

Particle Size Distributions and Particle Size Alterations in Microemulsions

B. W. MÜLLER* and R. H. MÜLLER

Received December 20, 1982, from the Department of Pharmaceutics, Christian-Albrechts University of Kiel, Gutenbergstrasse 76-78, D-2300 Kiel 1, West Germany. Accepted for publication June 7, 1983.

Abstract □ Several component diagrams for different alcohols as cosurfactants and potassium oleate as the surfactant were investigated. Between the regions of the water-in-oil microemulsion and the micellar solution (reverse micelles), and diagrams showed a zone of instability which was determined by particle size analysis by means of photon correlation spectroscopy (PCS). The polydispersity of the internal phase was determined at the instant of microemulsion formation and after fixed intervals of storage. At the edge of the microemulsion region, a rapid increase in particle size due to coalescence followed by breaking was observed. If a slow transition toward the region of solubilization could be seen by a slow decrease of the droplet size, coalescence was observed after storage. In the middle of the microemulsion region, the particle radius, however, was almost constant for a long interval. The velocity of the microemulsion formation depended on the alcohol used. In some cases a very fast formation (milliseconds) was observed; in others, macroemulsions were formed which became transparent within a few hours or days. PCS was used to follow the dynamic process of formation and breaking of such systems with droplet diameters of 5-200 nm by obtaining the mean hydrodynamic diameters. Distribution curves were calculated by the Laplace transform of the correlation function. The practicability of the method was demonstrated with mono- and polydispersed latex suspensions and microemulsions.

Keyphrases □ Particle size analysis--microemulsions, photon correlation spectroscopy, latex suspensions, Laplace transforms □ Emulsions--particle size analysis, photon correlation spectroscopy, Laplace transforms □ Stability--microemulsions, particle size analysis, photon correlation spectroscopy, Laplace transforms

Three types of microemulsions seem to exist: discrete micelles, microdroplets, and bicontinuous structures. To obtain an insight into the dynamics of microemulsion formation and breaking, photon correlation spectroscopy (PCS) can be used for the first two types (1). To eliminate measuring errors, interparticle effects and multiple scattering must be avoided by using low concentrations of the noncontinuous phase (5-10%). Under these conditions, PCS might be a valid method of measuring mean particle diameters and, in combination with a Laplace transform of the data, estimating the particle size distributions. By following alterations after spontaneous emulsification, it should be possible to develop thermody-

namically stable microemulsions with drugs and to find essential differences between a solubilization and a microemulsion if the system contains microdroplets or micelles.

THEORETICAL SECTION

The polydispersity of microemulsions and model suspensions was analyzed using PCS. With this method, the intensity autocorrelation function of the light, scattered by particles of the suspension, is obtained. The autocorrelation function $g(\tau)$ may be obtained from the intensity autocorrelation function $g^2(\tau)$:

$$g^2(\tau) = 1 + |g(\tau)|^2 \quad (\text{Eq. 1})$$

$g(\tau)$ contains information about the size distribution.

In the case of monodispersed particles, the autocorrelation function $g(\tau)$ is given by:

$$g(\tau) = g_0 \exp(-\gamma\tau) \quad (\text{Eq. 2})$$

where $\gamma = 2Dk^2$, D is the diffusion constant, and k is the line width constant. The decaying of the correlation function depends on the Brownian motion of the particles and may be derived from the statistics of the scattered light. In polydispersed suspensions and emulsions the autocorrelation function $g(\tau)$ can be derived from the size distribution $G(\gamma)$ by the Laplace transform:

$$g(\tau) = g_0 \int_0^\infty \exp(-\gamma\tau) \cdot G(\gamma) d\gamma \quad (\text{Eq. 3})$$

Ostrowsky (2) introduced a method for the inversion of this formula based on eigenvalues and eigenfunctions of the Laplace transform. The inversion problem in general has an "ill-conditioned nature" (3). This means that there is an upper limit of information about the size distribution function in the experimentally obtained correlation function. Using the method of Ostrowsky, the most complete information about the polydispersity may be obtained. Ostrowsky points out that the truncation of the correlation function due to noise leads to a band-limited Laplace transform. This is due to the fact that the maximum information about the polydispersity may be obtained by a discrete spectrum with exponentially spaced samples:

$$\begin{aligned} \gamma_{n+1} &= \gamma_1 \exp(n\pi/\omega_{\max}) \\ g(\tau)_{\omega_{\max} \rightarrow \infty} &= \sum_{n=1}^N \frac{\pi}{\omega_{\max}} \cdot G_D(\log \gamma_n) \cdot \exp(-\gamma_n \tau) \end{aligned} \quad (\text{Eq. 4})$$

