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Comparison of oil-in-water emulsions manufactured by microfluidization and homogenization

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The purpose of this study was to compare drug-free model submicron oil-in-water (o/w) emulsions manufactured by high-speed homogenization and microfluidization. The study was aimed at evaluating the influence of these two manufacturing processes on the stability of the emulsions with respect to emulsifier concentration. Stability was defined in terms of dispersed droplet diameter growth over time. The study was also directed towards identifying the minimum emulsifier concentrations required by either processing method within the same model o/w systems to produce emulsions viable throughout the study period of three months. The Microfluidizer™ 110L was found to be more effective than the homogenizer in producing stable o/w submicron emulsions using triglycerides of caprylic/capric acid as the oil phase and combinations of emulsifiers (polyoxyethylene sorbitan oleate with high HLB and sorbitan monooleate with low HLB) at low emulsifier concentrations. Submicron emulsions prepared by the microfluidization process had smaller droplet diameters and exhibited less droplet diameter growth over time compared to high-speed homogenization. At emulsifier concentrations below 20% w/w, the droplet diameter or stability of the dispersed phase of the sub-micron emulsions prepared by either process was found to be dependent on the emulsifier content.

1. Introduction

A number of studies indicate that oil-in-water (o/w) emulsions could be a suitable delivery system to improve the bioavailability [1–3] and enhance the therapeutic activity of poorly water-soluble drugs [4]. Yet, few drugs are available for therapeutic use in the emulsion dosage form, and factors affecting their commercialization are potential lack of stability and short shelf life [5], lack of information about manufacturing cost, regulatory acceptance and safety [6]. Safe lipid emulsions for intravenous infusion became available for clinical nutrition only since the mid 1970s, prior to which experimental formulations were known to cause serious adverse reactions in patients [7]. Concerns about compatibility and emulsion stability upon addition of additives limit the potential of commercially available intravenous nutrient emulsions to function as a delivery system for drugs with poor water solubility [8].

With the exception of microemulsions, emulsions are considered thermodynamically unstable formulations because of the interfacial tension, the large surface area of the dispersed phase and the differential densities of the two phases. The dispersed droplets tend to coalesce in order to reduce the excess surface free energy, causing instability with eventual phase separation. Research on stabilization of emulsions has focused on the type and concentration of emulsifying agents and processing techniques that reduce dispersed droplet diameter and hence delay the aggregation of droplets. Microemulsions are translucent or transparent in appearance and form spontaneously upon mixing the ingredients without the input of any external energy. Submicron emulsions [9, 10], which also have nanometer range dispersed droplet diameter but a lower surfactant content compared to microemulsions, appear white in color, are considered thermodynamically unstable [11], and may lead to aggregation, coalescence and eventual phase separation [12]. The rate of droplet aggregation in submicron emulsions is usually much slower compared to coarse emulsions and depending on the processing conditions and surfactant combinations used to reduce the interfacial free energy, the physical stability of submicron emulsions may be greatly improved.

Formation of submicron emulsions is conventionally achieved using high frequency agitation by various techniques, including the processes of homogenization and microfluidization. Although a number of published articles report the stability of submicron emulsions and the effect of processing parameters on stability, few studies have specifically compared the efficacy of high-speed homogenization with microfluidization. Homogenization using high-shear mixers equipped with specialized stirrer assemblies is based on the principle of generation of a high shear stress and thus reduction of droplet diameter of the dispersed phase. The microfluidization process involves flow of the liquid mixture at a very high pressure through micro channels toward an impingement area. Precisely controlled emulsification forces generated by this technique include high shear (laminar flow), turbulence (iner-

tial flow), and cavitation (vapor bubble implosion) [13], with these mechanical forces acting together to reduce mean droplet diameter of the dispersed phase.

