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Experimental design and partial least squares in the study of complex mixtures: microemulsions as drug carriers

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Summary

Four-component non-aqueous microemulsions, containing lecithin, taurodeoxycholic acid, ethyl oleate and 1,2-propylene glycol have been studied with chemometric techniques to emphasize the role of the components on the release of a drug. Since microemulsions can be obtained only for particular proportions of the constituents, their realm of existence was determined by using Doehlert experimental design. Successively, a mixture design technique was applied to select the set of microemulsions for the measurement of the diffusion rate of a model lipophilic drug, retinol, through a hydrophilic membrane. The drug permeability was modelled as a function of the mixture composition by partial least squares (PLS). The results emphasize the role of the cosurfactant and the oil phase of the system on the drug permeation behaviour from the waterless microemulsion.

Introduction

Microemulsions (Bourrel and Schechter, 1988) are isotropic systems of infinite stability, usually constituted by four-component mixtures including a surfactant, a cosurfactant, an oil and water; surfactant and cosurfactant are principally located at the surface separating the two immiscible liquids to stabilize their mutual dispersion. Either oil/water (o/w) or water/oil (w/o) microemulsions can be prepared. Microemulsions have been recently proposed as carriers in pharmaceutics to achieve the substained/controlled release of several drugs (Müller and Kleinebudde, 1988; Gasco et al., 1990, 1991).

Recently, o/w microemulsions were proposed by Ritschel et al. (1989, 1990) as carriers for the oral administration of cyclosporine A with very interesting results. In a previous work (Pattarino et al., 1992), o/w-type non-aqueous microemulsions constituted of lecithin as surfactant, taurodeoxycholic acid as cosurfactant, ethyl oleate as disperse phase and one of six different glycols as continuous phase were studied. The results showed that the permeation of a model drug, retinol, through a hydrophilic membrane can be related to the qualitative and quantitative composition of the microemulsion, whose internal phases behave as reservoir for the lipophilic drug.

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Chemometric methodologies currently provide effective tools for the study of pharmaceutical dosage forms (Gould, 1984). The aim of the present work was to apply these methodologies also to extremely complex systems, like microemulsions, that have not been investigated using such procedures. Waterless microemulsions consisting of four biocompatible substances (lecithin, taurodeoxycholic acid, ethyl oleate and 1,2-propylene glycol) were studied in order to assess their suitability as carriers for the oral administration of retinol.

Since microemulsions can be obtained only for particular proportions of the components, the system was previously investigated using Doehlert experimental design in order to determine the realm of existence of microemulsions. Successively, mixture design was used to select a set of microemulsions from which the permeation of retinol through a hydrophilic membrane was measured. The rate of diffusion of the drug was then modelled as function of the mixture's percentual composition by PLS.

Materials and Methods

Materials and instruments

Ethyl oleate (EO), retinol and 1,2-propylene glycol (PG) were from Fluka. Taurodeoxycholic acid (TDC) was from Sigma, Lecithin (L), from Merck, was purified (Hanahan et al., 1951) and stored at $-30°C$ until used.

The liquid chromatographic instrument was a Series 250 from Perkin Elmer equipped with a 150×4.6 mm HS-5 C8 (Perkin Elmer) column. A multi-cavity microdialysis cell from Scienceware, equipped with a dialysis membrane (cut-off, 12000) (Sigma), was used in the diffusion experiments.

The calculations were performed on an Epson AX3 series personal computer by SCAN program (SCAN).

Experimental design

A Doehlert design was used for the search of the microemulsion realm of existence. This experimental design (Doehlert, 1970) has already been used for optimization and regression, it provides the interesting feature that variables and experiments can be easily added to the first design without any loss of information.

Mixture design was used for planning the mixtures to be tested for building reliable regression models. From Cornell (1991) a mixture experiment is defined as an experiment where the response is assumed to depend only on the relative proportions of the components present in the mixture and not on the amount of the mixture itself. This is what was expected for the microemulsions under investigation. In this perspective, in order to be able to calculate a model non-linear in the composition variables, we chose the simplex-centroid design, augmented with the introduction of three interior experiments. Such a design can be used to calculate up to a complete third degree model and provides additional information for validating the model itself. Moreover, this design is characterized by the need and proper distribution of the information throughout the experimental region and is especially suitable for detecting the curvature of the response surface in the interior of the triangular region.

