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Mixture experiment methods in the development and optimization of microemulsion formulations

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ABSTRACT

Microemulsion formulations represent an interesting delivery vehicle for lipophilic drugs, allowing for improving their solubility and dissolution properties. This work developed effective microemulsion formulations using glyburide (a very poorly-water-soluble hypoglycaemic agent) as a model drug. First, the area of stable microemulsion (ME) formations was identified using a new approach based on mixture experiment methods. A 13-run mixture design was carried out in an experimental region defined by constraints on three components: aqueous, oil and surfactant/cosurfactant. The transmittance percentage (at 550 nm) of ME formulations (indicative of their transparency and thus of their stability) was chosen as the response variable. The results obtained using the mixture experiment approach corresponded well with those obtained using the traditional approach based on pseudo-ternary phase diagrams. However, the mixture experiment approach required far less experimental effort than the traditional approach. A subsequent 13-run mixture experiment, in the region of stable MEs, was then performed to identify the optimal formulation (i.e., having the best glyburide dissolution properties). Percent drug dissolved and dissolution efficiency were selected as the responses to be maximized. The ME formulation optimized via the mixture experiment approach consisted of 78% surfactant/cosurfacant (a mixture of Tween 20 and Transcutol, 1:1, v/v), 5% oil (Labrafac Hydro) and 17% aqueous phase (water). The stable region of MEs was identified using mixture experiment methods for the first time.

1. Introduction

Microemulsions (MEs) are thermodynamically stable, transparent, isotropic dispersions composed of oil and water stabilized by an interfacial film of surfactant molecules, suitably combined with a cosurfactant [1]. Recently, MEs have attracted a great interest as a potential drug delivery vehicle, mainly due to their ability to incorporate a wide range of drugs with different lipophilic properties. The advantages of MEs include improved drug solubilization and enhanced absorption properties. These advantages result from using the already dissolved form of the drug in the formulation and the resulting small droplet size, which provides a large interfacial surface area [2–4]. However, developing powerful ME formulations requires extensive pre-formulation studies. In fact, both (1) the amount and hydrophobicity of the drug to solubilize and (2) the nature, combination and proportions of the other formulation components (oil and aqueous phases, surfactant and cosurfactant) greatly affect the emulsification process [5,6]. Therefore, when designing such systems, a thoughtful evaluation of both the type and the relative proportions of the formulation components is necessary. Such an evaluation provides for selecting the most effective components and determining their optimal combination able to create a stable, fluid and reproducible ME. Furthermore, incorporating poorly water-soluble drugs into MEs represents a further formulation challenge, because their hydrophobicity prevents them from being dissolved in most commonly used solvents.

ME formulations are generally developed using pseudo-ternary phase diagrams, which allow identifying the most suitable components and their best relative proportions for obtaining physically stable systems [5]. Pseudo-ternary phase diagrams are constructed by progressive titration of the component mixtures, and thus they require a series of time-consuming and repetitive experiments. However, methods for the design and analysis of mixture experiments provide an alternative approach to more effectively determine the feasible region of ME formulations. These methods also provide for determining the optimal formulation within the region of feasible formulations identified [7–10].

Very few articles about using mixture experiment methods to develop ME-based, drug-delivery systems have appeared in the literature. The few articles that have appeared are aimed at composition optimization [11–13]. In previous work, we demonstrated the advantages of mixture experiment methods to optimize a ME

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system for the electrokinetic chromatographic analysis of ketorolac and its impurities [14].

In the present work, we investigated the usefulness of such an approach for formulating stable drug delivery systems based on MEs, using glyburide (GLY) as a model drug. GLY is a derivative of the second generation of sulfonylurea antidiabetics, commonly utilized as oral hypoglycaemic agents. The very low aqueous solubility of GLY gives rise to problems of poor and variable bioavailability and bio-inequivalence of its commercial dosage forms [15]. Several methods have been exploited to improve the solubility and dissolution properties of orally administered GLY, including drug amorphization [16], complexation with cyclodextrins [17], or solid dispersion in hydrophilic carriers [18–20]. As an alternative to these methods, we evaluated the effectiveness of the ME formulation approach to adequately increase the apparent solubility of this drug, and consequently its bioavailability.

We first used a mixture experiment to rapidly identify, for each combination of excipients, the stable emulsification region as an alternative to the classic pseudo-ternary phase-diagram approach. A second mixture experiment was performed within the stable region of excipients and the results were analysed to find the optimal formulation (in terms of the best drug dissolution properties).

2. Overview of mixture experiments

A mixture experiment involves combining components of an end-product in various proportions and measuring one or more response variables of the resulting end-product. The component proportions can sum to any constant ≤ 1, although traditionally the constant is 1.0 (because component proportions can always be scaled by the different constant so that the sum is 1.0). Often the proportions of the components are subject to lower and/or upper bounds and possibly multi-component constraints. In such cases, the mixture space generally is an irregular polyhedral region. Special methods for designing mixture experiments and analyzing the resulting data are discussed in the comprehensive book by Cornell [9].

