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Formulation and evaluation of o/w emulsions using experimental design

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Abstract

The aim of this work is to apply factorial design methods to reduce the number and time of the various steps required to formulate and evaluate stable emulsions. A preliminary study was performed to optimize the most accurate operating parameters. These operating conditions were applied to the determination of the required HLB of o/w emulsion. A second experimental design was then applied and emulsion stability was estimated by the average size of the droplets, the emulsion viscosity and the emulsion conductivity. In the case of average diameter, the best emulsion corresponds to the smallest particle size. For viscosity, a method is suggested to determine the required HLB. For conductivity, a graphic method was used. Experimental design allowed us to determine the required HLB with good approximation in five runs for the average diameter and viscosity studies while the conductivity study needed at least eight runs and confirmed the results obtained with the first two methods. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Experimental design; Emulsion stability; Required HLB; Viscosity; Conductivity; Paraffin oil

1. Introduction

Emulsion formulation requires tedious study in order to check the most important parameters to obtain stable emulsions. Optimizing a process implies determination of the experimental conditions giving optimal performance (Carlotti et al., 1993). In the preliminary study, the problem can be defined as maximizing the stability of emulsions of predetermined components, corresponding to optimized process conditions. In the second stage, the optimal operating conditions will be applied and the research will concern the optimal ratio of

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emulsifiers, corresponding to the determination of the critical hydrophilic-lipophilic balance (critical HLB) of the emulsion.

The dilemma in formulation of an emulsion lies in the fact that the success of such unstable systems can be judged only after a long time. Usually, when stability problems occur, formulation tests may be extended for a very long period. Accelerated testing could be applied to avoid this problem. Many methods are in use to evaluate destabilization processes, but none is actually recognized (Curt, 1994). Emulsion stability is estimated by the average size of the droplets and the variation of emulsion viscosity. The smaller the emulsion droplet size and the smaller the viscosity variations, the better the stability of the system (Friberg and Goldsmith, 1968). Conductivity measurements have been performed in order to confirm the required HLB determination of the other methods.

2. Materials and methods

2.1. Materials

The following materials were used: Paraffin oil (Cooper, France); Gelucire 48/09 (Gattefosse, France); Sodium lauryl sulfate (Na-LS) (Cooper, France); Montanox 20 (Tween 20) (SEPPIC, France); Montane 60 (Span 60) (SEPPIC, France).

2.2. Methods

2.2.1. Instruments

The following were used: A Polytron type PT 20.00 homogenizer, a Brookfield Sinchroletric viscometer, a WTW (LF537) conductivity meter and the microscope with micrometer was from Wild Instruments.

2.2.2. Preparation of emulsions

The emulsions were prepared by the phase inversion technique. An excess of 5% water was added because of water loss due to evaporation.

The water phase containing the emulsifier was heated to 75°C ± 0.5 and added to the oily phase at 70°C ± 0.5 while stirring with a Centri deflocculated paddle. The emulsion was mixed at the same temperature for a fixed time and then the emulsion container placed in a water bath at 20°C and mixed at the same speed until its temperature reached 25°C. The emulsion was homogenized for 2 min at 15 000 rev./min, or this stage was omitted.

2.2.3. Optical microscopy (Groves and Freshwater, 1968)

The diameter of the droplets was measured with an optical microscope (× 400) equipped with a calibrated eyepiece micrometer. The mean diameter was calculated on the basis of at least 100 droplets with the formula: $d_m = \Sigma\, d_i n_i / \Sigma\, n_i$ where n_i is the number of droplets with diameter d_i.

All measurements were taken after 24 h.

2.2.4. Rheological study (Seillier, 1970)

Viscosity measurements (Brookfield) were made at constant temperature (25 ± 0.1°C) at 100 rev./min. The runs were carried out 1 h and/or 24 h after the preparation.

2.2.5. Conductivity measurements

Conductivity was measured according to Latreille and Paquin (1990). Conductivity is often used to determine the nature of the emulsion and to control its stability during time (Kato et al., 1985). In the case of o/w emulsions, different macro- and microscopic studies have shown that the optimal emulsion could be determined with the conductivity vs HLB curve, as the critical HLB corresponds to greater slope variation (Puisieux and Seillier, 1983).

