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Emulsion Formulations: Study of the Influence of Parameters with Experimental Designs

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ABSTRACT

Parameters of formulation of emulsions were performed by the aid of experimental designs. The aim of the work was to investigate five factors at two levels: temperature of manufacture, time of phase introduction, rate of homogenization, mode of cooling, and operators. The study of the flow behavior of the emulsions corresponds to the quantitive response. This work allowed us to show the dominating influence of the factor "mode of cooling." The progressive cooling at room temperature gives a better stability than the brutal cooling with a water bath corresponding to a shorter homogenization. A further aim was to study the influence of an additional factor: the nature of the surfactant with the best mode of cooling, progressive cooling. In that case, we can conclude that the main factor, the nature of the surfactant, influences the response.

INTRODUCTION

Many authors have studied the influence of different parameters in emulsification process (1–3), but a classical approach to many experimentations is to investigate the effects of one experimental variable while keeping all others constant. In our work important parameters can be identified and their relative importance assessed. Several factors may affect lipophilic/hydrophilic (L/H) emulsion properties. In screening experiments, the aim of which is to investigate possibly influential and possibly interacting factors, two-level factorial experiments are a powerful tool. We chose a 2^5 design considering that the potential for detection of interesting effects or interactions is much greater with four or five factors than with less. Considering the large size of the experi-

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ment, designs are unreplicated and a different approach of data treatment is adopted (4). First, temperature of manufacture, rate of homogenization, time of phase introduction, mode of cooling, and operators' influence were studied by a two-level full factorial design. Then, with the best conditions found in the preliminary, we studied the influence of the surfactant nature.

MATERIALS AND METHODS

Materials

Nonionic surfactants: sorbitan monooleate (Montane 80, Seppic), polyoxyethylene (20) sorbitan monooleate (Montanox 80, Seppic), polyoxyethylene (2) oleyl ether (Brij 92, ICI), and polyoxyethylene (20) oleyl ether (Brij 98, ICI). Two binary mixtures, Montane 80/Montanox 80 and Brij 92/Brij 98, are used proportionately to 45–55 (W-W) and 70–30 (W-W) respectively.
Paraffin liquid, cinematic viscosity 40°C 70 cSt (Primol 352, Esso).
Glycerol monostearate (Prolabo).
Water freshly distilled.

Instruments

Emulsions were prepared with a homogenizer (Turbotest 33/300, Rayneri). The flow behavior of emulsions was studied with a Rheometer (Rheomat 30, Contraves).

Preparation of L/H Emulsions

The method of phase inversion is used to prepare emulsions (5). The oily phase is composed of 20% paraffin liquid, 5% surfactant mixture, and 1% glycerol monostearate. Both the aqueous and oily phase were warmed at a temperature near 75°C. The aqueous phase is then added to the oily phase and mixed by a homogenizer. About 300 g of emulsion were prepared for each test.

Experimental Design

In a first step we used a 2^5 experiment: five factors—X_1, X_2, X_3, X_4, X_5—each at two levels (+1, −1), were investigated; 32 trials were necessary for this full factorial design (6). A factor is an assigned variable and the levels of the factor are the values assigned to the factor. In a second step a 2^4 experiment was studied, with four factors—X_2, X_3, X_4, X_6—each also at two levels (+1, −1).

Studied Factors

The choice of factors to be included in the experimental design is considered carrefully and the procedure is as follows:

The mode of cooling is designated as factor X_1: Two modes of cooling are tested. The first one is a progressive cooling (PC) corresponding to 45 minutes of homogenization at room temperature (25°C); the second one is a brutal cooling (BC) with a cold water bath (15°C) corresponding to 30 minutes of homogenization.
Time of phase introduction (X_2): The aqueous phase is added in oily phase at once (3 seconds) or drop by drop (60 seconds).
Rate of homogenization (X_3): The intensity of agitation may be important, and we used two levels corresponding to 300 rpm and 500 rpm.
Temperature of manufacture (X_4): Two temperatures are selected: 70°C and 80°C.
Operators (X_5): Two skilled operators (op1 and op2) realized the experimental design.
Surfactants (X_6): Montane 80/Montanox 80 (S1) and Brij 92/Brij 98 (S2).