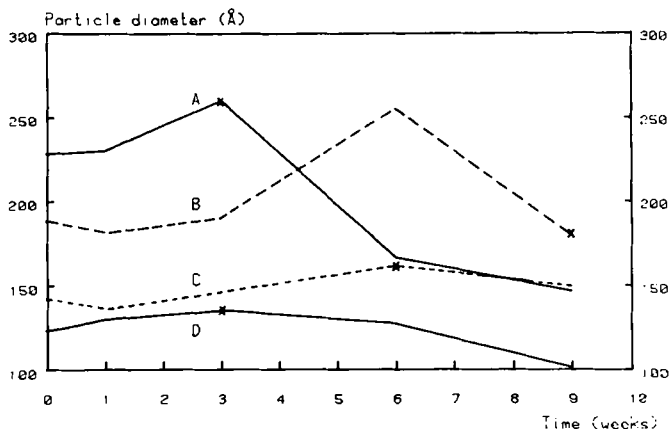


Figure 1—Particle sizes versus time in microemulsions with pentanol as cosurfactant. Composition: 3.6% water, 2.0% potassium oleate, and pentanol, q.s. to 100% with paraffin oil. Pentanol content: (A) 20.0%; (B) 22.0%; (C) 26.0%; (D) 30.0%.

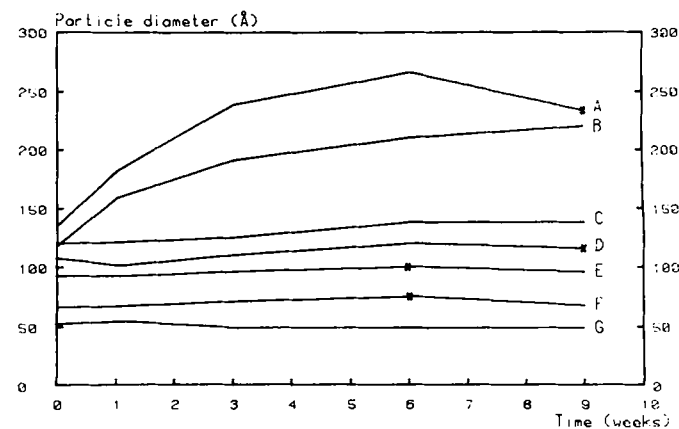


Figure 2—Particle sizes versus time in microemulsions with pentanol as cosurfactant. Composition: 3.6% water, 3.0% potassium oleate, and pentanol, q.s. to 100% with paraffin oil. Pentanol content: (A) 14.0%; (B) 16.0%; (C) 20.0%; (D) 24.0%; (E) 28.0%; (F) 36.0%; (G) 40.0%.

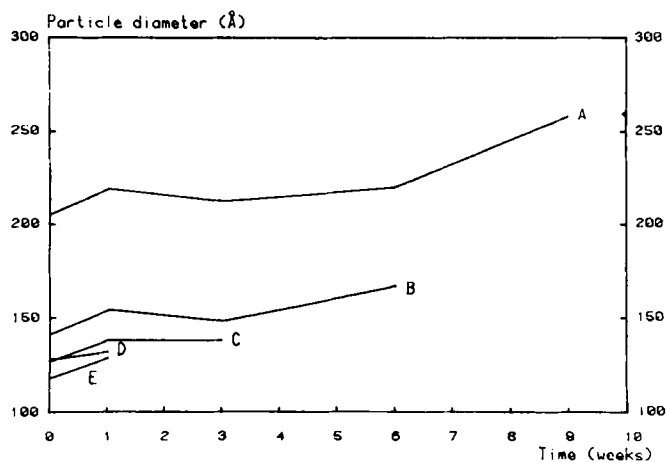


Figure 3—Particle sizes versus time in microemulsions with hexanol as cosurfactant. Composition: 3.6% water, 2.0% potassium oleate, and hexanol, q.s. to 100% with paraffin oil. Hexanol content: (A) 10.0%; (B) 12.0%; (C) 14.0%; (D) 16.0%; (E) 18.0%. (Broken systems, marked by x in the Figs. 1 and 2, were not investigated.)