3. Results and discussion

3.1. Determination of operating conditions

A factorial design was performed to optimize the stability of emulsions prepared with Na-LS as surfactant. All the emulsions contained 20% gelucire 48/09, 1% Na-LS and 79% distilled water. According to Tornberg and Hermansson (1977) and after preliminary studies, the variables se-

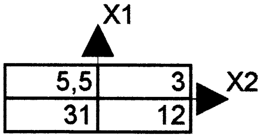
Table 1
Independent variables and their levels

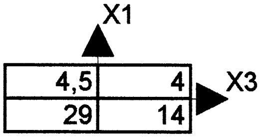
	Level (-)	Level (+)
Mixing rate (rev./min) (X_1)	500	900
Homogenization (X_2)	No	Yes
Mixing time (min) (X_3)	10	20

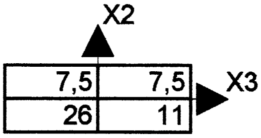
lected for investigation are listed in Table 1. Table 2 summarizes the runs and response values.

The main effects of the variables and their interactions were calculated and are listed in Table 3 (response Y_1) and Table 4 (responses Y_2 and Y_3).

Because of strong interactions, the variables were interpreted in pairs (interaction diagram; Sado and Sado, 1991).





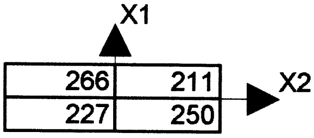


It is necessary to mix at 900 rev./min and homogenize the emulsion, but one can see that the energy given to the system by mixing is enough to obtain droplets with small diameter.

When X_1 is at its highest level, mixing time has no significant influence on the diameter of the droplets. When the emulsion is homogenized, mixing time has no effect.

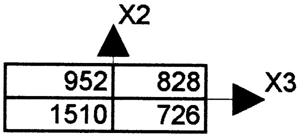
Homogenization allows the droplet size to decrease, but a 900 rev./min mixing seems to be enough. Moreover, the last technique reduces foam during the process.

Mixing time has a strong effect on viscosity (Y_2), viscosity decreases when mixing time increases. Interaction between X_1 and X_2 is relatively important, and these variables have to be interpreted together.



Emulsions will be better with X_1 and X_2 at their highest levels, but viscosity data show no significant difference, so it would be difficult make conclusions at this stage.

The viscosity measured after 24 h (Y_3) has to be minimum to be acceptable, because little variation generally tends to give stable emulsions. Mixing time has a strong effect on viscosity, but because of b_{23}, homogenization ought to be considered.



Between these two factors, mixing time is the main influence, because of greater variations due to the level change of X_3. Viscosity principally depends on mixing time; when the emulsion is

Table 2
Factorial design: experimental matrix

	X_1	X_2	X_3	X_1X_2	X_1X_3	X_2X_3	$X_1X_2X_3$	Y_1 (μm)	Y_2 (cP)	Y_3 (cP)
1	−	−	−	+	+	+	−	46	266	1847
2	+	−	−	−	−	+	+	6	252	1172
3	−	+	−	−	+	−	+	12	232	1040
4	+	+	−	+	−	−	−	3	244	864
5	−	−	+	+	−	−	+	16.5	188	716
6	+	−	+	−	+	−	−	5	280	736
7	−	+	+	−	−	+	−	12	267	960
8	+	+	+	+	+	+	+	3	178	696

Y_1: Average diameter of droplets; Y_2, Y_3: Viscosity measured at 1 and 24 h, respectively.

Table 3
Main effects calculated for response Y_1

Variable	X_1	X_2	X_3	X_1X_2	X_1X_3	X_2X_3
Average diameter	−8.7	−5.4	−3.8	4.2	3.6	3.8

Table 4
Main effects calculated for responses Y_2 and Y_3

Variable	X_1	X_2	X_3	X_1X_2	X_1X_3	X_2X_3
Viscosity (1 h) · Y_2	0.125	−8.13	−10.1	−19.4	0.625	2.375
Viscosity (24 h) · Y_3	−137	−114	−227	26.9	75.9	165

mixed for 20 min, droplets are well dispersed, giving higher stability.

These results allow us to set the different parameters:

1. Mixing rate has to be held at 900 rev./min;
2. Mixing time has to be held at 20 min.

Homogenization has no significant effect on responses, so it will be avoided. Furthermore this procedure will minimize the formation of foam.