The purpose of this study was to formulate submicron emulsions and study the influence of the processing technique (homogenization vs. microfluidization) on the initial droplet diameter and rate of droplet aggregation in a model o/w emulsion system with respect to surfactant concentration. The study was aimed at evaluating the effectiveness of these two manufacturing processes that are commonly used for small-scale and large-scale emulsification. The effects of surfactant concentration on the dispersed droplet size together with the effect of the specific manufacturing process on the physical stability have been compared. The study was also directed towards identifying the minimum surfactant concentration required by either process within the same model o/w system to produce an emulsion with a slow rate of particle size growth during storage over a study period of three months.

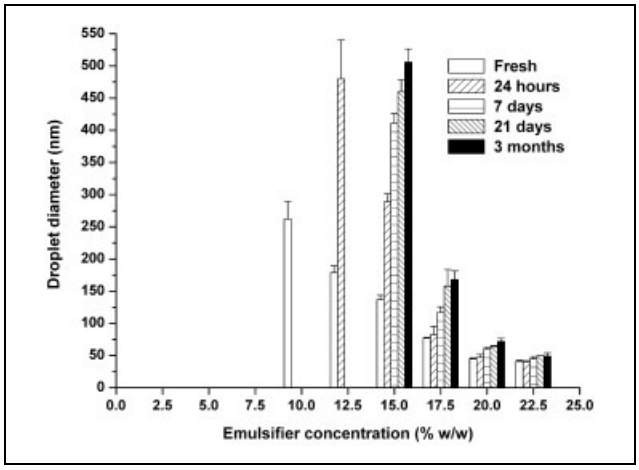

Fig. 1: Effect of emulsifier concentration on droplet diameter of high-speed homogenized submicron emulsions; mean ± SEM nm, (n = 9)

2. Investigations, results and discussion

2.1. Effect of emulsifier concentration on droplet diameter and stability

A mixture of surfactant (polyoxyethylene sorbitan oleate) and co-surfactant (sorbitan monooleate) was used in the formulations in order to ensure the integrity of the tightly packed amphiphile film at the interface [14]. As expected, a decrease in the droplet diameter of the sub-micron emulsions was observed with increase in emulsifier content in case of both the manufacturing processes, due to increased lowering of interfacial free energy. The initial droplet diameter and the rate of droplet aggregation were also dependent on the total emulsifier content. Mean value of polydispersity indices, which are dimensionless numbers calculated from a simple fit of a parabola to the photon correlation data, have been reported for the initial particle size data and particle size at 3 months (Table). A polydispersity index value between 0 and 1 indicates a unimodal size distribution, with a value of 0 describing an ideal monodisperse system.

Fig. 1 shows the effect of emulsifier content on droplet diameter of homogenized emulsions during storage over a period of three months. Data is presented only from 10% w/w to 22.5% w/w emulsifier concentration because below 10% w/w emulsifier concentration, the homogenized emulsions cracked within a few minutes of preparation and thus no further studies could be conducted on these emulsions. At 24 h, it was observed that the emulsions containing 12.5% w/w emulsifier had creamed and were not considered viable for further investigations as very gentle swirling did not produce reproducible droplet diameter sizes at the three zones of sampling – top, middle and bottom layers of the emulsion, with the diameter at the top layers being significantly higher than the bottom layers, indicating irreversible creaming. At the 3-month sample time point, the emulsions with 15% w/w emulsifier concentration were also visually observed to have slightly creamed but were instantly re-dispersible with gentle swirling.