where ω_{\max} is the band limit. These discrete spectra points are computed from the autocorrelation function by minimizing the equation:

$$\Delta = \sum_{i=1}^M \left[g(\tau_i) - \sum_{n=1}^N \frac{\pi}{\omega_{\max}} \cdot G_D(\log \gamma_n) \cdot \exp(-\gamma_n \tau_i) \right]^2 \quad (\text{Eq. 5})$$

The continuous polydispersity spectrum can be derived from the development of the discrete spectrum:

$$\tilde{G}(\gamma) = \sum_{n=1}^N G_D(\log \gamma_n) \sqrt{\frac{\gamma_n}{\gamma}} \frac{\sin \omega_n \cdot \left(\log \frac{\gamma}{\gamma_n} \right)}{\omega_n \cdot \left(\log \frac{\gamma}{\gamma_n} \right)} \quad (\text{Eq. 6})$$

This polydispersity spectrum was calculated by a computer program which includes the method of Ostrowsky (2). It must be pointed out that in addition to this method, there are other possibilities using other primary information about the polydispersity spectrum (4, 5).

EXPERIMENTAL SECTION

Materials—The microemulsions were prepared using fresh twice-distilled water, potassium oleate¹, liquid paraffin oil², and pentanol³, hexanol³, or heptanol³. The potassium oleate was taken from one homogeneous batch and was used without further purification. The alcohols were used as received.

To determine the accuracy of the polydispersity analysis by means of the Laplace transform of the correlation function, model suspensions were prepared by mixing latex particles of various sizes using monodispersed latex suspensions⁴ (10% by weight). The stock suspensions were diluted with distilled water and mixed in different ratios. The suspensions were passed through membrane filters⁵ (0.22–0.8 μm) to remove as many particle impurities as possible from the samples; the filters were attached to 10-mL disposable syringes⁶.

A commercial oil-in-water emulsion was tested as an example of a pharmaceutical product; it was composed of 200 g of soybean oil, 15 g of soy oil phosphatide, and 50 g of xylitol, q.s. to 1000 mL with water. Because the concentration of the dispersed phase was too high for PCS measurements, a dilution with twice-distilled water was necessary (50 μL of the emulsion was diluted to 100 mL). To avoid contamination with dust, the samples were prepared using the laminar flow technique.

Apparatus—For the measuring apparatus used in this experiment (1), the refractive indexes were determined by a refractometer⁷. To measure the viscosity of the dispersion media, a viscometer⁸ with light barriers as an automatic timing device was used. The thermostated bath had a constant temperature of $\pm 0.05^\circ\text{C}$.

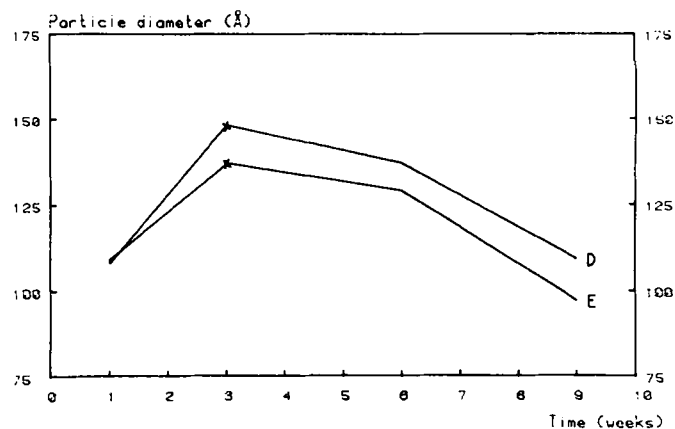
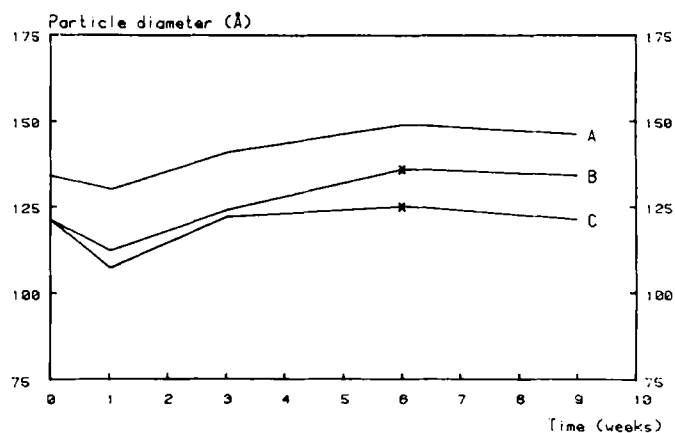


