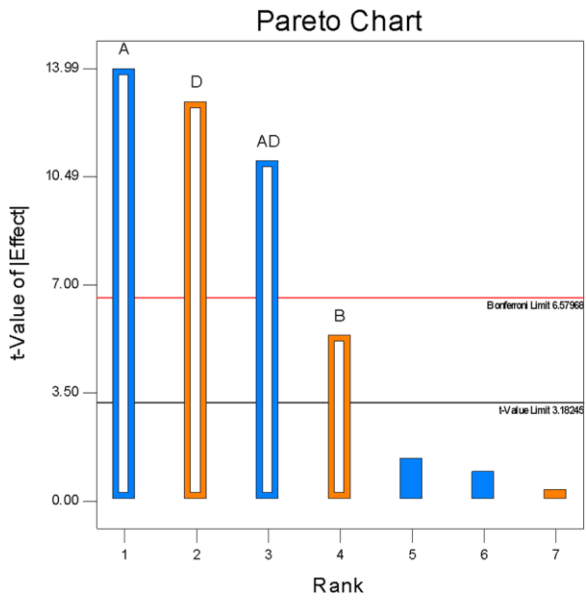
Figure 1. Photomicrograph of multiple emulsion ME1, 72 h after preparation

(magnification 400×)



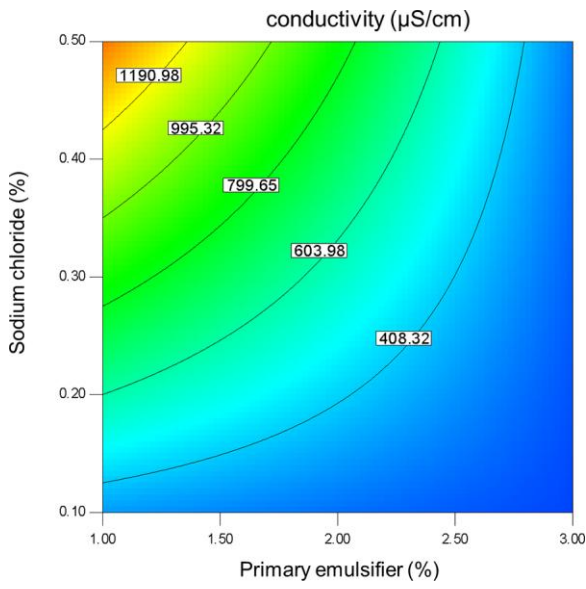
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Figure 2. Significance of the input parameters influence on the conductivity values ($\mu\text{S}/\text{cm}$)



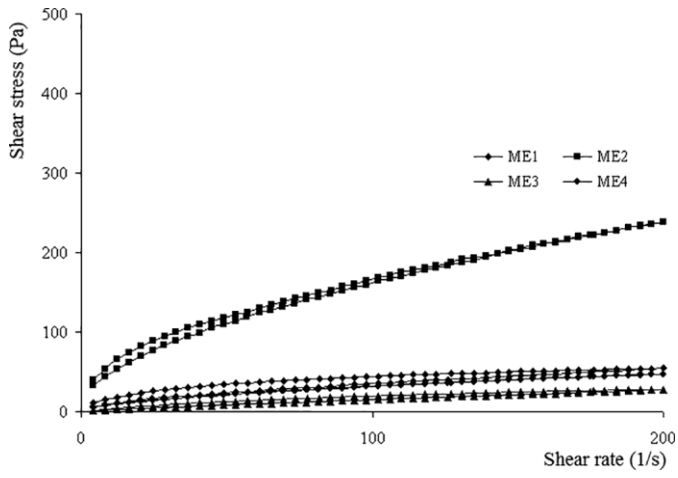
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Figure 3. Influence of the primary emulsifier and sodium chloride concentration on the conductivity ($\mu\text{S}/\text{cm}$)