As shown in the Table, at the 15% w/w emulsifier concentration, the droplet diameter of the homogenized emulsions increased from the initial size of 137 nm to 506 nm at the three month sampling time point, indicating more than 250% growth in mean droplet diameter size. The polydispersity index for the same formulation changed to 1.48 at three months from the initial polydispersity index of 0.47, indicating that the droplet size distribution of the emulsion was no longer unimodal and contained multiple populations of varying particle sizes. Even though the mean droplet diameter of 506 nm at three months is acceptable for parenteral use, the large SEM value and the trend of particle size growth indicates short-term stability for this formulation. The droplet diameter growth decreased with further increase in emulsifier concentration, growing about 120% and 60% for the 17.5% w/w and 20% w/w emulsions, respectively, over the storage period of three months. The mean polydispersity indices for both the emulsions remained relatively unchanged, indicating the lack of formation of multiple populations of varying droplet diameter sizes. At 22.5% w/w emulsifier concen-

Table: Particle size data for emulsions prepared by high-speed homogenization and microfluidization; (n = 9)

Total emulsifier content, (% w/w)	Microfluidizer				Homogenizer			
	Initial droplet diameter (nm) mean ± SEM	Droplet diameter at 3 months (nm) mean ± SEM	Mean initial poly-dispersity index	Mean 3-month poly-dispersity index	Initial droplet diameter (nm) mean ± SEM	Droplet diameter at 3 months (nm) mean ± SEM	Mean initial poly-dispersity index	Mean 3-month poly-dispersity index
2.5	73 ± 16.59	106 ± 11	0.945	0.7125	–	–	–	–
5.0	69 ± 6.3	95 ± 2.5	0.249	0.2243	–	–	–	–
7.5	60 ± 3.4	81 ± 1.2	0.239	0.2087	–	–	–	–
10.0	40 ± 1.4	54 ± 2.4	0.2050	0.2643	262 ± 27.2	–	1.103	–
12.5	40 ± 0.7	52 ± 2.3	0.0460	0.2183	179 ± 10.5	–	0.5845	–
15.0	39 ± 0.5	50 ± 1.1	0.0312	0.2370	137 ± 6.5	506 ± 20.5	0.4745	1.4821
17.5	42 ± 0.3	49 ± 1.9	0.2615	0.2663	77 ± 1.4	168 ± 13.4	0.4475	0.5203
20.0	44 ± 0.95	44 ± 1.6	0.0645	0.1900	45 ± 1.3	72 ± 4.7	0.2455	0.2440
22.5	42 ± 0.7	43 ± 1.9	0.0325	0.0588	41 ± 1.6	49 ± 4.9	0.2050	0.2053

tration, the homogenized emulsions showed about 20% increase of mean droplet diameter, growing to 49 nm from the initial diameter of 41 nm, with the polydispersity index remaining unchanged. Despite the 20% increase in particle size over a period of three months, it ought to be recognized that 49 nm is still a very small size for emulsion droplet diameter. The effect of the concentration of emulsifier on the initial droplet diameter was found to be statistically significant for the 10–20% w/w emulsifier containing homogenized emulsions. The mean initial droplet diameter size of the 22.5% w/w emulsifier containing emulsions (41 nm) was not statistically different from the 20% w/w emulsifier containing emulsions (45 nm) but at the three month sample time point, the droplet diameter of the 20% w/w emulsions increased to 72 nm compared to 49 nm for the 22.5% w/w emulsifier containing emulsions, which was a significant difference. The only homogenized formulations that were truly comparable to their microfluidized counterparts were the emulsions containing 15, 17.5, 20 and 22% w/w of emulsifier since at lower concentrations the homogenized emulsions were not found to be viable at three months.

Fig. 2 shows the effect of emulsifier content on the droplet diameter and stability of the microfluidized emulsions. In general, the Microfluidizer™ produced emulsions with very small droplet diameters at the low emulsifier concentrations where the homogenizer was unable to produce viable emulsions. Using microfluidization, the lowest emulsifier concentration that produced an emulsion that remained viable at three months was 2.5% w/w, where the initial droplet diameter of 73 nm increased to 106 nm at three months. The polydispersity index for the 2.5% w/w emulsion remained below 1 at the initial stage as well as at three months, indicating a capacity to retain the unimodal size distribution character with storage. However, the 3-month data shows a large standard error of mean (SEM), indicating ongoing aggregation of droplets. In case of microfluidized emulsions, the effect of emulsifier concentration on the initial droplet diameter was statistically significant from 2.5 w/w to 10% w/w emulsifier containing emulsions. Beyond 10% w/w emulsifier concentration, the initial droplet diameters of emulsions produced by the Microfluidizer™ were not significantly different, with each formulation averaging around 40 nm. Increasing emulsifier concentration further from 10% w/w significantly influenced the rate of particle size growth as observed at three months, except for the 20% w/w and 22.5% w/w formulations, which did not significantly differ in mean droplet diameter size at the three month time point.