The goal of mixture experimentation is often to find an optimum blend of components that provides desired or optimal values of one or more response variables. This goal is typically achieved by developing a mixture experiment design, forming the experimental mixtures according to the design, measuring the response variables, developing response–composition models using the experimental data and applying response–optimization methods. The advantages of this approach are that responses can be predicted (with uncertainties) throughout the experimental region, and different optimum formulations can be developed for different optimization goals.

3. Materials and methods

3.1. Materials

Materials used in the work included micronized GLY (from Guidotti Laboratori S.p.A.), Labrafil® 1944, Labrafac® Hydro WL1219, Transcutol® and Labrasol® (kind gifts of Gattefossé), Tween®20 (from ICI Group, USA), and PEG 400, propylene glycol and oleic acid (from Sigma–Aldrich, USA). Distilled water was used throughout the study.

3.2. Mixture design and data analyses

The NEMRODW software package [21] was used to generate the two mixture experiment designs described subsequently. Mixture experiment models were developed and other statistical analyses of the data were performed using the Design-Expert [22] software package. The second mixture experimental design was replicated (i.e., all tests in the design were performed twice), with the tests performed in a randomized order.

Mixture experiment models were developed relating the response variables to proportions of pseudo-components. For component proportions (x_i) with lower bounds (L_i), pseudo-component proportions (z_i) were calculated as $z_i = (x_i - L_i)/(1 - \Sigma L_j)$. Note that $\Sigma z_i = 1$. The pseudo-components are combinations of the original components, which rescale the constrained composition region so that the minimum allowable proportion of each pseudo-component is zero. This transformation may provide for more precisely estimating model coefficients compared to using the original component system [10,12].

3.3. Dissolution studies

Dissolution studies of GLY in ME formulations were performed by the dialysis method [23]. A ME amount corresponding to 5 mg GLY was put into a dialysis bag, which was firmly sealed with a dialysis clamp and placed in 30 mL of pH 7.4 phosphate buffer thermostated at 37 °C and maintained under stirring. At time intervals, samples were withdrawn and spectrometrically assayed for drug content at 302 nm. Drug dissolution efficiency (DE) was calculated according to Khan [24] from the area under the dissolution curve at time t (approximated using the trapezoidal rule) and expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time.

4. Screening excipients and preparing microemulsions

4.1. Screening excipients

In the screening stage of the study, solubility of GLY in several excipients was determined to select the most suitable options for oil (O), surfactant (S), cosurfactant (CoS) and aqueous (W) components to use for preparing MEs. In particular, the final objective was to solubilize the usual therapeutic dose of GLY (i.e., 5 mg) in the minimum volume of ME. With this goal, we determined the minimum amount of each examined component necessary to solubilize 5 mg of GLY.

Among the oil components considered (oleic acid, Labrafil 1944 and Labrafac Hydro) the latter showed the highest solubilizing power. Only 5 mL was needed to solubilize the pre-fixed amount of GLY, compared to more than 10 mL necessary with the other oils. As for the aqueous phase, PEG 400 and propylene glycol were tested as the possible water co-solvent. The former was selected based on its better efficacy in solubilizing the GLY (1 mL versus 4 mL).

Transcutol was selected as cosurfactant due to its excellent solubilizing ability (0.5 mL) for GLY. Labrasol and Tween 20 were tested as possible surfactants. Because they showed a similar solubilizing effect (1 mL), both surfactants were investigated in subsequent tests.

4.2. Preparing GLY microemulsions

Microemulsions were prepared using O = Labrafac Hydro, CoS = Transcutol, S = Labrasol or Tween 20 and W = water or a mixture of water and PEG 400. Briefly, GLY was solubilized in the CoS and afterwards the S was added. The resulting mixture was then added to the oil phase under stirring. Finally, the aqueous phase was added under vigorous stirring. The drug concentration was always 5 mg/10 mL of ME.

Table 1
Components[a] used in the four series of microemulsion formulations with glyburide[b].

ME Series	S	CoS	O	W
I	Labrasol	Transcutol	Labrafac Hydro	Water
II	Labrasol	Transcutol	Labrafac Hydro	Water-PEG 400
III	Tween 20	Transcutol	Labrafac Hydro	Water
IV	Tween 20	Transcutol	Labrafac Hydro	Water-PEG 400

[a] S, surfactant; CoS, co-surfactant; O, oil; W, aqueous phases.
[b] The concentration of glyburide was always 5 mg/10 mL.

5. Determining stable microemulsions

Two different approaches that were used to determine the composition subregion of stable microemulsions are discussed in Sections 5.1 and 5.2.

5.1. Pseudo-ternary phase diagram approach

Pseudo-ternary phase diagrams were constructed for homogeneous liquid mixtures of (1) O (Labrafac Hydro), (2) S (Labrasol or Tween 20):CoS (Transcutol) 1:1, v/v mixtures and (3) W (water or water:PEG 400 1:1 (v/v)), both in the absence and in the presence of the drug. Four series of formulations were considered based on four different combinations of the selected components (Table 1). For each series, pseudo-ternary phase diagrams were constructed using experimental data generated by progressive titration with each aqueous phase (under vigorous stirring) up to clouding of homogeneous mixtures of oil and surfactant:cosurfactant (1:1, v/v). Approximately 15 data points, each involving about 30 min, were obtained to determine each of the pseudo-ternary phase diagrams (Fig. 1).