Measurement of Responses: Rheological Properties of *Emulsions*

In order to show the flow behavior of emulsions at a constant temperature of 22 ±1°C we have drawn all the rheograms. To quantify the rheology of fluids, the shearing stress is measured as a function of the rate of shear and we used a model usually attributed to Oswald: the power law fluid model (7). The equation of the model may be written $\tau = KD^n$ with τ shearing stress, D rate of shear. In the logarithmic equation $\log \tau = \log K + n\log D$, where n is the slope, and $\log K$ is the intercept. Nevertheless, the flow curves obtained are very similar, and for all emulsions we have found the coefficient $n = 0.5 \pm 0.1$ corresponding to pseudoplastic fluids. Some differences were found between the emulsions for the coefficient K. This coefficient corresponds to the quantitative response used for the experimental design.

Execution of Experiment and Calculation

All experiments for the factorial design were performed in random order and the calculation was obtained by the NEMROD program (8).

RESULTS AND DISCUSSION

Factorial Design: 2^5

The table of the experiment is then drawn up in standard order (Table 1). A 2^5 type experimental design is first built in order to calculate a first-order polynomial including a constant, b_0 corresponding to the grand mean:

$$\begin{aligned}Y = {} & b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_5X_5 \\ & + b_6X_1X_2 + b_7X_1X_3 + b_8X_2X_3 + b_9X_1X_4 \\ & + b_{10}X_2X_4 + b_{11}X_3X_4 + b_{12}X_1X_5 + b_{13}X_2X_5 \\ & + b_{14}X_3X_5 + b_{15}X_4X_5 + b_{16}X_1X_2X_3 \\ & + b_{17}X_1X_2X_4 + b_{18}X_1X_3X_4 + b_{19}X_2X_3X_4 \\ & + b_{20}X_1X_2X_5 + b_{21}X_1X_3X_5 + b_{22}X_2X_3X_5 \\ & + b_{23}X_1X_4X_5 + b_{24}X_2X_4X_5 + b_{25}X_3X_4X_5 \\ & + b_{26}X_1X_2X_3X_4 + b_{27}X_1X_2X_3X_5 \\ & + b_{28}X_1X_2X_4X_5 + b_{29}X_1X_3X_4X_5 \\ & + b_{30}X_2X_3X_4X_5 + b_{31}X_1X_2X_3X_4X_5\end{aligned}$$

Table 1

Factorial Design (2^5): Variables, Their Levels, Matrix and Data of the Response K

Run	X_1	X_2	X_3	X_4	X_5	K	Factors	Levels	
								−	+
1	−	−	−	−	−	5.60	X_1 mode of cooling	BC	PC
2	+	−	−	−	−	19.68	X_2 time of phase introduction (sec)	3	30
3	−	+	−	−	−	6.11	X_3 rate of homogenization (rpm)	300	500
4	+	+	−	−	−	8.11	X_4 temp. of manufacture (°C)	70	80
5	−	−	+	−	−	2.36	X_5 operators	op1	op2
6	+	−	+	−	−	16.21			
7	−	+	+	−	−	4.17			
8	+	+	+	−	−	17.94			
9	−	−	−	+	−	14.17			
10	+	−	−	+	−	22.36			
11	−	+	−	+	−	9.48			
12	+	+	−	+	−	18.84			
13	−	−	+	+	−	5.10			
14	+	−	+	+	−	14.44			
15	−	+	+	+	−	3.83			
16	+	+	+	+	−	17.07			
17	−	−	−	−	+	7.60			
18	+	−	−	−	+	15.25			
19	−	+	−	−	+	6.74			
20	+	+	−	−	+	9.88			
21	−	−	+	−	+	4.39			
22	+	−	+	−	+	7.98			
23	−	+	+	−	+	3.86			
24	+	+	+	−	+	19.57			
25	−	−	−	+	+	9.49			
26	+	−	−	+	+	14.80			
27	−	+	−	+	+	9.10			
28	+	+	−	+	+	18.17			
29	−	−	+	+	+	5.85			
30	+	−	+	+	+	13.50			
31	−	+	+	+	+	6.90			
32	+	+	+	+	+	7.81			

Table 2

Main Effects and Interaction Effects Calculated from Factorial Design 2^5

b_j	Estimates	b_j	Estimates
b_1	4.27	b_{17}	0.21
b_2	−0.35	b_{18}	−0.65
b_3	−1.39	b_{19}	−0.94
b_4	1.11	b_{20}	0.36
b_5	−0.77	b_{21}	−0.44
b_6	−0.07	b_{22}	−0.46
b_7	0.60	b_{23}	0.11
b_8	1.06	b_{24}	−0.23
b_9	−0.33	b_{25}	0.37
b_{10}	−0.18	b_{26}	−1.14
b_{11}	−1.23	b_{27}	−0.27
b_{12}	−0.96	b_{28}	−0.87
b_{13}	−0.55	b_{29}	−0.24
b_{14}	−0.07	b_{30}	−0.62
b_{15}	−0.46	b_{31}	−0.56
b_{16}	0.65	$\mathbf{b_0}$	**10.83**

The contrasts (b_j) of the five factors and the contrasts of all interactions can be calculated and reported in Table 2.