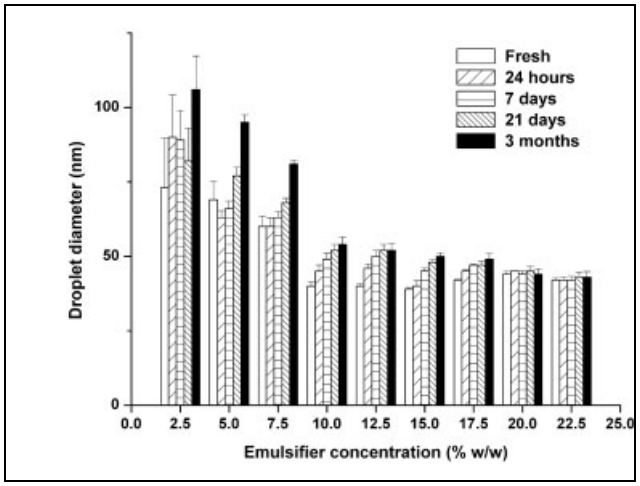

Fig. 2: Effect of emulsifier concentration on droplet diameter of microfluidized submicron emulsions; mean ± SEM nm, (n = 9)

2.2. Effect of processing method on emulsion formation and stability

No comparisons could be drawn between the two processes at low emulsifier concentrations except that microfluidization produced stable emulsions even at low emulsifier concentrations whereas emulsions of the same composition produced by high-speed homogenization either creamed or cracked. It is to be noted from Fig. 1 that only the emulsions with the highest surfactant concentrations (20 and 22.5% w/w) could be considered as likely to be stable for extended storage periods; at such high surfactant concentrations, the systems are expected to approach self-emulsification status and may not require the input of external energy for emulsification to occur. This indicates that the process of high-speed homogenization does not effectively contribute towards the stabilization of the emulsion systems studied. The influence of processing technique on the formulations becomes evident at the 10% w/w emulsifier concentration, where the homogenizer produced an initial droplet diameter of 262 nm compared to the 40 nm droplets produced by the microfluidizer. As mentioned before, the 10% w/w emulsifier containing emulsion produced by the homogenizer failed to remain viable for three months, whereas the same formulation remained viable after production in the microfluidizer with droplet diameter growing by about 50% over three months to 61 nm. As mentioned before, even though a 50% growth appears to be a large number, it ought to be recognized that 61 nm continues to be a very small droplet diameter and is well acceptable for parenteral use. Similar droplet sizes and growth patterns were obtained in case of the homogenized emulsions at the 20% w/w emulsifier concentration; hence, the difference between the two processing techniques becomes obvious.

The initial droplet diameter was significantly higher for the homogenized emulsions compared to their microfluidized counterparts for 10, 12.5, 15 and 17.5% w/w concentrations of emulsifier. By the homogenization process, the minimum emulsifier concentration necessary to form an emulsion was 10% w/w, and all emulsions below 17.5% w/w emulsifier content either creamed or cracked within three months. The process of microfluidization was superior to high-speed homogenization in preparing submicron emulsions that were viable for three months with total emulsifier content as low as 2.5% w/w. As shown in the Table, for the same emulsifier content, the droplet diameters produced by microfluidization were significantly smaller than that by homogenization. Fig. 3 compares the percentage droplet diameter growth at three months using both the processes. As shown in Fig. 3, the % growth of droplet diameter size was significantly higher for the homogenized submicron emulsions compared to their microfluidized counterparts. From the data presented in the Table, it is evident that increasing emulsifier content beyond 10% w/w and using the Microfluidizer™ did not alter the droplet diameter significantly for fresh emulsions, even though it did influence the rate of droplet diameter growth over three months (Fig. 3). At emulsifier concentrations of 20% w/w and 22.5% w/w, the initial droplet diameter produced by either processes were similar, indicating that the systems may be approaching self-emulsification status. The optical clarity (as observed visually as