3.2. Determination of required HLB

In this part, non-ionic emulsifiers were used. According to Seillier (1970), their ratios were fixed between 3% and 6%, typically 5%, in order to obtain stable emulsions at the required HLB. Optimized process parameters found in the preliminary study were applied at this stage. Even if these parameters were not optimal, in this case, we assume that they are suitable to characterize the required HLB of the emulsion which is the aim of this part.

The emulsions had the following composition: Paraffin oil, 20%; non-ionic emulsifiers, 5%; and water 75%. Emulsions were prepared according to Scheffé's model (Table 5).

The different emulsions were made in triplicate. The studied responses (Table 6) were the same as before, and conductivity measurements were added to determine the critical HLB; all of these parameters were measured at 24 h.

3.2.1. Particle size

Average diameter can be estimated by coefficients of the model ($b_1 = 8.5$; $b_2 = 6.7$ and $b_{12} = -15.6$; error $\leq 3\%$) and modeled as: $d_m = b_1X_1 + b_2X_2 + b_{12}X_1X_2$ and $\mathrm{HLB} = 16.7X_1 + 4.7X_2$.

According to the fact that the minimum droplet diameter corresponds to the most stable emulsion, the required HLB can be calculated by the derivative form of d_m.

$$RHLB = 4.7 + 6 \times (b_1 - b_2 + b_{12})/b_{12}$$

Table 5
Ratio of surfactants and corresponding HLB

Exp.	Tween 20	Span 60	HLB
1	1	0	16.7
2	0	1	4.7
3	1/2	1/2	10.7
4	2/3	1/3	12.7
5	1/3	2/3	8.7

Table 6
Responses obtained

Exp.	η (cP)	d_m (μm)	κ (μS/cm)
1	12	8.5	87
2	2820	6.7	39
3	30	3.7	88
4	19	4.3	89
5	81	3.7	75

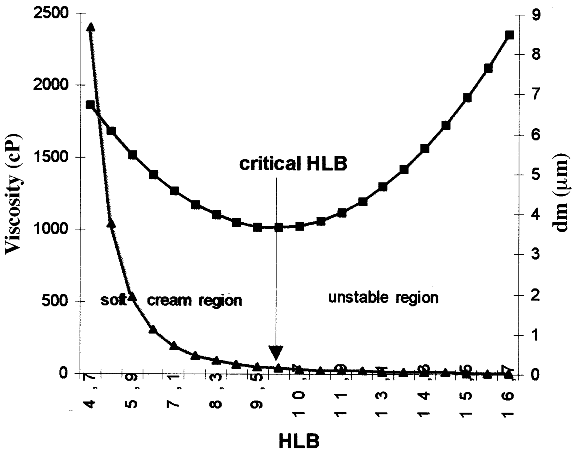


Fig. 1. Modeled curves of average diameter and viscosity vs HLB.

The average diameter of droplets reaches a minimum value for $\text{HLB} = 10 \pm 0.3$ (Fig. 1).

3.2.2. Viscosity study

The data obtained were modeled and plotted as a function of HLB. The viscosity of the emulsion decreased as HLB increased (Fig. 1). It was obvious from the viscosity vs HLB curve that viscosity abruptly decreased as HLB increased (soft cream region), after which there were small changes in viscosity with increasing HLB (unstable emulsions region). There was no singular point between these two fields (no minimum value), so optimal viscosity could not be found with Scheffé's model. However, the viscosity curve could be modeled as:

$$\begin{aligned}\log(\eta) &= 14.6 \times \text{HLB}^{-0.94}\\ &= 14.6 \times (12 \times x_1 + 4.7)^{-0.94} \quad r = 0.996.\end{aligned}$$

Because the curve is composed of two regions, the 'cream' region with a parabolic profile and the other with a linear profile, the derivative form of the modeled viscosity can be approximated by two lines, corresponding to each region (Fig. 2). The derivative form of the log(viscosity) has been transformed with a power function in order to minimize variations at the extremities of the HLB scale.

This artifact allows reduction of variations at low and high HLB values, so as to increase precision in the estimation of the break point. The intersection of these two lines corresponds to the critical HLB value. The precision is obtained by the different points which are not considered in the regression. The compromise is to obtain good correlation with good precision.