The absence of replication precludes a classic statistical analysis, so two proper tools are used for interpretation of data.

Graphical Treatment of Data

This method of analysis consists of constructing a normal plot of the contrasts (9–11). The effect-sparsity principle suggests that the active contrasts will tend to show up as outliers. If all effects represent noise, we should expect them to fit to a straight line. The N effects are ranked from the lowest to the highest value, where J = rank and $F(b_j) = (J - 0.5)/100$.

Figure 1 shows the effect b_1 as outstanding. The outlier main effect b_1 is the mode of cooling. The disadvantage of the normal plot method is that its interpretation is somewhat subjective, so that this interpretation is supplemented by another method to confirm the results.

Lenth's Proposed Method

Lenth (12) propose an effective alternative method for formal analysis of unreplicated factorials. This method is based on a simple formula for the standard error of the contrast estimates. The usual t procedures can be used to interpret the results. In the usual setting, the b_j values are independent realizations of N random variables, that is, the sampling distributions of the b_j are normal. Let

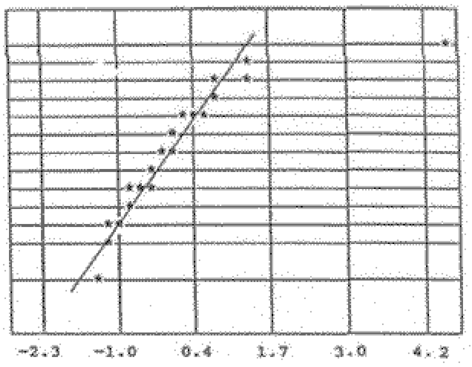

Figure 1. Experimental design 2^5. Normal plot of the effects.

$$s = 1.5 \times \text{median}\ (\ |b_j|\ )$$

The pseudo standard error (PSE) of the contrasts is

$$\text{PSE} = 1.5 \times \text{median}\ (\ |b_j|\ ) = 0.76$$

and in this case the median is taken over a restricted set of inlying $|b_j|$'s. A margin of error (ME) for b_j can be calculated with approximately 95% confidence.

$$\begin{aligned}\text{ME} &= \pm\, t_{(\alpha,\, df)} \times \text{PSE} = \pm\, t_{(0.975,\, 10)} \times 0.76\\ &= \pm\, 1.67\end{aligned}$$

Lenth uses degrees of freedom $df = m/3 = 31/3 = 10$ where m is the number of effects. With a large number of contrasts, one can expect one or two estimates of inactive contrasts to exceed the ME, leading to false conclusions. So a simultaneous margin of error (SME) is defined:

$$\begin{aligned}\text{SME} &= t_{(\gamma,\, df)} \times \text{PSE} = 2.69 \text{ with } \gamma\\ &= (1 + 0.95^{1/m})\end{aligned}$$

Estimates that fall inside the inner limit could be described as inactive. Only the contrast b_1 with a value of 4.27 is probably active because it falls outside the outer limits. Figure 2 shows the results graphically.

This second method confirms the first result, where only the main factor b_1 mode of cooling influences the response. There is a drastic difference in behavior between the progressive and the brutal mode of cooling shown by the study of flow behavior in Figure 3 (with only one operator). A supplemented study with micro-

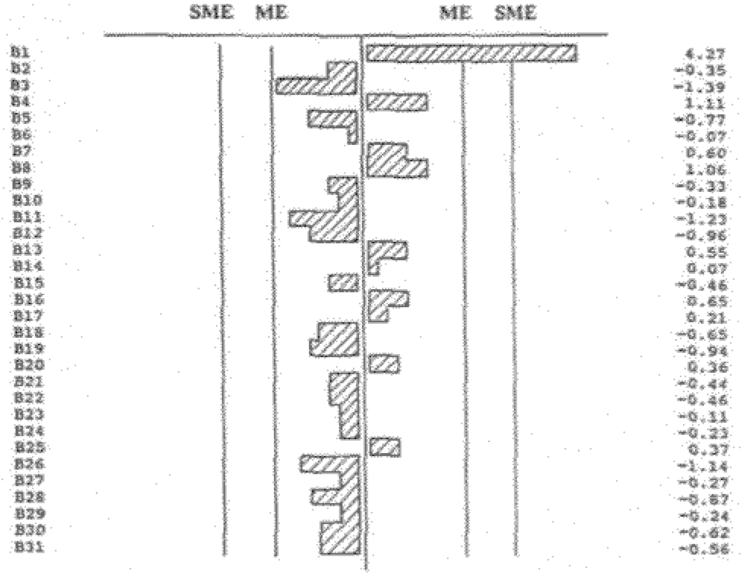

Figure 2. Experimental design 2^5. *Graphical treatment of Lenth's proposed method.*