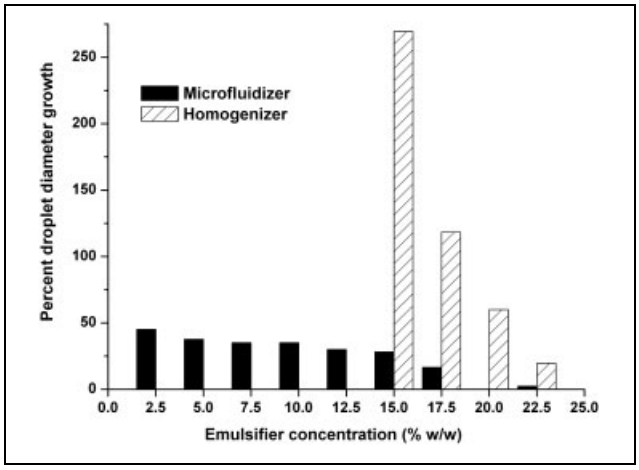

Fig. 3: Effect of emulsifier concentration on the percent droplet diameter growth in emulsions at the end of the three month storage period

well as evidenced by the intensity values in the Coulter N4 plus) of the emulsion systems improved at these surfactant concentrations, with the emulsions being translucent in appearance, also indicating that the systems may be approaching spontaneous emulsification status. However, the 20% w/w and 22.5% w/w emulsifier containing emulsions are not true microemulsions as the homogenized versions did exhibit continued droplet diameter growth over a period of three months, indicating the lack of thermodynamic stability. In case of true microemulsions, the emulsion properties should not be affected by storage or the type or duration of processing. A further increase in emulsifier concentration would almost certainly result in the formation of microemulsions, but such experiments were not conducted as this study focuses on submicron emulsions and the influence of processing parameters therein.

Apart from the smaller droplet diameters produced by the Microfluidizer™ as well as production of more stable emulsions compared to homogenization, one more significant advantage of the process of microfluidization over high-speed homogenization is the sample volume that may be critically important for pilot batches of expensive drugs. A 50 ml batch of emulsion was large enough to process in the Microfluidizer™ while the same sample size was too small for homogenization with Silverson SL2T, where the minimum processable volume proved to be 100 ml. However, the recovery of the processed emulsion was high for the homogenized product as a very small volume (about 2 ml) is lost due to adhesion to the stirrer assembly. The loss of sample was higher in the process of microfluidization as the first 3 ml collected from the initial run has to be rejected (because of potential mixing with the previously processed liquid that stayed within the interaction chamber) and also about 2 ml of the processed emulsion stays back in the interaction chamber at the end of the process and cannot be recovered. Thus a total loss of 5 ml volume amounted to a loss of 10% in case of the 50 ml sample size. As the volume of lost product is constant, the percentage loss would be reduced as the volume of processed fluid is increased.

In conclusion, Microfluidizer™ 110L was more effective than the Silverson™ SL2T homogenizer in producing stable o/w sub-micron emulsions employing triglycerides of caprylic/capric acid as the oil phase and combinations of emulsifiers (polyoxyethylene sorbitan oleate and sorbitan monooleate) at low emulsifier concentrations. The process of microfluidization was capable of imparting considerably smaller particle size and enhanced stability to the emulsion systems at low emulsifier concentrations, whereas the process of high-speed homogenization did not perform at the same level with identical systems. In general, submicron emulsions prepared by the microfluidization process were more stable over a period of three months than their counterparts prepared by high-speed homogenization. At the highest emulsifier concentrations, the emulsions appeared to approach self-emulsification status but were not true microemulsions. The dispersed phase droplet diameter of the sub-micron emulsions prepared by either process was positively correlated to the total concentration of the emulsifiers in the formulations and the age of the emulsions.