The line d_1, corresponding to soft cream region is:

$$y_1 = -0.211 + 0.096x \quad (r = 0.991)$$

while the line d_2, corresponding to unstable milk region is:

$$y_2 = 0.472 + 0.025x \quad (r = 0.990)$$

So, intersection of the lines defines the critical HLB at 9.6 with precision of 9.5–10.3.

In summary, five experiments can define the critical HLB value of the emulsion:

1. Average diameter: $9.7 < \text{HLB}_c = 10 < 10.3$;
2. Viscosity: $9.5 < \text{HLB}_c = 9.6 < 10.3$.

In order to confirm these results, conductivity measurements have been made.

3.2.3. Conductivity study

Determination of the variation profile for conductivity requires three further experiments: The emulsions were carried out at HLB values of: 5.9, 7.7 and 10.2. All experiments were done in triplicate with results shown in Fig. 3.

Graphical methods allow us to define the critical HLB value between 9.8 and 10.2 but because of profile estimation, error is relatively important. So, another experiment is necessary to minimize relative error, and this has to be between 9.5 and 10.2 HLB values. Viscosity and mean diameter studies defined $9.5 < \text{HLB}_c < 10.3$ and $9.7 < \text{HLB}_c < 10.3$, respectively, so the next experiment was run at $\text{HLB} = 10.0$. Results are shown in Table 7.

On account of the relative errors of the experimental results, the emulsion at $\text{HLB} = 10$ can be considered as the most stable one. So, in our experimental conditions, $\text{HLB} = 10$ corresponds to the required HLB of paraffin oil (Poré, 1992).

The conductivity study confirmed the results obtained from viscosity and average diameter studies. However this last study required three further experiments to obtain the required HLB value.

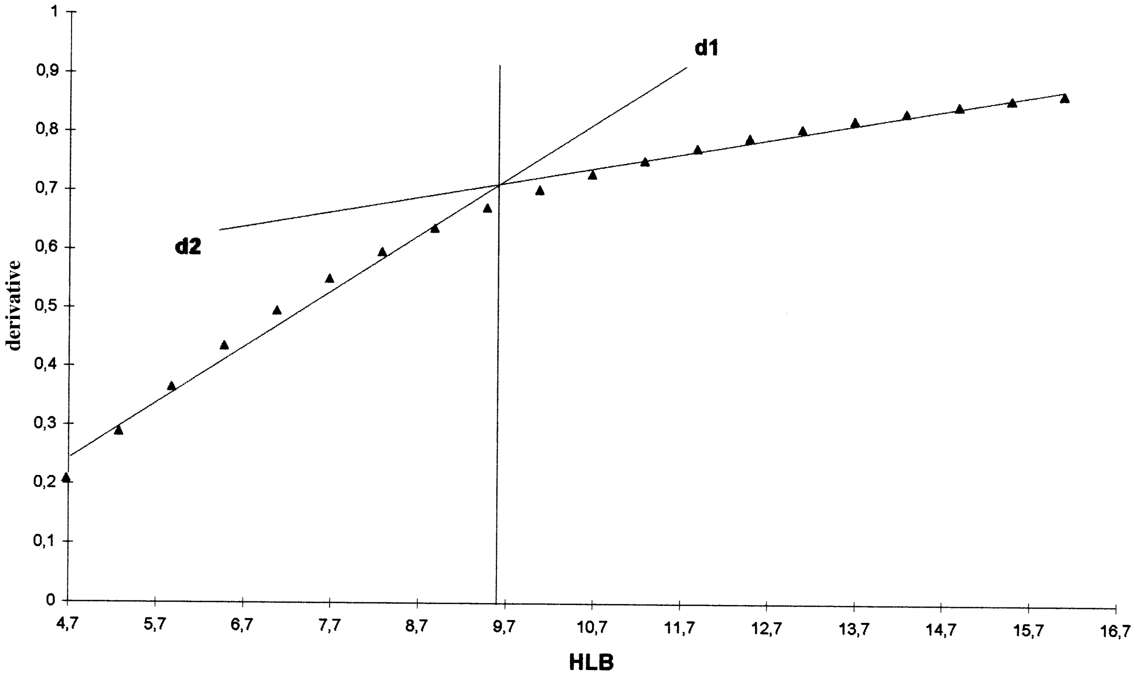


Fig. 2. Determination of the required HLB.