Microemulsion preparation

Microemulsion preparation was performed as follows: retinol was dissolved in EO and lecithin was added; TDC dissolved in 1,2-PG was then mixed for 10 min with the oil phase. The systems obtained were classified into microemulsion or thermodynamically unstable system categories respectively, according to the transparency criterion (Lagues et al., 1978).

Microemulsion domain

Two series of mixtures, each containing a fixed amount of lecithin (10 or 15%, respectively) were studied: the percentages of EO, TDC and PG were selected on the basis of the Doehlert design as depicted in Fig. 1A and B.

Diffusion studies

Diffusion studies were performed only on the microemulsions containing 15% of lecithin, because of their larger area of existence.

The diffusion experiments were carried out in a multi-cavity microdialysis cell designed for separate but simultaneous dialysis experiments. Each compartment held 1.0 ml and could be filled and sampled through separate ports. A cellulose membrane separated the diffusion cell compartments: the effective surface area of the membrane was 1.98 cm^2. The whole assembled cell was rotated at 120 rpm at 25°C. Samples (25 μl) were withdrawn from the acceptor compartment at regular time intervals for HPLC analysis (Cuesta-Sanz and Santa-Cruz, 1986). The acceptor compartment was simultaneously refilled with fresh acceptor phase.

The donor phase was represented by microemulsions selected according to the simplex centroid design shown in Fig. 2 and containing 6.0 mg/ml of retinol; as acceptor phase, a glycol solution of TDC at the same concentration present in the microemulsion was used.

Each drug diffusion experiment was performed in triplicate and the mean apparent permeability coefficients (K_p) of retinol were calculated (Trotta et al., 1990) from the data collected over 120 min.

Regression model

There are several model forms which can be calculated from the set of mixtures selected. Among them we chose the Cox-type models which consist of full rank polynomials, containing the offset (Cornell, 1991), In particular, second and third degree Cox models were considered.

The selected regression approach was partial least squares (PLS). The PLS algorithm has been widely described elsewhere and we shall not enter into details here (Wold, 1982; Geladi and Kowalski, 1986; Manne, 1987).

PLS is based on the decomposition of the original independent variable block in a new set of orthogonal variables (i.e., each other linearly independent), called latent variables (LV), obtained as linear combinations of the original ones. These variables are calculated with the aim of eliminating the collinearity present in the independent block and of correlating them as much as possible with the responses of interest. The maximum number of latent variables corresponds to the dimensionality of the original variable space. The proper number of latent variables, i.e., the number of latent variables which provides the best predictive ability is usually determined through cross-validation (Wold, 1978). This is a validation technique based on the prediction of the response for experimental points left out during the model building. From the difference between the predicted and the experimental responses, it is possible to calculate a sum of squared errors which is a measure of the model predictive ability: predictive errors sum of squares (PRESS). The selection of the proper number of latent variables is performed adding one latent variable at each step and calculating the PRESS. The number of LV selected is that of the model providing the minimum PRESS.

The problem which arises when using PLS consists mainly in the difficult interpretability of the regression model which is distributed on several latent variables, so that PLS models are more often used for prediction rather than for interpretation. On the other hand, some algorithms are now available which allow the calculation from the PLS model of a set of OLS type coefficients, which are of straightforward interpretation, though maintaining the good features of PLS (Clementi et al., 1989; Marengo and Todeschini, 1991) from which they have been derived.

A variables selection procedure was applied because the presence of independent variables which do not correlate with the response may influence the model reliability by introducing a noise effect.

The selection of the best set of independent variables was performed using a step-wise algorithm (Marengo et al., 1992). This is an iterative procedure in which at every cycle the variables left out are in turn reintroduced, and the variables at the moment present into the model are in turn left out. Then, the change in the model (i.e., addition of an absent variable or elimination of an existing one) provides the greatest increase in the model predictive ability. This procedure is repeated introducing or eliminating every time one variable, until no more improving action can

be detected (i.e., the PRESS cannot be further decreased).

The evaluation of the predictive ability of the model is based on the calculation of the PRESS. The PRESS was evaluated through a leave-one-out cross-validation procedure, similar to that used to determine the proper number of LVs. In this iterative procedure every experiment is in turn left out during the model building and then its response is predicted using the model calculated in its absence. The sum of squared errors calculated in this way is a measure of the model predictive ability.