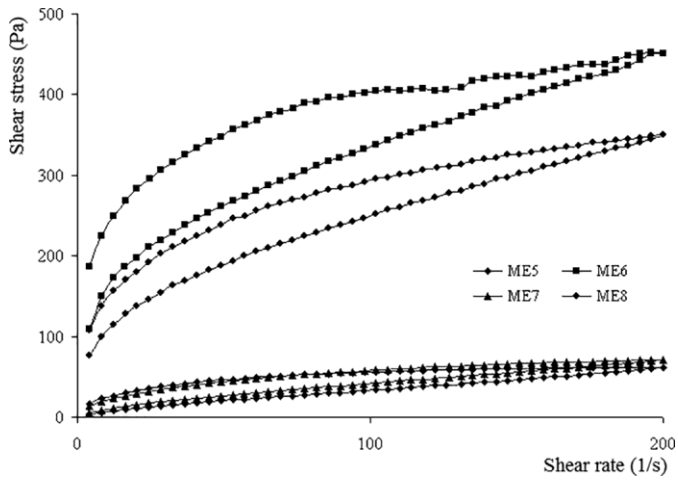
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Figure 4. Flow curves of the W/O/W multiple emulsions ME1 - ME4



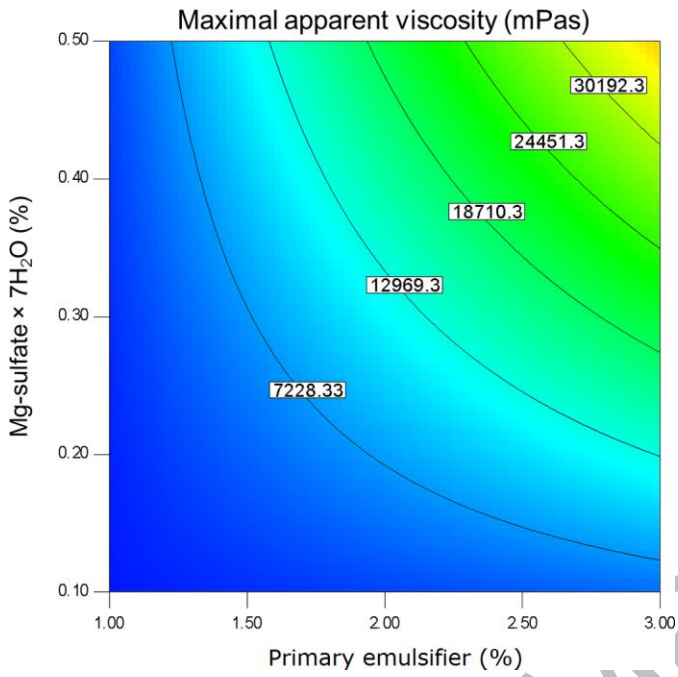
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Figure 5. Flow curves of the W/O/W multiple emulsions ME5 – ME8



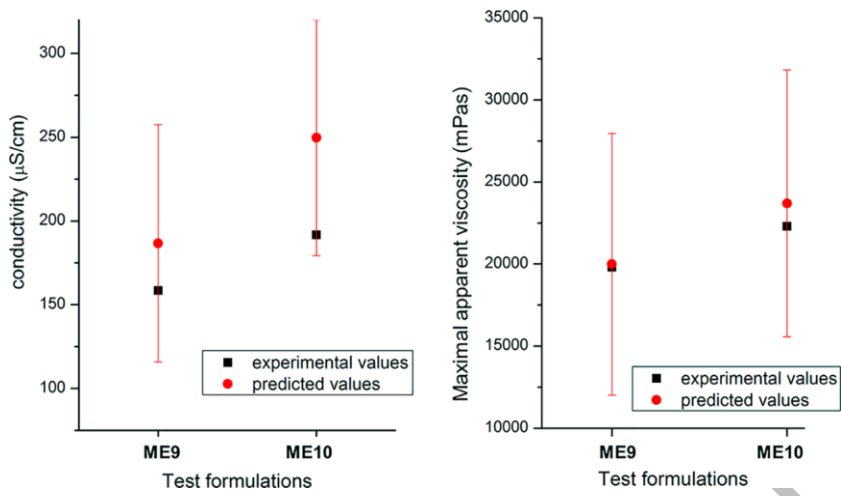
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Figure 6. Influence of the primary emulsifier and magnesium sulfate heptahydrate concentration on the maximal apparent viscosity (mPas)



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Figure 7. Comparison of experimentally obtained and predicted values for conductivity and maximal apparent viscosity of test formulations ME9 and ME10



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