For the four series of components, the shaded areas of the pseudo-ternary diagrams in Fig. 1 show the regions of stable (and transparent) ME formation. The unmarked areas indicate the turbid regions. In all cases, the areas of stable ME formation extended over a more or less limited area in the S/CoS-rich part of the phase diagram. Using Labrasol as surfactant gave a smaller zone of stable ME formation than Tween 20, probably due to the more hydrophilic nature of the latter component.

5.2. Mixture experiment approach

A mixture experiment approach was used to find the area of stable ME formation for each of the four ME series in Table 1. The ME transmittance percentage at 550 nm (T%), indicative of the ME transparency and thus of its stability, was selected as the response to be maximized. Based on subject-matter knowledge and preliminary experiments, lower and upper limits on the proportions (v/v) of the mixture components were chosen:

$$\begin{aligned} 0.05 \le x_1(\mathrm{S:CoS},\ 1:1, \mathrm{v/v}) \le 0.85 \\ 0.05 \le x_2(\mathrm{O}) \le 0.45 \\ 0.10 \le x_3(\mathrm{W}) \le 0.50 \end{aligned} \quad (1)$$

These constraints restricted the size of the experimental region as shown in Fig. 2. The components (x_i) were transformed into new variables called pseudo-components (z_i) [10] as described in Section 3.2.

To determine the region of stable ME formation for each ME series, a special-cubic mixture model of the form

$$\ln\left(\frac{T\%/100}{1-T\%/100}\right) = \beta_1 z_1 + \beta_2 z_2 + \beta_3 z_3 + \beta_{12} z_1 z_2 + \beta_{13} z_1 z_3 + \beta_{23} z_2 z_3 + \beta_{123} z_1 z_2 z_3 \quad (2)$$

was used to relate the logistic transformation of the response variable (T%) to the proportions of the pseudo-components (z_i). The logistic transformation on the left side of (2) is appropriate for response variables that vary from 0 to 1, so T% was divided by 100 to apply the transformation. The $\beta_i z_i$ terms are called the linear blending terms and the β_i are the expected logistic-transformed responses at the pure pseudo-components (i.e., they represent the expected response from a mixture with $z_i = 1$ and $z_j = 0$ for $j \neq i$). The quadratic terms $\beta_{ij} z_i z_j$ and the special-cubic term $\beta_{123} z_1 z_2 z_3$ are called nonlinear blending terms [9].

Table 2
13-run experimental plan and measured responses (Transmittance, %) for microemulsion Series I, II, III, IV.

Exp. #	S:CoS (v/v)	Oil (v/v)	Water (v/v)	I (T%)	II (T%)	III (T%)	IV (T%)
1	0.85	0.05	0.10	100	100	100	100
2	0.45	0.45	0.10	1.2	100	100	100[a]
3	0.45	0.05	0.50	0.6	1.0	0.6	100
4	0.05	0.45	0.50	0	0.3	0	0.1
5	0.65	0.25	0.10	99.5	100	100	100
6	0.65	0.05	0.30	100	100	100	100
7	0.25	0.45	0.30	0.1	0.7	0.1	1.5
8	0.25	0.25	0.50	0.1	0.3	0	0.2
9	0.45	0.25	0.30	0.2	0.8	0.2	3.6
10	0.65	0.15	0.20	100	100	100	100[a]
11	0.45	0.35	0.20	0.3	1.8	0.7	29.2
12	0.45	0.15	0.40	0.1	1.0	0.2	5.6
13	0.25	0.35	0.40	0.1	0.6	0.1	0.3

[a] Measured values greater than 100 were obtained, but were changed to 100 because that is the maximum possible value for T%.

The 13-run mixture experiment design generated by NEMRODW [21] is listed in Table 2. As seen in Fig. 2, the design consists of four vertices, four midpoints of edges, a center point, and four interior points of the constrained region. The MEs of the design in Table 2 were prepared (as described in Section 4.2) in a randomized order by the same operator. For each ME, the T% was measured, where T% = 100 indicates full limpidity and the highest stability of each ME. Table 3 presents the results of applying statistical regression analysis to fit model (2) for each series of data. The R^2 statistics in Table 3, which range from 0.838 to 0.929, quantify the proportion of variation in the left-hand side of (1) accounted for by the fitted model. Ideally the R^2 values should be somewhat larger. However, with only four to six of the T% values at or near 100% (depending on the ME series) and most of the rest of the values at or near zero, modelling T% is challenging. Still, the models fit sufficiently well to identify the regions of stable formulations.

Fig. 2 shows the contour plots of T%/100 produced using the fitted special-cubic mixture models in Table 3 for each series of ME. The contour plots clearly show the dependence of T% (i.e., of the ME

Table 3
Coefficients and summary statistics for special-cubic mixture models in the pseudo-components fit to the logistic transformation of percent transmittance (T%) for Series I to IV.

