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Influence of Two Salicylate Components on the Particle Size of an Oil-in-Water Emulsion with Nonionic Surfactants

G. Mestres,[1,*] F. Nielloud,[1] R. Fortune,[1]
J. M. Devoisselle,[1] R. Marti,[2] and H. Maillols[1]

[1]*Laboratoire de Technique Pharmaceutique Industrielle, Faculté de Pharmacie, Université Montpellier I, 34060 Montpellier, France*
[2]*Laboratoire d'Econométrie, Faculté des Sciences Economiques, Université Montpellier I, 34060 Montpellier, France*

ABSTRACT

This paper reports a study undertaken using techniques of static and dynamic light scattering to investigate the influence of sodium salicylate and methyl salicylate on droplet size of oil-in-water emulsions. The rates of changes were measured by determining the size and distribution of the oil droplet in the material. All emulsions showed a bimodal size distribution; the mean diameters and polydispersity were calculated from intensity. These data were analyzed with nonlinear regressions and bootstrap methodology. An amount of methyl salicylate component induced a decrease of mean diameter and standard deviation. On the contrary, sodium salicylate entailed the growth of all droplet populations and coalescence for the highest concentration.

Key words: *Bootstrap methods; Light scattering; Methyl salicylate; Oil-in-water emulsion; Particle size analysis; Sodium salicylate.*

INTRODUCTION

A preliminary work using experimental designs has shown the importance of formulation parameters of oil-in-water emulsions. Five factors were investigated: temperature of manufacture, time of phase introduction, rate of homogenization, mode of cooling, and operators. The best conditions found in this preliminary study with a ruggedness formulation method give a stable oil-in-water emulsion (1,2), and we studied the influence on this emulsion of additional drugs, two different salicylate compounds. Methyl salicylate and sodium salicylate are

*To whom correspondence should be addressed. Fax: (33) 67.04.88.74; e-mail: gmestres@pharma.univ-montpl.fr

two of the most commonly used analgesic and anti-inflammatory ingredients in commercially marketed over-the-counter dermatological products. They are formulated in such pharmaceutical preparations, and their topical application can procuce rubifacient action at the applied site (3,4). The purpose of this study is to determine the component which produce the most stable emulsion with the system Brij 92/Brij 98–water–paraffin oil. Light scattering spectroscopy and quasi-elastic light scattering spectroscopy (QELSS) have been used successfully to study fine emulsions.

MATERIALS AND METHODS

Materials

Two nonionic surfactants: oleyl ether (Brij 92®, ICI) and polyoxyethylene (20) oleyl ether (Brij 98®, ICI)
Paraffin liquid, kinematic viscosity 40°C 70 cSt (Primol®, Esso)
99% methyl salicylate and 99% sodium salicylate (Prolabo, Rhône-Poulenc)
Distilled water for emulsion formulation
Sterilized water (Versol®, Aguettant) for dilution.

Methods

Preparation of Emulsions

The method of phase inversion was used to prepare oil/water (o/w) emulsions (5). The oily phase is composed of 20% paraffin and 5% surfactants mixture (Brij 92/Brij 98, 70/30, p/p). Both the aqueous and oily phase were warmed to a temperature of 75°C. The aqueous phase was then added to the oily phase in 60 sec, and mixed by the use of a homogenizer (Turbotest 33/300, Rayneri) at 400 rpm with progressive cooling corresponding to 45 min of homogenization at room temperature (25°C). About 300 g of emulsion were prepared for each test. Five different emulsions are obtained: one without drug, corresponding to reference emulsion (Ref); one with 1% methylsalicylate and 3% (MeS); and one with 1% sodium salicylate and 3% (NaS). Methyl salicylate is incorporated in the oily phase and sodium salicylate in the aqueous phase before emulsification. All emulsions obtained were o/w and stored at 20 ± 1°C. No study can be completed with 3% sodium salicylate because in the first hours this emulsion breaks.

Emulsion Dilution

Previous works emphasized the requirement that emulsions be dilute. Emulsion are usually measured in a high state of dilution to ease interpretation in terms of independent particle scattering models (6,7). At elevated solute concentrations, diffusing particles interact with each other, modifying the diffusion coefficients (8). Different dilute solutions were studied, and for all experiments we used exactly 25 µl of each emulsion in 200 ml of water (Versol).