Moreover the PRESS can be easily transformed into a measure of the fraction of variance explained in prediction of R_p^2 through the following expression:

$$R_p^2 = 1 - \text{PRESS}/\text{SST}$$

where SST is the total sum of squares of the mean centered responses. This expression is analogous to that which expresses the usual R_A^2 coefficient except for the substitution of the sum of squared errors in fitting with the PRESS.

The use of a stepwise variables selection procedure would be very dangerous using OLS because of overfitting problems. The use of PLS and of cross-validation makes the results more stable and reliable.

Results and Discussion

The study was performed at two different fixed percentages of lecithin, i.e., 10 and 15%, as described in Materials and Methods. The first step of the study consisted in the search for the microemulsion realm of existence.

The starting experimental region explored was selected on the basis of previous results (Pattarino et al., 1992) which showed that waterless microemulsions could be obtained with TDC, EO and PG approximately in the following proportions: TDC 17.5%; EO 7.0%; PG 60.5% (for lecithin at 15.0%); TDC 14.5%; EO 9.5%; PG 65.0% (for lecithin at 10.0%).

Since the lecithin percentage was held constant, the problem was reduced to a three-component mixture, with only two really independent variables. This means that, in this case, a Doehlert design requires six experiments located on the vertices of a hexagon plus one experiment in the center of the hexagon.

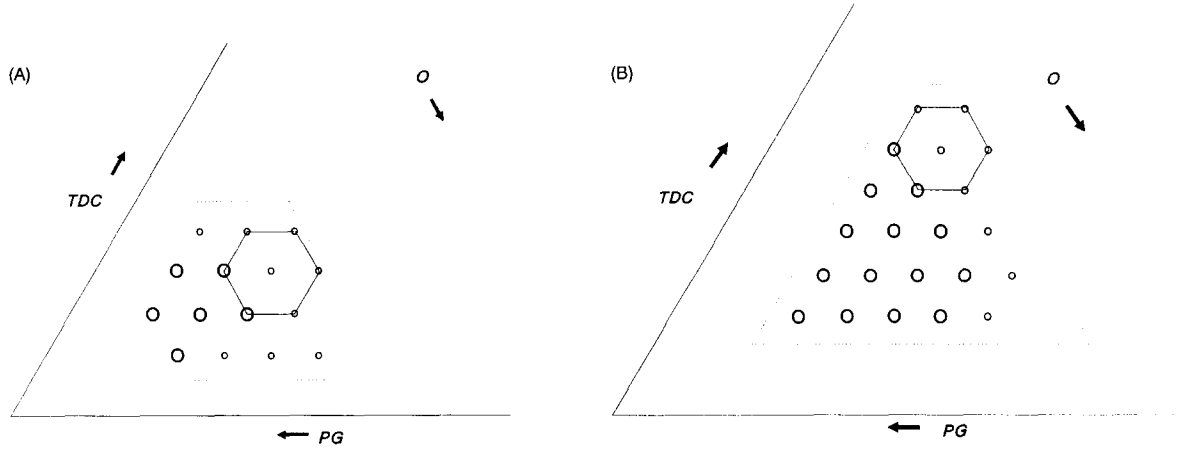

Fig. 1. (A) Experimental design for microemulsion realm of existence searching (lecithin percentage; 10%). (B) Experimental design for microemulsion realm of existence searching (lecithin percentage: 15%). The small circles represent unstable systems while the large circles represent microemulsions.

The first seven mixtures tested for the existence of microemulsions are reported in Fig. 1A and B. The starting Doehlert design was extended, adding at every successive step the new mixtures necessary to complete the design in a neighbouring mixture region, in the proper direction (dashed line).

The expansion of the Doehlert design was constrained according to pharmaceutical requirements, since microemulsions of pharmaceutical interest must be characterized by particular ranges of the components. In this case the adopted constraints were:

$L = 15\%$

$9.0\% < \text{TDC} < 20.0\%, 7.0\% < \text{EO} < 17.0\%, 50.5\% < \text{PG} < 68.5\%$

$L = 10\%$

$7.5\% < \text{TDC} < 25.0\%, 4.5\% < \text{EO} < 15.0\%, 53.0\% < \text{PG} < 70.5\%$

When the constraints, or a region of instability were reached, the search in that direction was stopped.