Logit (T%) model term in pseudo-components	Model coefficients estimated from data			
	Series I	Series II	Series III	Series IV
S:CoS (z_1)	16.33	15.98	15.78	15.30
Oil (z_2)	−24.22	−7.02	1.02	21.66
Water (z_3)	−54.86	−32.30	−52.92	11.28
$z_1 z_2$	−5.72	22.37	8.60	−18.96
$z_1 z_3$	62.46	29.18	63.88	−2.73
$z_2 z_3$	111.05	63.28	56.72	−88.67
$z_1 z_2 z_3$	−122.58	−316.33	−324.87	−298.26
Summary statistics				
R^2	0.859	0.838	0.896	0.929

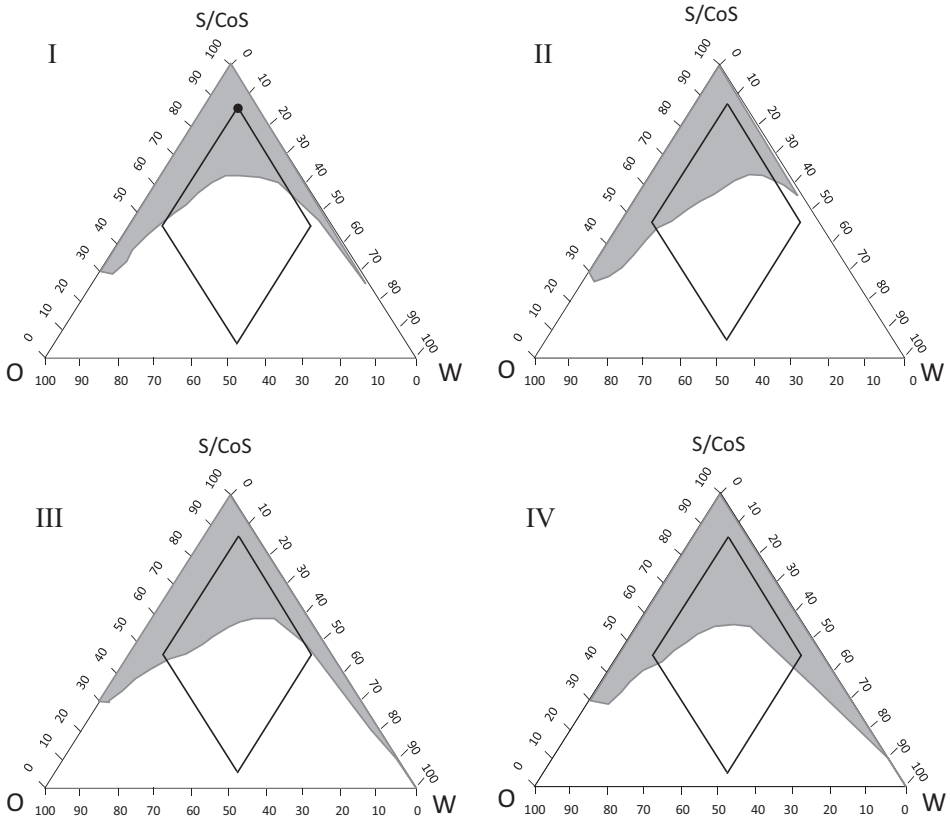

Fig. 1. Pseudo ternary phase diagrams for formulation Series I, II, III and IV. Stable regions of microemulsion formulations are shown as shaded areas. The different surfactant:cosurfactant (S:CoS), oil (O) and aqueous (W) phases used for their preparation are reported in Table 1. The diamond-shaped subregion is the constrained experimental region shown in Fig. 2.

stability) on the ME composition. For the four series of ME investigated, the most important component was S:CoS (a high proportion of that component generally increased stability).

We decided to identify the formulations in Fig. 2 with model-predicted *T*%/100 values > 0.999 as the regions of stable MEs. These regions shown in Fig. 2 are in very good agreement with those identified in Fig. 1 via classic pseudo-ternary phase diagrams. For ease in comparing Fig. 1 to Fig. 2, the experimental region of Fig. 2 has been superimposed on the pseudo-ternary phase diagrams in Fig. 1.

The contour plots in Fig. 2 also show important differences among the examined series. For example, obtaining stable ME formulations using Labrasol as the surfactant and water as the aqueous phase (Series I), required a very high percentage of S:CoS ranging from 85% (the highest examined value) to a minimum of approximately 60%. On the other hand, for ME formulations where Labrasol was the surfactant and water-PEG 400 was the aqueous phase (Series II), as well as for those where Tween 20 was the surfactant and water the aqueous phase (Series III), an antagonistic effect between O and S:CoS was observed. In this case, a high proportion of the first component required a low proportion of the second one, and vice versa. Finally, for ME formulations in which Tween 20 was the surfactant and water-PEG 400 mixture was the aqueous phase (Series IV), there was a strong antagonism between the oil and aqueous phases. Hence, stable MEs were possible for formulations with (1) S:CoS above ~63% and (2) using high values of the oil phase and low values of the aqueous phase and vice versa. In summary, stable ME regions were found in all series, with some similarities and some differences depending on the series. The stable regions were very similar for Series II and III, avoiding some of the unique aspects of the stable regions for Series I and IV. Hence, Series II and III were chosen for finding MEs with good drug dissolution properties.

6. Optimizing the GLY microemulsion

This section discusses the optimization stage of the study, where the goal, was to identify MEs with good drug dissolution properties for Series II and III. A mixture experiment approach was used again, this time focusing on the region of stable ME formulations.