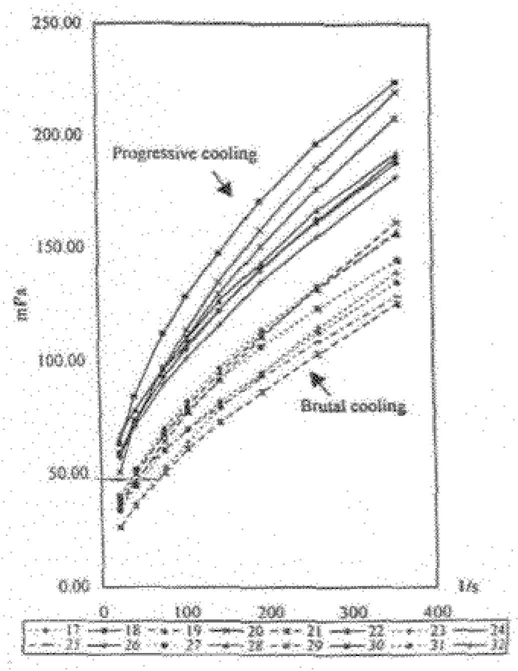

Figure 3. Flow behavior of emulsions. One operator and two modes of cooling.

scopic controls also shows a size particle polydispersity only with the brutal cooling. This globule size dispersity influences flow properties, thus lowering the response of viscosity and giving a bad stability. On the contrary, with progressive cooling the spread of size about mean diameter is narrow and stability is better. The other factors do not influence the response and one can note that the operators' effect is very weak. All these results led us to choose the progressive cooling for the following study, and allowed us to work with only one operator.

Factorial Design: 2^4

The second part of this study has been devoted to investigate one more factor: the nature of the surfactant. The table of the experiment is then drawn up in standard order (Table 3).

$$\begin{aligned} Y' = {} & b'_0 + b'_1X_2 + b'_2X_3 + b'_3X_4 + b'_4X_6 \\ & + b'_5X_2X_3 + b'_6X_2X_4 + b'_7X_3X_4 + b'_8X_2X_6 \\ & + b'_9X_3X_6 + b'_{10}X_4X_6 + b'_{11}X_2X_3X_4 \\ & + b'_{12}X_2X_3X_6 + b'_{13}X_2X_4X_6 + b'_{14}X_3X_4X_6 \\ & + b_{15}X_2X_3X_4X_6 \end{aligned}$$

The contrasts (b'_j) of the four factors and the effects of all interactions can be calculated and reported in Table 4.

Table 3

Factorial Design (2^4): Variables, Their Levels, Matrix, and Data of the Response K

							Levels	
Run	X_2	X_3	X_4	X_6	K	Factors	−	+
1	−	−	−	−	1.876	X_2 time of phase introduction (sec)	3	30
2	+	−	−	−	1.714	X_3 rate of homogenization (rpm)	300	500
3	−	+	−	−	1.259	X_4 temp. of manufacture (°C)	70	80
4	+	+	−	−	0.726	X_6 surfactant's mixture	S1	S2
5	−	−	+	−	2.48			
6	+	−	+	−	2.56			
7	−	+	+	−	1.051			
8	+	+	+	−	2.080			
9	−	−	−	+	15.25			
10	+	−	−	+	9.88			
11	−	+	−	+	7.98			
12	+	+	−	+	19.57			
13	−	−	+	+	14.80			
14	+	−	+	+	18.17			
15	−	+	+	+	13.50			
16	+	+	+	+	7.81			

Graphical Treatment of Data

The normal plots of the effects are given in Figure 4. This graphical treatment shows the effect b_4 as outstanding. b_4 corresponds to the effect of the surfactants.

Lengh's Proposed Method

In this method PSE = 0.74, ME = $\pm t_{(0.975,\ 5)} \times 0.74 = \pm 1.90$ and SME = 2.95 with $\gamma = (1 + 0.95^{1/15})$. Only the main contrast b_4 (effect of the surfactants) with the value of 5.83 is probably active and Figure 5 shows the result graphically. For the factor surfactants, the low level is Montane 80/Montanox 80, and the high level is Brij 92/Brij 98. For the binary mixture, Montane 80/Montanox 80, all emulsions have a lower reponse of viscosity and Figure 6 illustrates the rheological behavior of 2^4 experiments.