The mixtures added at each step to complete a new hexagon are also shown in Fig. 1A and B where the small circles indicate mixtures which did not provide microemulsions. The Doehlert design expansion was stopped when the boundaries of the region of interest were reached.

In this way, the regions of existence of oil-in-glycol microemulsions, compatible with the region of pharmaceutical interest could be defined, for the two percentages of lecithin.

As can be observed from Fig. 1A and B, the two regions are both approximately triangular, and the domain at 15% of lecithin is larger than that at 10% of the surfactant.

The next problem was to build up a regression model to relate the composition of the mixtures to the response of interest, i.e., the rate of diffusion of retinol from the microemulsion through a hydrophilic membrane.

The regression calculations were performed only on the mixtures with 15% lecithin as they showed a larger area of existence. The mixtures for measurement of the diffusion rate of retinol were selected according to the simplex centroid design augmented with three interior points, as

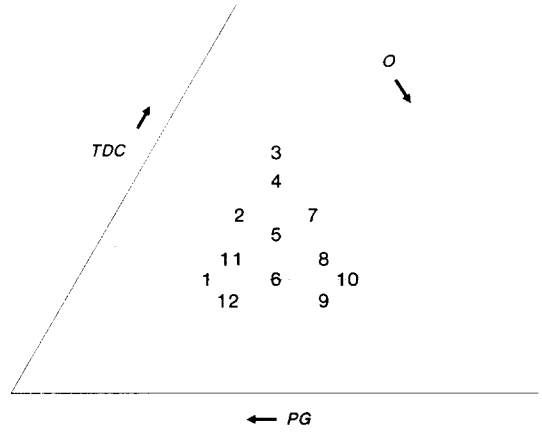

Fig. 2. Experimental design for mixture selection. The composition of each mixture selected and the corresponding measured K_p are listed in Table 1.

previously described. Since the realm of existence of microemulsions was larger than the region sampled by the simplex (triangular region), two more experiments (mixtures) outside the triangle were added, in order to extend the region of validity of the regression model.

The mixtures selected are represented in Fig. 2 and listed in Table 1, together with the observed apparent permeability coefficients.

Two calculations were performed on this dataset, using different sets of variables, i.e., Cox models of different degree.

In particular, the starting sets of variables corresponded to full rank second (set A) and third degree (set B) Cox's models.

The two sets of variables for Cox's models are:

SET A

EO, TDC, PG, EOxTDC, EOxPG, TDCxPG, EO^2, TDC^2, PG^2

SET B

EO, TDC, PG, EOxTDC, EOxPG, TDCxPG, EO^2, TDC^2, PG^2EOxTDC^2, EOxPG^2, TDCxEO^2, TDCxPG^2, PGxEO^2, PGxTDC^2, EOxTDCxPG

The results of the calculation using set A are reported in Table 2. The final model from set A, which explained 83.8% of the variance in prediction with one latent variable, contained two vari-

TABLE 1

Mixtures of the experimental design and corresponding observed permeabilities

Mixture	EO (%)	TDC (%)	PG (%)	K_p ($\times 10^{-6}$)
1	7.00	10.00	68.00	5.82
2	7.00	13.75	64.25	5.00
3	7.00	17.50	60.50	3.53
4	7.83	15.83	61.33	4.23
5	9.50	12.50	63.00	4.62
6	10.75	10.00	64.25	5.91
7	10.75	13.75	60.50	4.30
8	12.83	10.83	61.33	5.89
9	13.67	9.17	62.16	5.88
10	14.50	10.00	60.50	5.64
11	7.83	10.83	66.34	5.11
12	8.66	9.17	67.17	5.61

ables, TDC and TDC^2, whose OLS type coefficients are reported in Eqn 1. The predictive ability of the model is shown as a plot of predicted against experimental permeabilities in Fig. 3, while the numeric data are reported in Table 3. In Fig. 4, a contour plot of iso-permeability curves in the region of existence of the microemulsions is shown.

In the case of set B, the final model contained the four variables, reported in Table 4, together with the OLS type coefficients (Eqn 2) and the statistics about the final model. This model achieved the best predictive ability using all the four available LVs. This means that in this case the PLS and OLS models are identical. This model explains 87.4% of the varaince in prediction, 3.6% more than could be obtained with the variable set A. In Table 5, experimental, predicted and calculated permeabilities are reported. The same result is shown in graphical form in Fig. 5 as a plot of predicted vs experimental responses. In Fig. 6, a contour plot of iso-permeabilities, analogous to that of Fig. 4, is shown. As can be observed the two contour plots are similar in spite of the different model structures.