A 13-run mixture design was chosen for each of Series II and III within the following ranges of the component proportions (v/v)

$$\begin{aligned} 0.65 \le x_1(\mathrm{S:CoS}) \le 0.85 \\ 0.05 \le x_2(\mathrm{O}) \le 0.25 \\ 0.10 \le x_3(\mathrm{W}) \le 0.17 \end{aligned} \quad (3)$$

where S = Labrasol (Series II) or Tween 20 (Series III), CoS = Transcutol, O = Labrafac Hydro and W = H_2O (Series III) or H_2O:PEG 400 1:1 (v/v) (Series II). These ranges focus on a subregion of the region stable ME formulations of interest for both Series II and III. The design points are listed in Table 4 and shown in Fig. 3. They include the four vertices, four midpoints of edges, a center point and four interior points of the constrained region.

The goal was to find, for each series, the ME composition that maximized the dissolved percent (DP) and dissolution efficiency (DE) of the drug. The selected responses to be maximized were DP and DE at 30 and 60 min (DP30, DP60, DE30 and DE60). In this stage of the study, each experiment was duplicated (with all experiments done in random order) because of the expected high variability in dissolution values.

All MEs prepared according to the experimental design for each of Series II and III were stable, with *T*% values at or near 100%. The

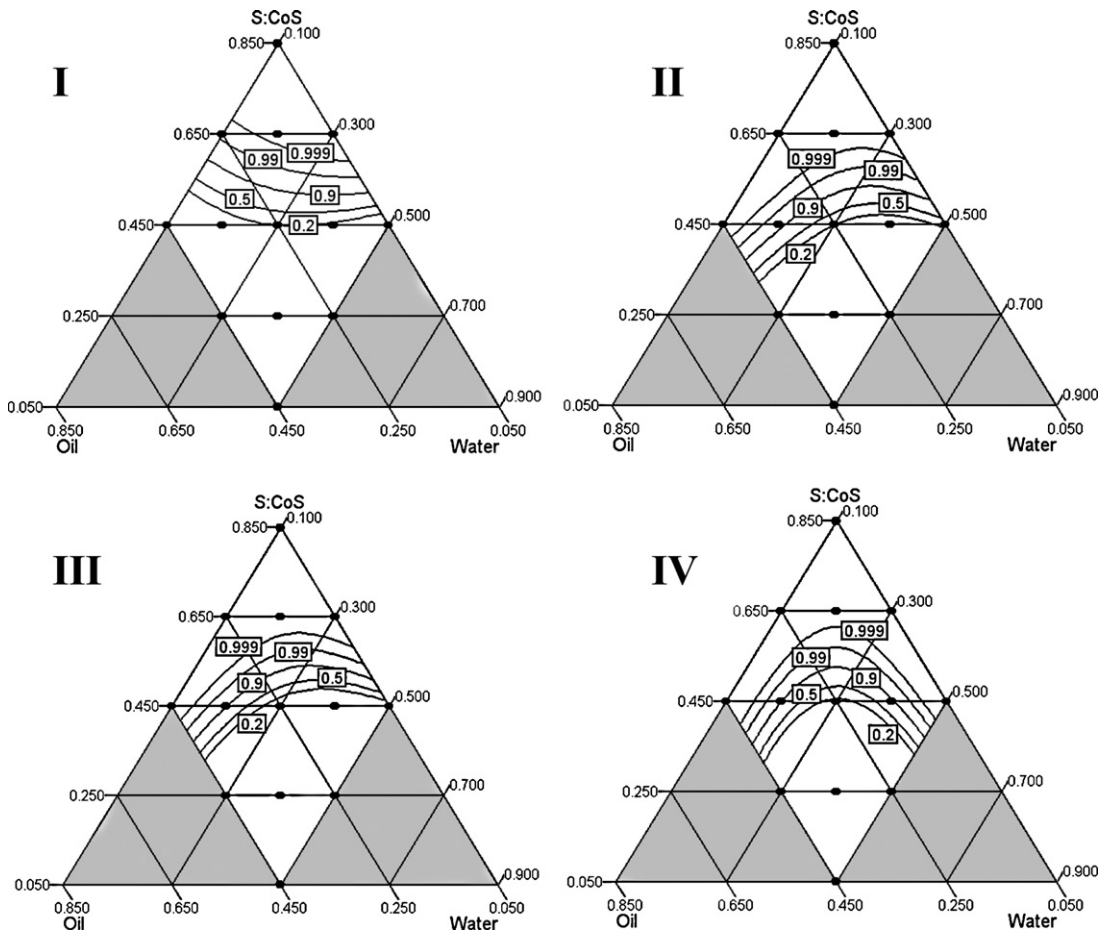

Fig. 2. Contour plots of % transmittance/100 for formulation Series I, II, III and IV in the experimental region defined by the lower and upper bounds on the proportions of surfactant:cosurfactant (S:CoS), oil (O) and aqueous (W) phases used for their preparation (see Table 1).

Table 4
Replicated 13-run experimental plan and measured responses (dissolved percent and dissolution efficiency at 30 and 60 min) for Series III microemulsions (see Table 1 for microemulsion composition).

