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Research Article

Validation of Fiber-Optic Doppler Anemometry (FODA) for Characterizing the Droplet Size of Emulsions

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Fiber-optic Doppler anemometry (FODA) is a useful tool to characterize polydisperse systems such as emulsions. The mean droplet radius of a model emulsion determined by FODA matched the mean radius determined by sizing droplets from a freeze-fractured freeze-etched scanning electron photomicrograph. The mean droplet radius determined by the Coulter technique was four times too large because the small droplets were not detected. The utility of FODA was further illustrated by its ability to accurately size droplets and monitor the coalescence upon aging of an intravenous lipid emulsion.

KEY WORDS: Fiber-optic Doppler anemometry (FODA); emulsion sizing; droplet interactions; intravenous lipid emulsion.

INTRODUCTION

A variety of methods has been used to characterize emulsions. Accurate characterization of emulsions is crucial because it is used to determine optimal formulation and processing conditions, as well as to assess stability. A common method of studying emulsions is by droplet sizing techniques. A relatively easy, but subjective and time-consuming method is by visualization with a light microscope. This method is limited to sizes greater than 1–2 μm because of poor imaging due to Brownian motion and poor resolution for very small sizes (1). It has long been recognized that an appreciable number of emulsion droplets are below the limits of detection of the optical microscope (2).

Another popular sizing technique is through the use of the Coulter principle (3). The instrument based on this principle can count large numbers of droplets but it is useful only for droplets larger than 0.5 μm. It is also limited by problems due to thermal and electrical noise (1). This technique has been used to study the effects of additives on the droplet size of intravenous emulsions (4,5) and the effect of different manufacturing processes on droplet size (6).

Fiber-optic Doppler anemometry (FODA) is a recently introduced technique for particle size determination in liquid media which is based on the measurement of the diffusion coefficient of the dispersed phase (7–9). Integration of the modified Lorentzian power spectrum yields the area under the curve (AUC), which is directly related to the number of freely diffusing particles in the sample (10). The utility of FODA in characterizing the particle size and monitoring particle interactions in suspensions has been demonstrated in several studies (10). The purpose of this study was to determine the validity of FODA for the study of emulsions.

MATERIALS AND METHODS

Standard monodisperse polymeric latexes (Dow) were diluted with distilled water. Sorbitan monooleate, polyoxyethylene sorbitan monooleate (Sigma), and olive oil (Ogden Food Products) were used as received. A commercial intravenous lipid emulsion (Intralipid 20%, Cutter) was used as received or diluted as indicated with distilled water.

A series of binary mixtures of a 40- and a 584-nm-radius latex, as well as binary mixtures of a 40- and 230-nm-radius latex, was prepared. The total latex concentration was kept constant at 1% (v/v), while the volume ratio of each of the two latexes was varied.

Two o/w emulsions containing 40% (w/w) olive oil and 5% (w/w) nonionic surfactants were prepared by dispersing the surfactant mixture in the oil and then layering this mixture over the water. Primary emulsification was achieved by stirring with a propeller mixer at 1500 rpm for 10 min. Secondary emulsification was effected at room temperature by sonication of 30 ml of the primary emulsion at setting 3 with a Model W-370 Sonicator Cell Disruptor (Heat Systems—Ultrasonics). A water-cooled jacketed beaker was used to prevent more than a 2°C temperature rise during insonation. The emulsions were gently stirred during sonication with a magnetic stirring device. Emulsion A was prepared using the optimal hydrophile–lipophile balance (HLB) for olive oil of 9 and a relatively long insonation period to produce smaller droplets. Thus a polyoxyethylene sorbitan monooleate (HLB 15.0) and sorbitan monooleate (HLB 4.3) mixture producing an overall HLB of 9 was used in conjunction with 3 min of sonication. Emulsion B was prepared to have larger droplets. Thus, the surfactant ratio was changed to give an

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HLB of 5, and 1 min of sonication was employed. Emulsions A and B were examined by FODA, freeze-etching scanning electron microscopy, and Coulter counter. Samples were prepared on the day of each examination.