Figure 4—Particle sizes versus time in microemulsions with heptanol as cosurfactant. Composition: 3.6% water, 2.0% potassium oleate, and heptanol, q.s. to 100% with paraffin oil. Heptanol content: (A) 8.0%; (B) 10.0%; (C) 12.0%; (D) 14.0%; (E) 16.0%.

RESULTS AND DISCUSSION

A series of four-component phase diagrams was established to determine the microemulsion regions. The quaternary systems, water-paraffin oil-potassium oleate as the surfactant and various alcohols as cosurfactants, were investigated; some of the results are published in the companion paper (1). The homologous series butanol, pentanol, hexanol, heptanol, octanol, and decanol was used. A "single-phase" microemulsion region could be observed only for pentanol, hexanol, and heptanol. In this report these systems were investigated to study the variations of the particle sizes as a function of the storage time.

Most of the microemulsions stored for a period of several weeks at room temperature were found to be unstable. The particle size alterations were extreme. The instability of the tested systems was due to their composition. These systems were located at the edge of the water-in-oil microemulsion region in the tetrahedral phase diagram. A basic difficulty with PCS studies is that the concentration of the dispersed phase cannot be higher than $\sim 10\%$ in order to avoid concentration faults due to interparticle forces and multiple scattering (1). Only low concentrations allowed the use of the Stokes-Einstein equation without a correction factor (so called "structure factor"). The systems examined were composed of 1.0–3.0% (by volume) potassium oleate and a constant 3.6% volume of water. The following assumptions were made:

1. Some of the alcohol molecules, together with the oleate, are needed to stabilize the amphiphilic layer between the aqueous core of the microdroplets and the dispersion medium.

2. The surrounding paraffin oil contains the remainder of the alcohol.

3. All oleate molecules are in the water phase and the bilayer of the emulsifier mixture (*i.e.*, the oleate concentration in the lipophilic continuous phase is neglected). Thus, the concentration of the dispersed phase cannot be higher than $\sim 7\%$ (3.6% water + a maximal 3.0% potassium oleate) and the Stokes-Einstein equation is valid.

The experimental points in the compared systems were chosen to clarify the variation in particle diameter of the microemulsions for which the amount of alcohol changes at constant potassium oleate concentrations. The results are presented in the form of curves of particle size against time. All concentrations are given as volume percent.

¹ Depuratum grade; Roth, Karlsruhe, FGR.

² Pharmacopeia quality DAB 8.

³ "Pro synthesis" grade, >98% pure; Merck.

⁴ Dow Chemical.

⁵ Millipore Corp.

⁶ Lipofundin S 20%, Ch. No. 117381; Braun AG, Melsungen, FGR.

⁷ Carl Zeiss Abbé.

⁸ Ubbelohde.