Dynamic Light Scattering or Quasi-Elastic Light Scattering

Measurements were made using a SEMATech photogogniodiffusiometer (SEM-633) operating at 632.8 nm and with a He–Ne laser as the source of incident light. The SEMATech instrument is combined with a Correlator SEM Real Time Granulometry (RTG), using 12 channels, Log–Log, corresponding to 4096 channels. The cylindrical scattering cells were immersed in a thermostated bath (20 ± 1°C) of index-matching liquid (toluene). All data in the homodyne mode were collected at a scattering angle of 90°, at 20 ± 1°C, and analyzed using RTG software to obtain correlation function, translational diffusion coefficient, and emulsion droplet diameter.

Microscopic Observations

Emulsions were observed with a Zeiss Axiostrop equipped with PanNeofwar (100 × 1.3), and photomicrographs were obtained in ordinary light.

RESULTS AND DISCUSSION

The size distribution of its droplets is a most important parameter in characterizing any emulsion (9). Quasi-elastic light scattering is used to obtain particle size distribution information from the time-dependent fluctuations of scattered light intensity caused by fluctuations of particles (Brownian motion). Intensity fluctuations of the scattered light are studied through their autocorrelation function. This function is exponential, and a typical example of experimental data is given in Fig. 1 (3% methyl salicylate). The diffusion coefficient of the scattering centers may be calculated from the decay of the correlation function; the hydrodynamic radius of the particle r_H can be determined from the

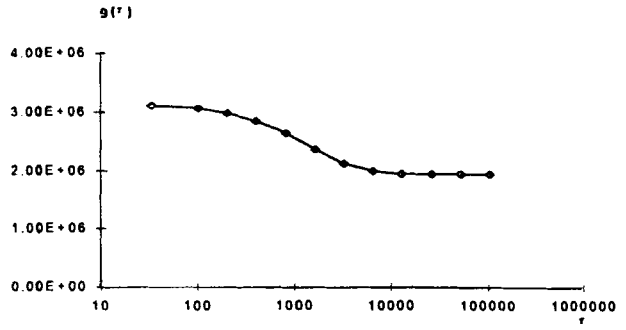

Figure 1. Normalized intensity-time correlation function $g(2)$ (τ). Log scale, emulsion MeS (3% w/w).

diffusion coefficient D using the Stokes-Einstein relationship:

$$D = k_B T/6\ \pi\ \eta\ r_H$$

where k_B is the Boltzmann constant, T is the absolute temperature, and η is the viscosity of the solvent (10,11). The four emulsions were analyzed regularly, after standing at 20°C for 2 months. The stability of emulsions was demonstrated by the lack of change on the diffusion coefficient over the period (70–75 days), and the values of D are reported in Fig. 2. Addition of a small quantity of methyl salicylate to emulsion results in an increase in the diffusion coefficient, which could be interpreted as a decrease in the hydrodynamic radius of the particle. The other emulsions are very similar to one another. With the cumulant technique, the mean diameter of the population of particles and a variance of this population are calculated. A polydispersity parameter is also given. In all cases this parameter is high and as a result, with polydisperse emulsions, the method of cumulants is not a rigorous approach. Another means of analysis is implanted in the RTG program [singular system analysis (SSA)]. It is the most powerful technique for polydisperse samples, introduced by Bertero and Pike (12). The RTG software gives the intensity, number, and mass of diffusant particles, and it is the most widely method used to determine droplet size distribution for polydisperse emulsions (13). We used the SSA with this type of emulsion, and the probability density of intensity was obtained.

For each formulation (Ref, 1% and 3% MeS, 1% NaS) at day 7, the particle size data are given by RTG correlator and show a bimodal distribution. By hypothesis this bimodal distribution corresponds to two normal laws with p and $q = (1 - p)$ proportions.

$$PD(x) = p\ PD_1(x) + (1 - p)\ PD_2(x)$$

where:

$$PD_i(x) = \frac{1}{\sigma_i\sqrt{2\pi}} \times e^{-\frac{(x-m_i)^2}{2\sigma_i^2}} \qquad i = 1, 2$$