3. Experimental

3.1. Materials

The surfactant, polyoxyethylene sorbitan oleate or polysorbate 80 (CRILLET™ 4NF, HLB = 15) and cosurfactant, sorbitan monooleate (CRILL™ 4NF, HLB = 4.3) were obtained by the courtesy of Croda Inc., NJ. The oil phase, consisting of triglycerides of caprylic/capric acid, (CAPTEX™ 355; 63% caprylic (C8), 32% capric (C10) and less than 2% caproic (C6)), was obtained by the courtesy of ABITEC Corporation, OH. Distilled filtered water obtained from the Nanopure® water system (0.2 μm, Barnstead) was used throughout the studies. All formulations were prepared and stored in dark at room temperature in borosilicate glass vials.

3.2. Methods

3.2.1. High speed homogenization

Oil-in-water emulsions containing 10% w/w triglycerides of caprylic/capric acid as the oil phase and varying concentrations of emulsifiers (polyoxyethylene sorbitan oleate and sorbitan monooleate), ranging from 2.5% w/w to 22.5% w/w, were prepared using homogenization. Although the total emulsifier content varied for the different batches, the surfactant to cosurfactant ratio was maintained at 6 : 1 throughout the study. This particular ratio was selected following a number of test runs conducted to produce stable emulsions, the results of which are not presented for the sake of clarity. 100 ml batches of coarse emulsions were prepared by accurately weighing the components and magnetically stirring the mixture for 5 min at 30 °C in glass beakers. The coarse emulsion was processed in the Silverson™ SL2T high-speed homogenizer (Silverson Machines, East Longmeadow, MA) at 6000 rpm for 20 min in an ice bath the beaker to prevent excessive increase of temperature during processing. The Silverson™ SL2T high-speed homogenizer is a high shear rotor/stator mixer that produces multi-stage mixing/shearing action as materials are drawn through the specially designed workhead The temperature of the emulsion attained during processing was 30 ± 2 °C. Emulsions were stored in amber USP 1 glass containers at room temperature.

3.2.2. Microfluidization

The oil-in-water emulsion formulations prepared by homogenization were duplicated by microfluidization. The batch size was reduced from 100 ml to 50 ml because microfluidization allowed successful formulation with lesser volume of material compared to homogenization. Coarse emulsions produced by magnetic stirring for 5 min at 30 °C were processed through a Microfluidizer™ 110L (Microfluidics™ Corp., Newton, MA) at room temperature. The inlet air pressure was set at 60 psi and the individual batches were processed through the device for 20 discrete volume cycles and collected into glass beakers. Running cold water around the metal coil dissipated the heat produced during the microfluidization process and the temperature of the emulsion attained during processing was 30 ± 5 °C. Cooling with a stationary ice-jacket similar to the homogenization process failed because the emulsion within the capillary coil congeals due to any sudden drop in temperature, aborting the run. The process parameters were selected after several trial runs (the results of which are not presented for the sake of clarity) so that mechanical droplet size minimization should be complete and observed changes in droplet distribution would reflect only on the constituent ratios in the emulsion system. Emulsions were stored in amber USP 1 glass containers at room temperature.