Exp #	Des #	S:CoS (v/v)	Oil (v/v)	Water (v/v)	DP30	DP60	DE30	DE60
1	1	0.6500	0.2500	0.1000	17.03	31.76	10.45	17.48
14	1	0.6500	0.2500	0.1000	18.70	31.96	9.88	17.28
2	2	0.6500	0.1800	0.1700	16.93	31.24	8.76	16.55
15	2	0.6500	0.1800	0.1700	17.77	31.69	8.97	17.19
3	3	0.8500	0.0500	0.1000	22.42	36.54	9.98	19.28
16	3	0.8500	0.0500	0.1000	22.84	37.65	10.07	20.08
4	4	0.7800	0.0500	0.1700	29.90	53.35	20.35	30.79
17	4	0.7800	0.0500	0.1700	28.80	52.98	19.63	29.35
5	5	0.6500	0.2150	0.1350	16.69	34.07	6.58	16.36
18	5	0.6500	0.2150	0.1350	17.05	34.81	6.71	17.15
6	6	0.7500	0.1500	0.1000	23.90	34.13	16.35	23.30
19	6	0.7500	0.1500	0.1000	23.47	34.54	15.26	22.82
7	7	0.7150	0.1150	0.1700	22.30	39.75	12.98	23.41
20	7	0.7150	0.1150	0.1700	22.50	40.23	13.23	22.37
8	8	0.8150	0.0500	0.1350	23.50	51.35	13.95	26.95
21	8	0.8150	0.0500	0.1350	23.00	52.63	12.79	25.39
9	9	0.7325	0.1325	0.1350	24.57	45.62	11.44	22.06
22	9	0.7325	0.1325	0.1350	24.99	44.13	11.46	23.48
10	10	0.6912	0.1913	0.1175	22.03	42.63	15.51	28.11
23	10	0.6912	0.1913	0.1175	22.30	41.82	14.63	26.58
11	11	0.6912	0.1563	0.1525	18.29	44.23	12.35	22.55
24	11	0.6912	0.1563	0.1525	18.90	43.51	13.39	23.12
12	12	0.7912	0.0913	0.1175	26.37	46.82	16.17	26.56
25	12	0.7912	0.0913	0.1175	27.60	47.56	15.57	27.28
13	13	0.7562	0.0913	0.1525	24.50	48.72	14.03	25.54
26	13	0.7562	0.0913	0.1525	24.28	49.53	13.98	26.55

best results in terms of drug dissolution properties were obtained for ME formulations of Series III, whose responses are reported in Table 4. Table 5 lists the experimental-plus-measurement uncertainties in the four responses (DP30, DP60, DE30, DE60) based on the measured responses of the replicate pairs of the 13 formulations tested.

The scatterplot matrix graph of the design formulations and the four responses given in Fig. 4 show that S:CoS and oil are strongly negatively correlated, which is a result of the constrained region specified in (3). Fig. 4 also shows that all pairs of DP30, DP60, DE30 and DE60 are positively correlated, with the strongest correlation occurring for DE30 and DE60. This suggests that formulations that are good or optimal with respect to one response are likely to be good and close to optimal with respect to other responses.

Several mixture experiment model forms (p. 28 and 71 of Ref. [9]) were considered, with the special-quartic mixture (SQM)

Table 5
Estimates of experimental-plus-measurement uncertainty based on the measured responses for two replicates each of 13 formulations for Series III microemulsions.

Response	DF[a]	SD[b]
DP30	13	0.54
DP60	13	0.58
DE30	13	0.47
DE60	13	0.73

[a] Degrees of freedom. There is one DF for each of the 13 replicate pairs of test formulations.
[b] Standard deviation.