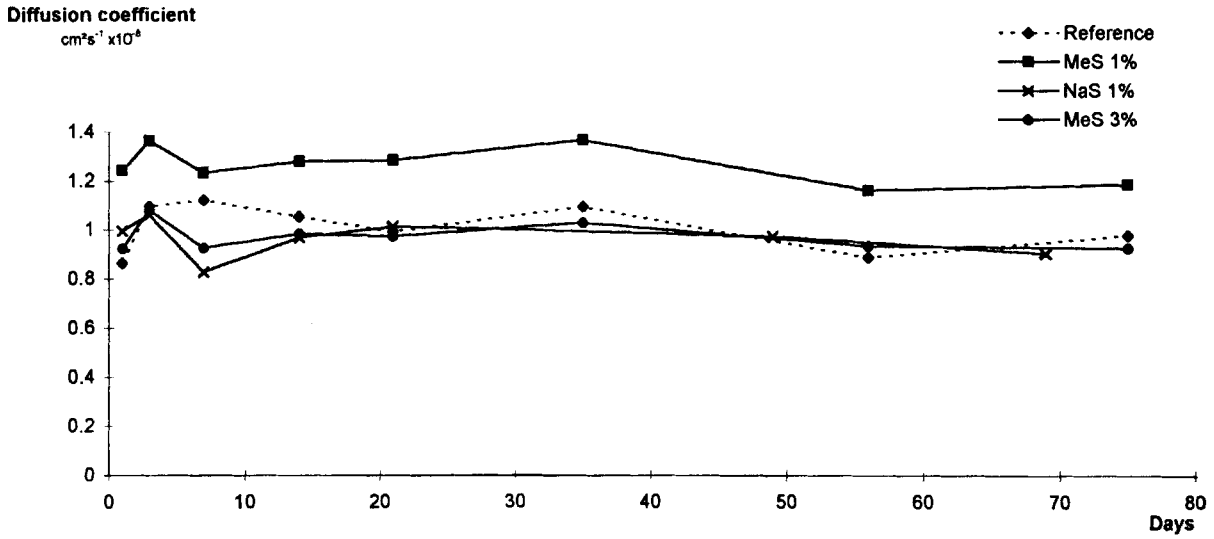

Figure 2. Diffusion coefficient as a function of time, reference emulsion, emulsion with methyl salicylate (1%, 3% w/w), and with sodium salicylate (1% w/w).

Table 1

Characteristics of Each Emulsion

Emulsion Characteristics	Methyl Salicylate 1%	Methyl Salicylate 3%	Reference	Salicylate
		First Population		
Probability (p)	0.45	0.61	0.51	0.52
Standard deviation	0.07	0.03	0.06	0.07
Mean (nm)	233.1	276.8	250.3	288.4
Standard deviation	15.9	9.9	24.7	20.8
Standard deviation	46.7	60.9	70.6	88.3
Standard deviation	9.6	7.4	15.9	17.2
		Second Population		
Probability (q)	0.55	0.39	0.49	0.48
Standard deviation	0.07	0.03	0.06	0.07
Mean (nm)	1037	1163	1753	3314
Standard deviation	49	68	173	363
Standard deviation	211	226	469	935
Standard deviation	38	43	108	239

A nonlinear regression associated with bootstrap methodology (14–17) is used to estimate bimodal distribution parameters and their first and the second moments, Table 1. The main advantages of this technique is that it allows an analysis with high coverage accuracy in small to moderately sized samples. For every series, a simulation study with $N = 100$ resampling is a reasonable estimate (18). Nonlinear regression and bootstrap calculations were obtained by the Mathcad program (19), and Fig. 3 corresponds to an example of experimental points and calculated curve. For purposes of comparison, the curves of all emulsions obtained by nonlinear regression are illustrated in Fig. 4.

We can observe two populations of droplet diameters. By and large the first population of salicylate emulsions is roughly not different from the reference