3.2.3. Emulsion droplet size analysis and stability studies

The diameter of the dispersed phase oil droplets in the emulsions was analyzed using the principle of photon correlation spectroscopy using a

Coulter N4Plus™ sub-micron particle sizer (Beckman Coulter, FL) equipped with a 632.8 nm 10 mW helium-neon laser source. Routine analysis was done at 90° using both unimodal (cumulant) fit that yields mean droplet diameter and standard deviation in nm and Size Distribution Processor (SDP) analysis that resolves the components of polydispersed samples. Representative batches of emulsions were also sized at 14.9°, 20.6°, 30.4°, 40.2°, 50.4° and also using the Coulter Z2 particle sizer (Beckman Coulter, FL – electrical zone sensing technique, 1–120 μM) and Mastersizer 2000 (Malvern Instruments, UK – laser light scattering technique, 0.02 to 2000 μM) in addition to regular observation under optical microscope to ascertain the presence of any bigger droplets in the sample. The droplet diameters of the sub-micron emulsions prepared by homogenization and microfluidization were recorded immediately after preparation. Emulsion stability was studied as a function of droplet size, where a growth in particle size with time would indicate aggregation and coalescence of the droplets of the internal phase. The emulsions were stored at room temperature and 1 ml samples were withdrawn at 24 h, 7 d, 21 d and 3 months and analyzed for the droplet diameter. Mean droplet diameter for three separate batches of emulsions of identical composition are reported along with the standard error of mean (SEM). For every separate batch of emulsion, three random samples were drawn from the surface, middle and bottom region of the undisturbed emulsion and the droplet diameter determined. This resulted in $n = 9$ (3×3) samples for each composition of emulsion.

3.2.4. Data analysis

Statistical analyses of data were performed using SigmaStat™ (SPSS Inc, NC) software using analysis of variance (ANOVA). Tukey's multiple comparisons procedure was used to determine significance among all possible pairs of treatments and interactions. Tukey's procedure controls the experiment-wise error rate at the $\alpha = 0.05$ level.

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References

1 Palin, K. J.; Philips, A. J.; Ning, A.: J. Pharm. Sci. **33**, 99 (1986)
2 Kararli, T. T.; Needham, T. E.; Griffin, M.; Schoenhard, G.; Ferro, L. J.; Alcon, L.: Pharm. Res. **9**, 888 (1992)
3 Constantinides, P. P.; Welzel, G.; Ellens, H.; Smith, P. L.; Sturgis, S.; Yiv, S. H.; Owen, A. B.: Pharm. Res. **13**, 210 (1996)
4 Sasaki, H.; Takakura, Y.; Hashida, M.; Kimura, T.; Sezaki, H.: J. Pharm. Dyn. **7**, 120 (1984)
5 Humberstone, A. J.; Charman, W. N.: Adv. Drug Deliv. Rev. **25**, 103 (1997)
6 Collins-Gold, F.; Feichtinger, N.; Warnheim, T.: Modern Drug Discovery **3**, 44 (2000)
7 Driscoll, D. F.; Adolph, M.; Bistrian, B. R. In: Rombeau, J. L.; Rotandelli, R. (eds): Parenteral Nutrition. W B Saunders 2000
8 Driscoll, D. F.: Nutr. Clin. Pract. **10**, 114 (1995)
9 Wagner, J. G.; Gerard, E. S.; Kaiser, D. G.: Clin. Pharmacol. Ther. **7**, 610 (1966)
10 Carrigan, P. J.; Bates, T. R.: J. Pharm. Sci. **62**, 1476 (1973)
11 Becher, P.; Emulsion theory and practice, 2nd ed., Reingold, New York, NY 1965
12 Washington, C.; Athersuch, A.; Kynoch, D. J.: Int. J. Pharm., **64**, 217 (1990)
13 Korstvedt, H.; Bates, R.; King, J.; Siciliano, A.; Drugs & Cosmetics Industry, November, 36 (1984)
14 Rieger, M. R.; Emulsions, in Lachman, L.; Lieberman, H. A.; Kanig, J. L. (eds): The Theory and Practice of Industrial Pharmacy, 3rd ed., p. 502, Lea and Febiger, Philadelphia, PA 1986