TABLE 2

Best model obtained with PLS in terms of R_p^2 with respect to the number of LVs (set A variables) (the OLS type equation was obtained from the PLS model)

LV	R_p^2	R^2
1	83.8	87.4
2	79.1	87.5

$K_p = 7.5533 - 0.1374 \cdot \mathrm{TDC} - 0.0052 \cdot \mathrm{TDC}^2$

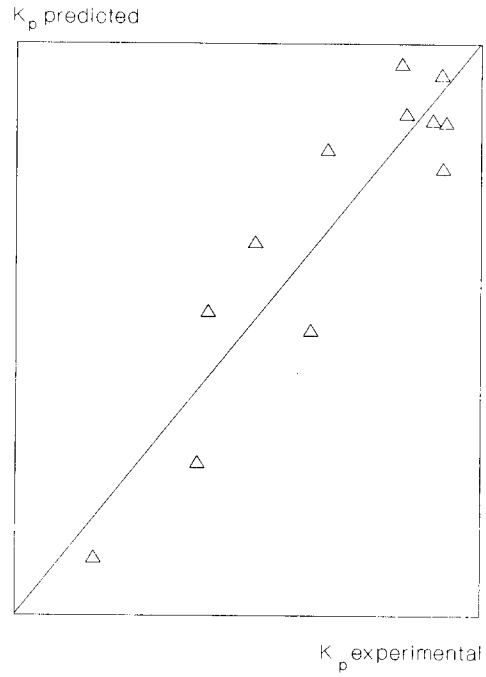

Fig. 3. Plot of predicted vs experimental K_p values from the final PLS model obtained with set A variables (Eqn 1, Table 2).

TABLE 3

Experimental, predicted and calculated permeability coefficients by means of the best PLS model obtained with set A variables

Mixture	K_p exp. ($\times 10^{-6}$)	K_p pred. ($\times 10^{-6}$)	K_p calc. ($\times 10^{-6}$)
1	5.82	5.63	5.65
2	5.00	4.63	4.67
3	3.53	3.55	3.54
4	4.23	4.00	4.06
5	4.62	5.05	5.02
6	5.91	5.62	5.65
7	4.30	4.72	4.67
8	5.89	5.40	5.45
9	5.88	5.85	5.85
10	5.64	5.66	5.65
11	5.11	5.49	5.45
12	5.61	5.90	5.85

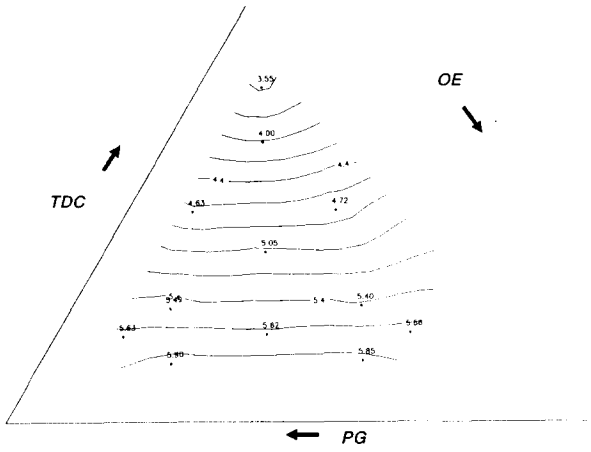

Fig. 4. Contour-plot of iso-permeability curves obtained from the final PLS model with set A variables (Table 2).

TABLE 4

Best model obtained with PLS in terms of R_p^2 with respect to the number of LVs (set B variables) (the OLS-type equation was obtained from the PLS model)

LV	R_p^2	R^2
1	25.2	53.8
2	83.5	90.2
3	79.0	90.2
4	87.4	94.3

$K_p = 6.6102 + 28.6985 \cdot EO \cdot TDC - 0.3377 \cdot EO \cdot TDC \cdot PG - 0.3392 \cdot EO^2 \cdot TDC - 0.6688 \cdot EO \cdot TDC^2$

TABLE 5

Experimental, predicted and calculated permeability coefficients by means of the best PLS model with set B variables