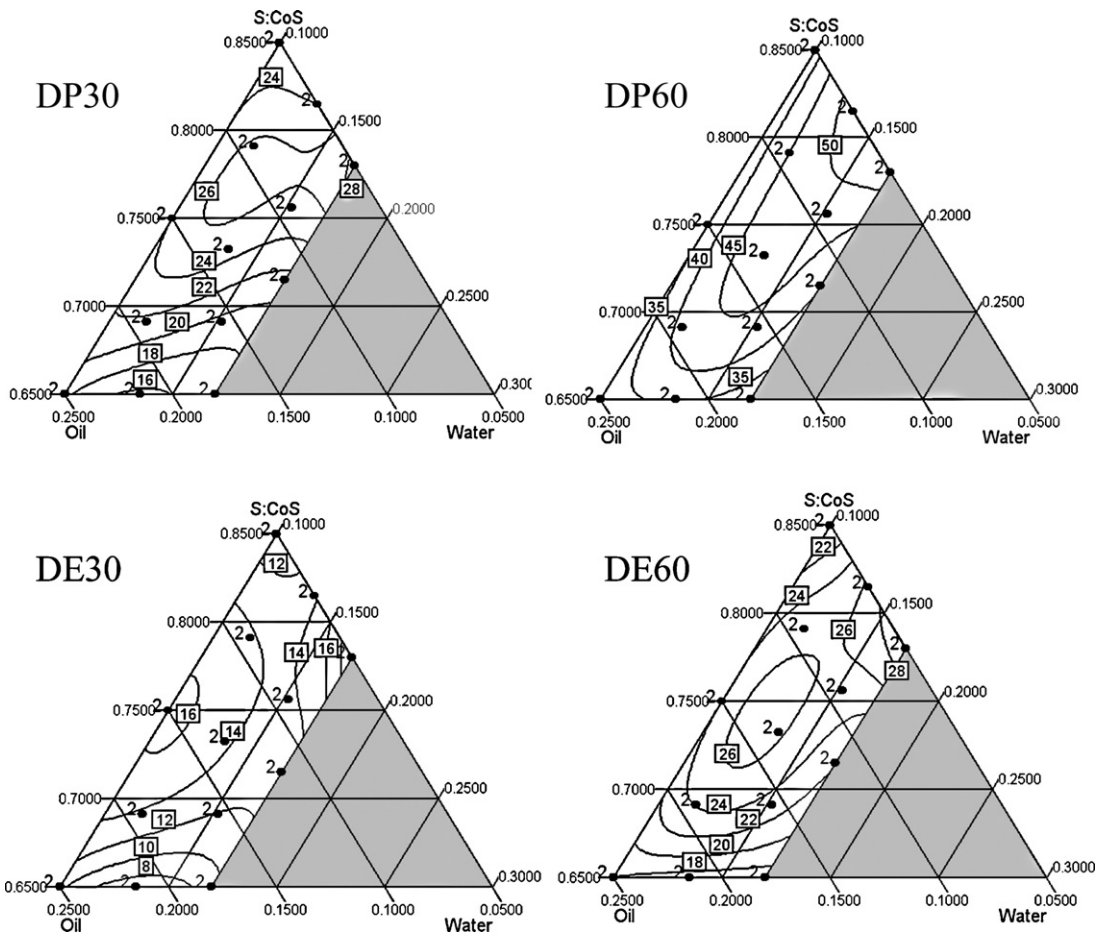

Fig. 3. Contour plots of dissolved percent (DP) and dissolution efficiency (DE) at 30 and 60 min of glyburide (GLY) from Series III microemulsion formulations in the experimental region defined by lower and upper bounds on the percentages of surfactant:cosurfactant (S:CoS), oil (O) and aqueous (W) phases used for their preparation (see Table 1).

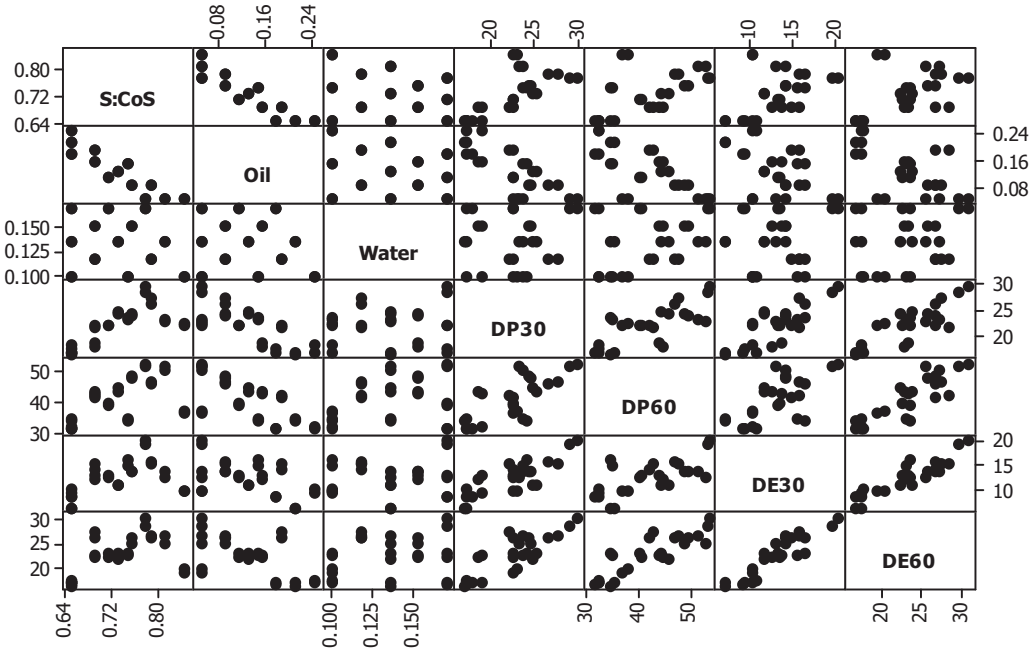

Fig. 4. Scatterplot matrix graph of experimental design formulations and DP30, DP50, DE30 and DE60 responses for Series III.

Table 6
Coefficients and summary statistics for complete or reduced special-quartic mixture models in the pseudo-components fit to DP30, DP60, DE30 and DE60 for Series III.