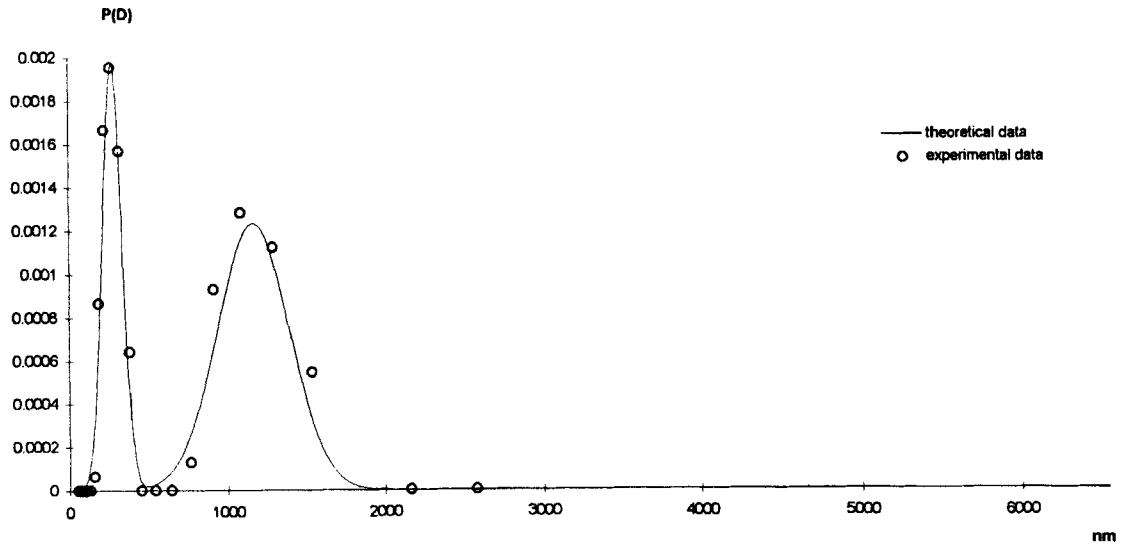

Figure 3. Emulsion MeS (3% w/w) experimental data and curve obtained by nonlinear regression.

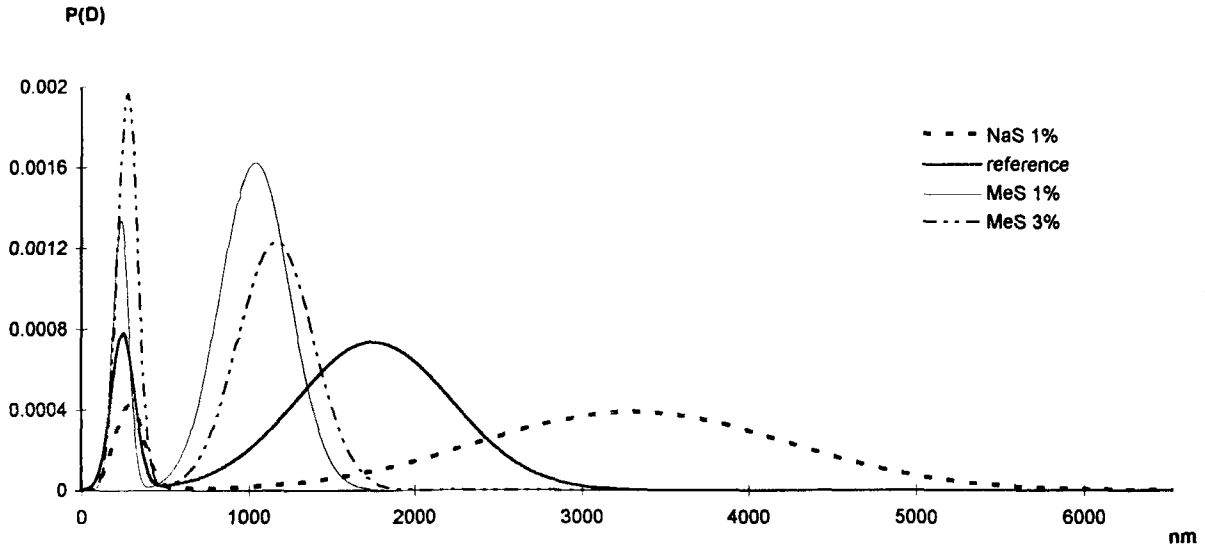

Figure 4. Comparison between curves obtained by nonlinear regression of all emulsions [reference, with methyl salicylate (1%, 3% w/w) and with sodium salicylate (1% w/w)].

emulsion, averaging 250 nm, with a small spread of standard deviation. It can be noted that the standard deviation between 1% MeS and 1% NaS increases from 46 to 88. On the contrary, the addition of salicylate component has an important effect on the second population. Both methyl salicylate concentrations entail a reduction of the mean particle diameter in comparison with the reference emulsion. Furthermore, the standard deviation decreases approximately by one-half. Nevertheless, this effect is more important with 1% of MeS. Sodium salicylate varies in location with substantially higher mean droplet size, and in spread with an important standard deviation (twice the value of reference emulsion). It can be seen that the presence of 1% concentration of sodium salicylate promotes aggregation of oil droplets. This phenomenon is confirmed by the destabilization of 3% NaS emulsion. The destabilizing effect of sodium salicylate observed at low electrolyte concentration is not observed in the presence of methyl salicylate. A microscopic observation (Fig. 5) illustrates these results.

Stability and resistance to creaming are influenced by both relative size and size distribution, and these results predict the behavior of the products. So, MeS emulsions are in all cases ≤ 1 μm, predicting the most stable emulsions, and very polydispersed NaS emulsion will entail unstability. All emulsions stocked at 20° ± 1°C were macroscopically controlled every month during 1 year. The reference emulsion was stable during 180 days, the NaS emulsion was less stable (90 days), and the two MeS emulsions were more stable (>360 days).