Mixture	K_p exp. ($\times 10^{-6}$)	K_p pred. ($\times 10^{-6}$)	K_p calc. ($\times 10^{-6}$)
1	5.82	5.54	5.67
2	5.00	4.69	4.74
3	3.53	3.46	3.51
4	4.23	4.43	4.32
5	4.62	4.97	4.83
6	5.91	5.41	5.56
7	4.30	4.27	4.28
8	5.89	5.67	5.81
9	5.88	5.91	5.90
10	5.64	5.83	5.71
11	5.11	5.51	5.43
12	5.61	5.84	5.78

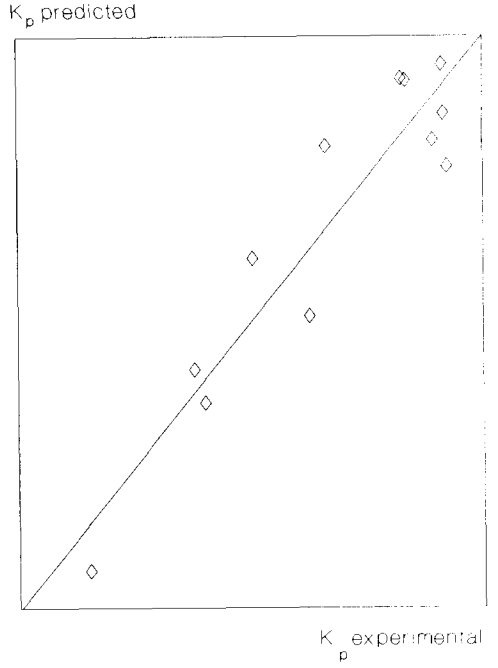

Fig. 5. Plot of predicted vs experimental K_p values from the final LPS model obtained with set B variables (Eqn 2, Table 4).

As proposed in the previous work (Pattarino et al., 1992), different equilibria involving retinol and microemulsion components can affect the retinol permeation process: transport of the free drug, diffusion of drug aggregates, interaction of

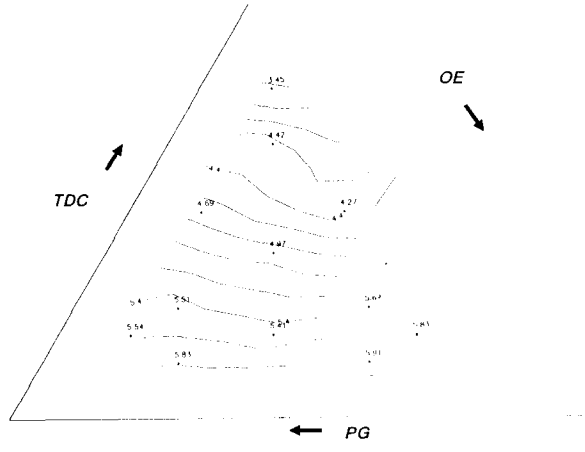

Fig. 6. Contour-plot of iso-permeability curves obtained from the final PLS model with set B variables (Table 4).

the drug with the components of the microemulsion interphase, micellization of the drug present in the continuous phase and, primarily, drug partition in the internal phase of microemulsion.

In the present work, the resulting model, obtained with the first set of variables (set A), indicates that the cosurfactant plays a very important role in the permeation of the drug through the dialysis membrane: as can be observed from the contour plot of iso-permeabilities (Fig. 4), at fixed proportion of EO, increasing percentages of cosurfactant lowered the K_p value of retinol. As previously shown (Pattarino et al., 1992), TDC can form micelles in polar organic solvents: also in the external glycol phase of our microemulsion, the cosurfactant could form micelles, in association with lecithin (mixed micelles) or not, thus reducing the free-diffusible amount of retinol.

The model obtained with the set B shows that the permeability coefficient of retinol is a function of terms that are products of different variables (Table 4).

Inspection of Fig. 6 accounts for this feature: at constant TDC levels (> 12%), the permeability coefficient of the drug decreases significantly as the percentage of oil rises, and when the oil is held at a fixed proportion, the permeation rate decreases for increasing percentage of the cosurfactant.

These findings confirm the reservoir effect of the internal phase of the microemulsion, according to our previous results (Pattarino et al., 1992) and demonstrate the influence of micellization of the drug on its releasing behaviour. Both calculated models showed good predictive abilities of the retinol permeability from microemulsions through a hydrophylic membrane. Therefore, they could be used to select proper mixture compositions to achieve a desired drug release performance.

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