Table 4

Main Effects Caculated from Factorial Design 2^4 and Their Interactions

b'_j	Estimates	b'_j	Estimates
b'_1	0.270	b'_9	−0.358
b'_2	−0.797	b'_{10}	−0.062
b'_3	0.262	b'_{11}	−1.544
b'_4	5.826	b'_{12}	+0.458
b'_5	0.530	b'_{13}	−0.647
b'_6	−0.421	b'_{14}	−0.861
b'_7	−0.899	b'_{15}	−1.709
b'_8	0.218	b'_0	7.544

CONCLUSION

When a method of formulation is developed to be used in different environments, it is necessary to know possible consequences of this. In the cases studied here, the effects of changing conditions [time of phase intro-

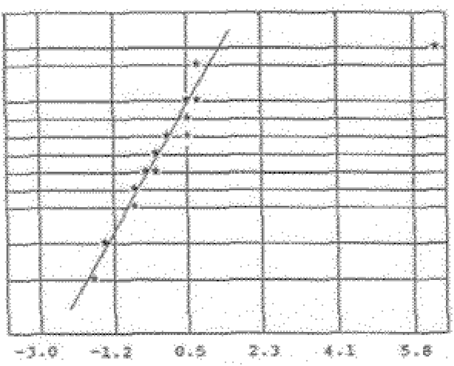

Figure 4. Experimental design 2^4. Normal plot of the effects.

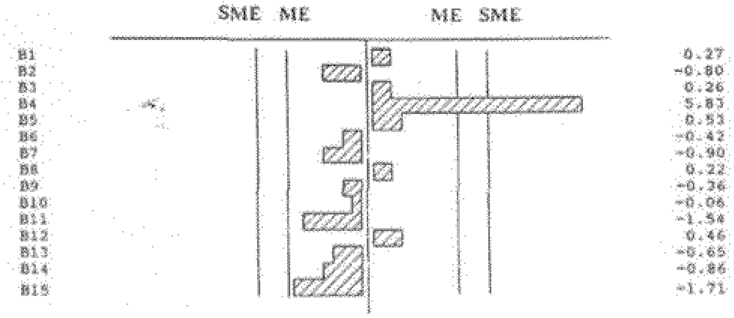

Figure 5. Experimental design 2^4. Graphical treatment of Lenth's proposed method.

duction (3–30 secondes), rate of homogenization (400–500 rpm), and temperature of manufacture (70–80°C)] have no influence on the response. The effect of factor operator is also insignificant. Nevertheless, some factors show a more important influence. A progressive mode of cooling leads to emulsions with higher viscosity, better homogeneity, and better stability than a brutal mode of cooling does. Moreover, the factor *nature of the surfactant* prevails over the other factors and the changing conditions of manufacture become negligible. We must also keep in mind that the binary mixture Montane 80/Montanox 80 has a low viscosity. And as the brutal mode of cooling entails a lowering of viscosity, one might expect that it causes the instability of the emulsion with Montane 80/Montanox 80 made in those conditions. In conclusion, we could say that independently of the nature of the surfactant, the progressive cooling seems to be a better method.

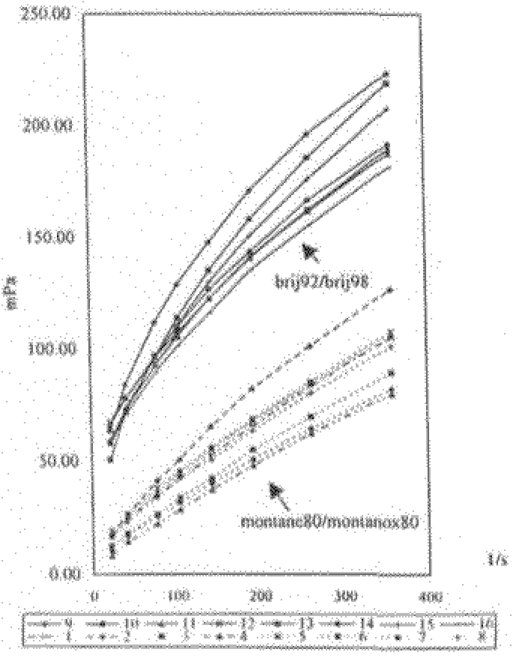

Figure 6. Flow behavior of emulsions at progressive cooling.

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