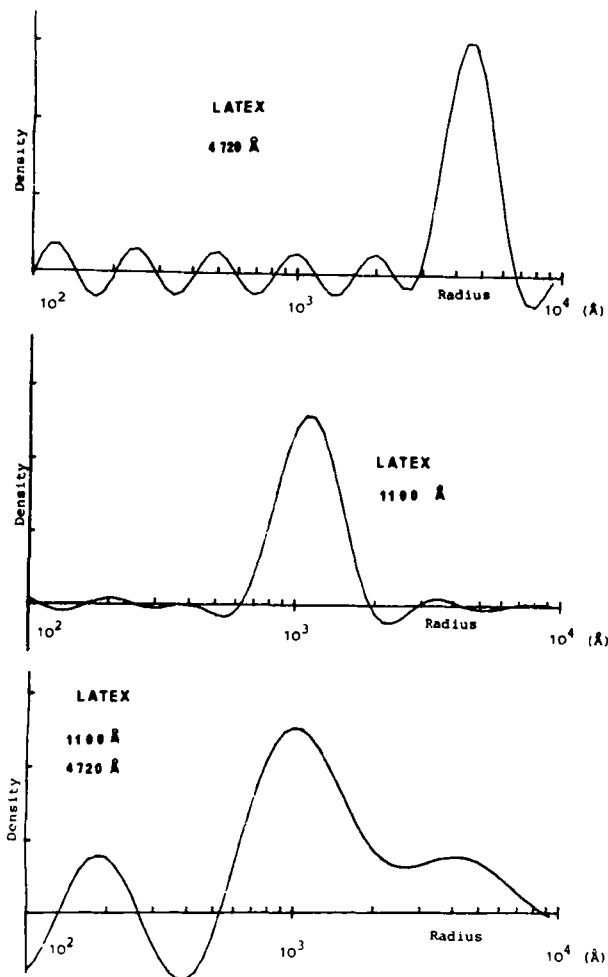


Figure 5—Distribution curves of monodispersed latex suspensions (radii: 1100 and 4720 Å) and their mixture at the ratio of 5:1.

The results obtained with pentanol are shown in Figs. 1 and 2. The phase diagram of the pseudoternary system of paraffin oil, pentanol, potassium oleate, and a constant water fraction of 3.6% showed a zone of instability between the microemulsion and the micellar solution [see Fig. 2 in the previous paper (1)]. A continuous transition from the microemulsion to the solubilization region was found only if the system contained ~3% potassium oleate. In the diagrams for the other alcohols, no continuous transition according to the swollen micelles theory was observed.

Figure 1 shows the particle sizes along a composition line with a constant concentration of 2.0% potassium oleate. Microemulsion A is formed with the smallest possible amount of alcohol, microemulsion D with a maximum amount of alcohol. Both systems are located at the edge of the microemulsion region. Microemulsion formation with a lower or higher concentration of pentanol is not possible. System A is located at the edge bordering on the macroemulsions with a low pentanol concentration. It showed a nearly constant particle size of ~230 Å for 1 week. After 3 weeks it was broken (marked by x in the curve) and an aqueous phase had formed at the bottom of the specimen cell. There was, however, still a microemulsion in the upper phase with a particle size of 260 Å, which decreased during storage. An analogous behavior was found for microemulsion D, which is located at the edge of the microemulsion region towards the solubilization region. The values obtained for compositions B and C proved that the systems in the middle of the microemulsion region were more stable.

The particle size alteration is more obvious in Fig. 2, and the reason for the alterations can easily be explained as follows. Figure 2 shows the particle sizes during the continuous transition from the microemulsion region (systems A-F) to the solubilization region (system G). After 6 weeks of storage, the "single-phase" systems in the zone of instability (systems E and F) were broken and a separation between microemulsions and micellar solutions occurred. After 3 additional weeks, systems D and A were also broken and the microemulsion field was narrowed from both sides. Microemulsion A showed a rapid increase in particle size from 135 Å to 266 Å due to the coalescence of the dispersed phase. After 9 weeks the system was broken, but a microemulsion