4. Conclusion

The formulation of emulsions requires a large number of runs according to process and chemical parameters. Experimental design can be applied to reduce the number of steps in the two cases. For operating parameters, eight experiments lead to better regulation of the process.

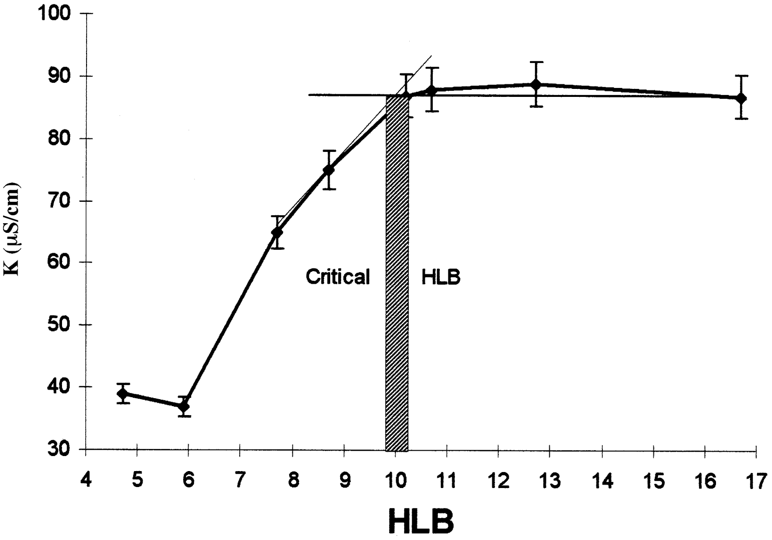


Fig. 3. Determination of the required HLB (graphical method).

Table 7
Experimental and calculated responses

Responses	Experimental	Calculated	Error (%)
d_m (μm)	3.6	3.65	1.4
Viscosity (cP)	40	47	4.4
κ (μS/cm)	85	—	—

For the required HLB determination, the average diameter of droplets can give a good approximation of this value in five experiments according to Scheffé's model. The viscosity cannot be modeled by this method but the method suggested here can give a good approximation of the required HLB in five experiments.

The conductivity study requires at least three further runs but allows better approximation and can be helpful when other responses cannot be modeled. In the case of different lipidic substances, because of the large scale of HLB, it is possible that some emulsions (high HLB values) will be separated into two phases after 24 h, so average diameter will be impossible to model.

Further investigations are in progress to study on the one hand the influence of the concentration of the emulsifier and the oil, and on the other, the process parameters, on the required HLB values.

References

Carlotti, M.E., Pattarino, F., Gasco, M.R., Brusasca, P., 1993. Optimization of parameters in the emulsification process by two different methods. Int. J. Cosm. Sci. 15, 245–259.

Curt, C., 1994. Revue: méthodes d'évaluation de la stabilité des émulsions. Principes, applications, avantages et inconvénients. Sci. Aliments. 14, 699–724.

Friberg, S.E., Goldsmith, L.B., 1968. Theory of emulsions. In: Lieberman, H.A., Rieger, M.M., Banker, G.S. (Eds.), Pharmaceutical Dosage Forms: Disperse Systems, vol. 1. Dekker, New York, pp. 49–90.

Groves, M.J., Freshwater, D.C., 1968. Particle size analysis of emulsion systems. J. Pharm. Sci. 57, 1273–1291.

Kato, A., Fujishige, T., Matsudomi, N., Kobayashi, J., 1985. Determination of emulsifying properties of some proteins by conductivity measurements. J. Food Sci. 5, 56–67.

Latreille, B., Paquin, P., 1990. Evaluation of emulsion stability by centrifugation with conductivity measurements. J. Food Sci. 6, 1666–1672.

Poré, J., 1992. Emulsions, micro-emulsions, émulsions multiples. Tech. Ind. des Corps gras, pp. 39–53.

Puisieux, F., Seillier, M., 1983. Galenica: Agents de surface et Emulsions. Tech. Doc. Lavoisier.

Sado, G., Sado, M.C., 1991. Les plans d'expériences: de l'expérimentation à l'assurance qualité, Afnor technique (Ed.), Paris.

Seillier, M., 1970. Les emulsions. Etude du HLB critique d'une huile de vaseline. These, Paris.

Tornberg, E., Hermansson, M., 1977. Functional characterization of protein stabilized emulsions: effect of processing. J. Food Sci. 2, 468–472.