Model term in pseudo-components	Model coefficients estimated from data			
	DP30	DP60	DE30	DE60
S:CoS (z_1)	22.41	37.46	10.30	19.82
Oil (z_2)	18.43	31.43	10.44	17.99
Water (z_3)	72.20	−65.18	70.72	21.28
z_1z_2	14.68	3.43	23.54	20.62
z_1z_3	−48.78	228.42	−50.41	43.73
z_2z_3	−84.40	146.44	−98.51	−9.83
$(z_1)^2z_2z_3$	336.16	–[a]	–[a]	–[a]
$z_1(z_2)^2z_3$	168.41	443.21	240.27	319.48
$z_1z_2(z_3)^2$	−744.73	−562.85	−541.58	−569.32
Summary statistics				
RMSE	1.05	1.47	1.33	1.87
R^2	0.946	0.971	0.893	0.861
$R^2_{Predicted}$	0.870	0.951	0.832	0.772
LOF p-value	0.0002	<0.0001	<0.0001	<0.0001

[a] Coefficient was not statistically significant with at least 90% confidence.

model

$$y = \beta_1 z_1 + \beta_2 z_2 + \beta_3 z_3 + \beta_{12} z_1 z_2 + \beta_{13} z_1 z_3 + \beta_{23} z_2 z_3 + \beta_{1123} z_1^2 z_2 z_3 + \beta_{1223} z_1 z_2^2 z_3 + \beta_{1233} z_1 z_2 z_3^2 \quad (4)$$

providing the best fit to the data for each of the four responses (y = DP30, DP60, DE30 and DE60) in Table 4. The results of fitting complete or reduced SQM models to the four responses are summarized in Table 6. The statistics reported in Table 6 include root mean squared error (RMSE), R^2, $R^2_{Adjusted}$, $R^2_{Predicted}$ and the LOF p-value. RMSE estimates the experimental uncertainty in measuring a response if the model does not have a statistically significant lack of fit (LOF). R^2 compares predicted and measured response values for the formulations in the experimental design, and is the proportion of variation in a response accounted for by the fitted model. $R^2_{Predicted}$ is calculated similarly to R^2, except each data point is eliminated in determining the model-predicted value for that data point. Hence, $R^2_{Predicted}$ is a form of model cross-validation. Finally, LOF p-value is the probability of incorrectly declaring that the fitted model has a significant LOF. Small p-values (e.g., less than 0.05) indicate a model has a statistically significant LOF.

Based on R^2, the response models that best fit the data are DP60 (0.971), DP30 (0.946), DE30 (0.893) and DE60 (0.861). Somewhat lower values for $R^2_{Predicted}$ indicate that some data points are influential in the model fits (which is understandable given there are only 13 design points). All four of the models have highly statistically significant LOF (p-values of 0.0002 or less). However, the models fit the data well enough to understand the composition effects on the responses and to select an optimal formulation.

Contour plots produced using the Table 6 models for Series III ME are shown in Fig. 3. Because of the strong correlations among DP30, DP60, DE30 and DE60, they are all maximized using high percentages of W and S:CoS and a low percentage of O. Hence, the use of a multi-criteria optimization strategy was considered unnecessary and the composition of design point #4 in Table 4 was selected as optimum. Thus, the most effective formulation in terms of both DP and DE was a 78% mixture of Tween 20 and Transcutol (1:1, v/v), 5% Labrafac Hydro and 17% water.

To confirm that the selected formulation was acceptable, five ME batches were prepared using this formulation. The five batches produced stable, fluid and transparent dispersions. They also showed a satisfying and reproducible drug-release profile with average ± standard deviation values of 33.6 ± 0.9 for DP30, 58.5 ± 1.2 for DP60, 25.5 ± 1.1 for DE30 and 37.0 ± 0.7 for DE60. These mean values are all larger than the mean values from the two replicates of experiment 4 during testing (29.4 for DP30, 53.2 for DP60, 20.0 for DE30 and 30.1 for DE60). However, higher drug releases are desirable, so the confirmation tests for the optimal formulation were considered successful.

7. Conclusions

Mixture experiment design, modelling, and optimization methods were applied to find the region of stable MEs and develop an optimum ME formulation. During the "stable formulation" stage of the study, the same mixture design was used for each of four series of tests corresponding to different choices for the excipients. By examining contour plots produced from fitted mixture experiment models, it was possible for each formulation series, to (1) identify the subregion of stable GLY MEs and (2) select the most efficient combination of excipients. The region of stable formulations identified by the mixture experiment approach compared very well with the region identified using traditional pseudo-ternary phase-diagrams. This application of mixture experiment methods is the first in literature for finding stable regions of MEs and it demonstrates the effectiveness of the approach in developing efficient and stable ME formulations using a reduced number of experiments with respect to those required by the traditional titration approach.

Furthermore, the mixture experiment approach was used to develop predictive models for four GLY dissolution responses as functions of the proportions of the ME components. These models were then used to identify (within the region of stable formulations for the selected excipient combination) the ME composition with optimal (i.e., high) values of DP and DE values for GLY. The mixture approach also identified (1) the most important formulation variables and (2) non-linear blending effects of the ME components on GLY dissolution.

In summary, we highly recommend the use of mixture experiment design, modelling, and optimization methods to (1) identify the stable region of MEs for a given set of excipients and (2) develop models for predicting drug dissolution responses with the region of stable MEs, identify stable ME formulations with optimal drug dissolution responses. Although not illustrated in this article, we also recommend applying mixture experiment methods to optimize the ME composition in ME electrokinetic chromatography.

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