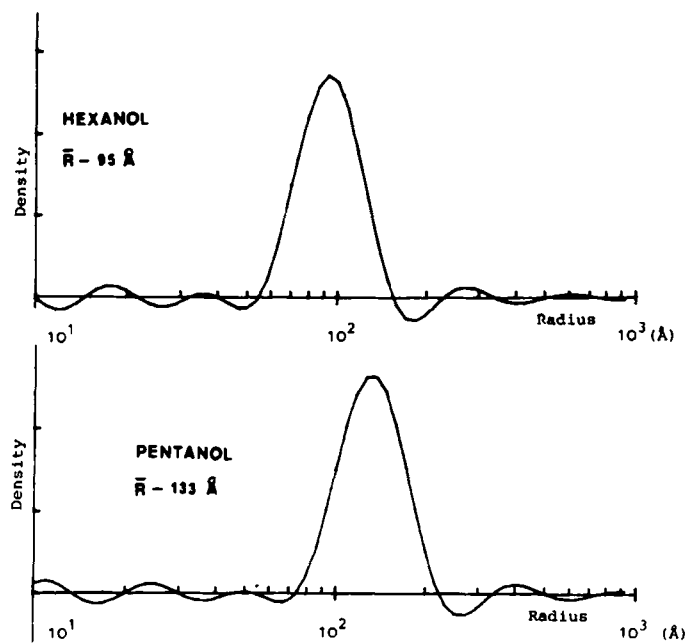


Figure 6—Distribution curve of two microemulsions produced with equal volume fractions of hexanol and pentanol. Composition: 3.6% water, 1.5% potassium oleate, and 13.0% alcohol, q.s. to 100% with paraffin oil.

still remained in the upper phase with a particle size of 233 Å. Relatively large unstable microdroplets had coalesced, and an aqueous subphase had formed. The remaining microdroplets in the upper phase increasingly coalesced in proportion to the length of the storage. After a period of 1 year, only a micellar solution remained, whereas the volume of the aqueous subphase had increased. Microemulsion D had a nearly constant particle size for some weeks. An increase before phase separation was not observed, as this occurred between two measurements. Systems E and F in the unstable zone at the edge of the micellar solution region showed a very slow increase in particle size. For micellar solution G, no alteration was observed. Pentanol as cosurfactant caused rapid microemulsion formation, but also rapid coalescence.

The use of hexanol as cosurfactant also results in rapid microemulsion formation, within milliseconds, but the coalescence is less distinct. Figure 3 shows that the particle size is almost constant for a long time, and the systems are more stable compared with those using pentanol. Another important difference is that only the microemulsions in the unstable zone toward the micellar solution region break at the beginning of the storage period (Fig. 3, systems D and E). The microemulsions on the opposite side (Fig. 3, system A) are, on the other hand, stable in contrast with those formed with pentanol.

Heptanol as cosurfactant causes very slow microemulsion formation. During the formation process the smallest possible particle size was not reached at

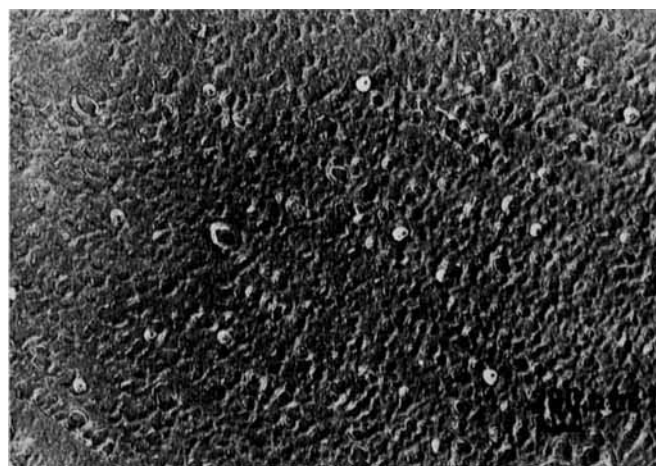


Figure 7—Electron micrograph of an oil-in-water emulsion after 5 d of storage. The narrow distribution of the particle sizes is obvious. The determined mean radius (25 nm) is almost the same as yielded by photon correlation spectroscopy.

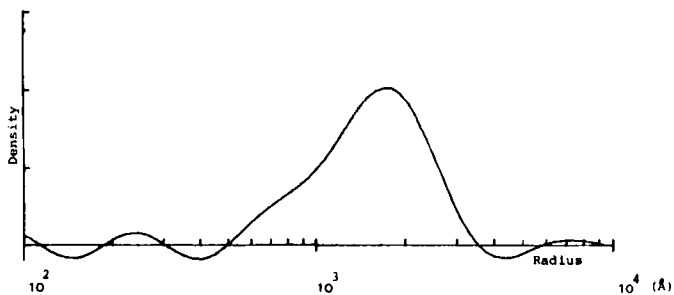


Figure 8—Distribution curve of an oil-in-water emulsion for infusion.

once but, rather, after 1 week of storage (Fig. 4, curves A, B, and C). In some cases, macroemulsions were first formed, becoming transparent after a few days (Fig. 4, curves D and E). These emulsions showed the same instability as those of hexanol and broke rapidly. In general, the systems with heptanol were as unstable as those with pentanol, showing a rapid increase in particle size.