CONCLUSION

Laser light scattering was used to monitor particle size and polydispersity of several oil-in-water emulsion formulations. The emulsions evaluated in this study produce a bimodal distribution, and the introduction of salicylate components has a dramatic effect on the mean particle diameters and the spread of the distribution. The growth of droplets is important with 1% NaS emulsion. This salting effect is conducive to rapid coalescence of the oil droplets with 3%. This fast coalescence of droplet was observed during the first hour after preparation. Accordingly, with a small amount of this electrolyte, the electrostatic forces are strongly diminished, and particles can approach closely and form agglomerates. This result is in agreement with those reported by other authors (20). On the other hand, at low methyl salicylate concentration the particles turned out to be smaller than the initial emulsion reference droplets. This work cannot show a significant change in particle size between the two concentrations (1% and 3%) of methyl salicylate. With nonionic surfactants such as Brij 92–Brij 98, only

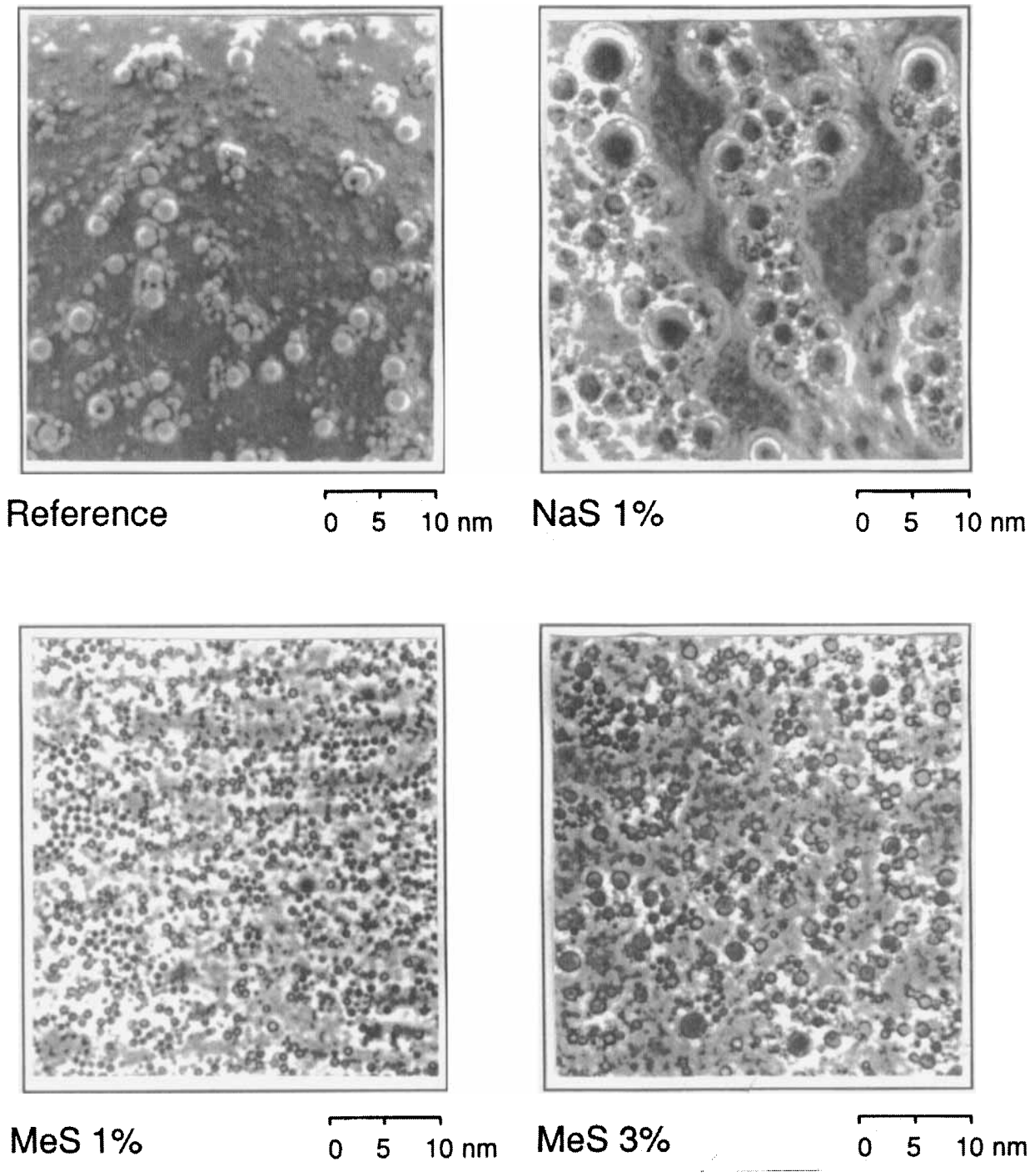

Figure 5. Photomicrographs in ordinary light of the emulsions without drug (Reference), with NaS 1%, and with MeS 1% and 3%.

MeS emulsions have a droplet size ≤ 1 μm and the greatest stability.

REFERENCES

1. F. Nielloud, J. P. Laget, R. Fortune, G. Marti-Mestres, and H. Maillols, Influence du protocole opératoire sur les caractéristiques d'une émulsion L/H réalisée avec des surfactifs polymériques non ioniques, Int. J. Cosmetic Chem., 17, 175–186 (1995).
2. F. Neilloud, G. Marti-Mestres, J. P. Laget, C. Fernandez and H. Maillols, Emulsion formulation, study of the influence of parameters with experimental designs, Drug Dev. Ind. Pharm., 22(2), 159–166 (1996).
3. T. Yano, T. Kanetake, M. Saita, and K. Noda, Effect of *l*-menthol and *dl*-camphor on the penetration and hydrolysis of methyl salicylate in hairless mouse skin, J. Pharmacobio-Dyn., 14, 663–669 (1991).
4. H. Maruta, K. Minami, T. Yamagata, and K. Noda, Percutaneous absorption of methyl salicylate from medicated plasters in mice and human, Kurume Med. J., 24, 131–137 (1977).
5. K. Shinoda and H. Kunieda, Phase properties of emulsion: PIT and HLB, in *Encyclopedia of Emulsion Technology: Basic Theory*, Dekker, New York, 1983, pp. 337–367.
6. R. S. Farinato and R. L. Rowell, Optical properties of emulsions, in *Encyclopedia of emulsion technology*, Vol. 1, Becher P, New York, 1983.
7. A. M. Bellocq and D. Roux, Phase diagram and critical behavior of a quaternary microemulsion system, in *Microemulsions: Structure and Dynamics*. CRC Press, Boca Raton, FL, 1987.
8. G. D. Phillies, Quasi elastic light scattering, Anal. Chem., 62, 1049A–1057A, (1990).
9. C. Orr, Emulsion droplet size data, in *Encyclopedia of Emulsion Technology*, Vol. 1, Dekker, New York, 1983, pp. 369–404.
10. R. J. Hunter, *Introduction to Modern Colloid Science,* Oxford University Press, Oxford, 1992.
11. B. J. Berne and R. Pecora, *Dynamic Light Scattering*, Krieger, Malabar, FL 1990.
12. M. Bertero and E. R. Pike, Exponential sampling method for Laplace and other dilationally invariant transforms. 1. Singular-system Analysis, Manual SEMATech, Nice, France, 1994.
13. C. Bezot, *Analyse des fonctions de correlation en diffusion de la lumière,* Coultronics, France, 1986.
14. L. A. Thombs and W. R. Schucany, Bootstrap prediction intervals for autoregression, *J. Am. Stat. Assoc.*, 85, 486–492 (1990).
15. M. W. Lutz, T. P. Kenakin, M. Corsi, I. A. Menius, C. Krishnamoorthy, T. Rimele, and P. H. Morgan, Use of resampling techniques to estimate the variance of parameters in pharmacological assays when experimental protocols preclude independent replication. An example using schlid regression, J. Pharmacol. Toxicol. Methods, 34(1), 37–46 (1995).
16. D. A. Fredman, Bootstrapping regression models, Ann. Stat., 9(6), 1218–1228 (1981).
17. T. J. Diciccio, M. A. Martin, and G. A. Young, Fast and accurate approximate double bootstrap confidence intervals, Biometrika, 79(2), 285–295 (1992).
18. A. Antoniadis, J. Berruyer, and R. Carmona, Regression non linéaire et applications, Ed. Economica, Paris, 1992.
19. Logiciel Mathcad Plus 5.0 for Windows, Mathsoft, 1986–1994.
20. R. Schuhmann, W. Mehnert and R. H. Müller, Coulter counter based coalescence assay for the determination stability after addition of electrolyte, Pharm. Ind., 55(7), 701–704 (1993).