In summary, pentanol and hexanol in combination with potassium oleate cause rapid microemulsion formation. The smallest possible particle size is reached at once in contrast with heptanol, where this takes some days. Pentanol and heptanol form unstable systems with a rapid increase in particle size; hexanol, however, shows more constant particle sizes during storage. Hexanol and heptanol have in common that the microemulsion systems at the edge of the solubilization region break at the beginning of the storage time, whereas those bordering the macroemulsion region only coalesce after an induction period. A simultaneous breaking on both sides of the microemulsion region occurred using pentanol. The tested microemulsions showed the same instability due to coalescence as found with macroemulsions, because they are located at the edge of the microemulsion region.

The particle size distribution can be determined with the polydispersity analysis by the Laplace transformation of the correlation function as described above. The efficiency of the method was tested using monodispersed latex suspensions and their mixtures. The two upper curves of Fig. 5 show the distributions of monodispersed latex suspensions with radii of 1100 and 4720 Å. As the particles are not exactly monodispersed and the Laplace transform inherently has some uncertainty, two relatively narrow logarithmic normal distributions are obtained. The distribution of the smaller particles is not as narrow as that of the larger ones because the signal-noise ratio is small, which results in a higher standard deviation of the values and a wider distribution. The mixing of these two types of particles in a ratio of 5:1 results in a distribution curve where the peak heights reflect the particle proportion. Most of the commercial apparatuses, however, calculate only an unsatisfactory polydispersity index.

All the tested microemulsions showed a narrow particle size distribution. Two microemulsions prepared with equal volume fractions of hexanol and pentanol are given as an example (Fig. 6). The mean diameters calculated using the Stokes-Einstein equation were 113 Å for the pentanol system and 80 Å for that with hexanol. This corresponded exactly to the peak maxima in Fig. 6. The measured distributions were confirmed by electron microscopic studies. The structure of microdroplets and the obtained particle sizes were verified (Fig. 7) using the freeze-fracture technique (6).

The described polydispersity analysis can be applied in the pharmaceutical industry. An example is process control of the liposome production, especially small unilamellar vesicles of diameters <100 nm. The liposome size can hardly be controlled during production. Up to now these analyses were made by separating the different liposome sizes into monodispersed systems using a Sephadex column and measuring each system, e.g., with a particle counter. A polydispersity index of ≤ 2 confirmed the successful separation. A higher polydispersity index indicates a bad separation and does not allow any further statement about the distribution. Such analyses can be carried out faster with the method described herein. An illustration of the practicability is the determination of particle size distributions in oil-in-water emulsions for parenteral use. The tested emulsion⁶ had a mean diameter of 446 nm. The corresponding distribution curve shows that it is a polydispersed system (Fig. 8), and the droplet distribution is not logarithmic as is generally assumed for emulsions.

REFERENCES

- (1) B. W. Müller and R. H. Müller, *J. Pharm. Sci.*, **73**, 915 (1984).
- (2) N. Ostrowsky, D. Sornette, P. Parker, and E. R. Pike, *Optica Acta*, **28**, 1059 (1981).
- (3) J. G. McWhirter and E. R. Pike, *J. Phys. A*, **11**, 1729 (1978).
- (4) E. O. Schulz-DuBois, "Photon Correlation Techniques," Springer Series in Optical Sciences, Springer-Verlag, Berlin, Heidelberg, New York 1983.
- (5) S. W. Provencher, *Macromol. Chem.*, **180**, 201 (1979).
- (6) J. E. Rash and C. S. Hudson, "Freeze Fracture: Methods, Artifacts, and Interpretations," Raven, New York, N.Y., 1981.

ACKNOWLEDGMENTS

This study is part of the research project "Bestimmung Molekularer Parameter von Tensiden und Tensidkomplexen mit Hilfe der Streuung von Laserlicht" which is subsidized by the Deutsche Forschungsgemeinschaft. We want to express our sincere thanks for this grant. Many thanks are also due to P. Hille and J. Merz (Institut für Angewandte Physik, Christian-Albrechts-Universität Kiel, W. Germany) for their help in the data processing and the production of the hardware.