The procedure for the use of the fiber-optic Doppler anemometer (SIRA) was the same as previously described (10) except that a background water correction was not necessary.

A Model TA II Coulter counter (Coulter Electronics) was used with a 30-μm aperture tube and a 16-channel analyzer. The model emulsion was properly diluted and 10,000 droplets were counted. For each repeat analysis, fresh diluent and emulsion were used to minimize droplet coalescence.

For examination by freeze-fractured freeze-etched scanning electron microscopy the model emulsions were diluted to 1% (w/w) olive oil using water just prior to sample preparation. A small drop of the emulsion was placed on the sample holder peg and immediately immersed in liquid nitrogen slush (liquid nitrogen in contact with solid nitrogen) for approximately 1 min. The sample was then transferred to the specimen chamber of the cold-stage assembly (Hexland CT-100). The top portion of the frozen droplet was removed with a chilled fracturing knife. The sample was moved to the microscope (JEOL JSM-840) cold stage for sublimation of the frozen aqueous phase. The sublimation temperature was −110°C for 1 min, and the etching process was monitored by viewing under a 1-kV electron beam. After sublimation, the sample was moved to the preparation stage for sputter coating. The gold coating was applied for 4.5 min at 2 mA. Upon completion of the coating, the sample was placed in the microscope cold stage for imaging using a 10-kV electron beam.

RESULTS AND DISCUSSION

Emulsions are expected to be polydisperse rather than monodisperse. Thus, the first concern in using FODA to characterize the droplet size of emulsions was the response of FODA to polydisperse systems. This was investigated by studying binary mixtures prepared by mixing appropriate quantities of standard monodisperse latexes.

Accurate size measurements can be made with FODA only when the particles are freely diffusing, i.e., particle interactions are absent (10). The volume fraction at which particle interactions begin in monodisperse latex suspensions has been shown to be directly related to the particle size (10). Thus, the volume fraction at which particle interactions are initiated in the smallest-size latex to be used in the binary mixtures was determined. A series of dilutions of the 40-nm latex (Fig. 1) shows that the AUC is linear up to a volume fraction of 0.02. Thus, at concentrations less than 2% (v/v), the latex particles are freely diffusing. Therefore, size measurements were made at a 1% (v/v) concentration to ensure that the latex particles were not interacting. At this concentration the larger monodisperse latexes will also be free of particle interactions and the assumption is made that no interactions occur between particles in the binary mixtures.

Figure 2 shows the mean radius determined by FODA for a series of mixtures of 40- and 584-nm-radius latexes, in addition to the number-calculated and volume-calculated

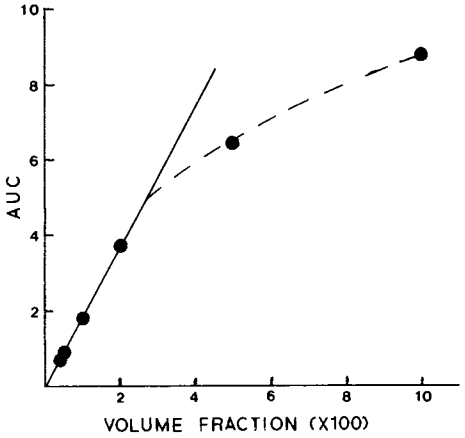

Fig. 1. Effect of the volume fraction on the area under the curve (AUC) of the modified Lorentzian power spectrum for a 40-nm-radius latex dispersion.

radius. For this series of binary mixtures, having nearly a 15-fold difference in size, the mean FODA radius is very close to the number-calculated radius. The number of particles per volume in the smaller latex is much greater than in an equivalent volume of the larger latex [approximately 2.53×10^{13} particles/ml for the 40-nm-radius latex and 1.20×10^{10} particles/ml for the 584-nm-radius latex at 1% (v/v)]. Therefore, the smaller latex particles will predominate in most of the mixtures and the greater part of the frequency shift of the backscattered light will be due to the smaller particles.

The area under the curve (AUC) results from FODA analysis of this series are shown in Fig. 2. A linear relationship exists between the volume fraction of the 40-nm-radius latex in the mixture and the AUC ($R^2 = 0.96$). When the

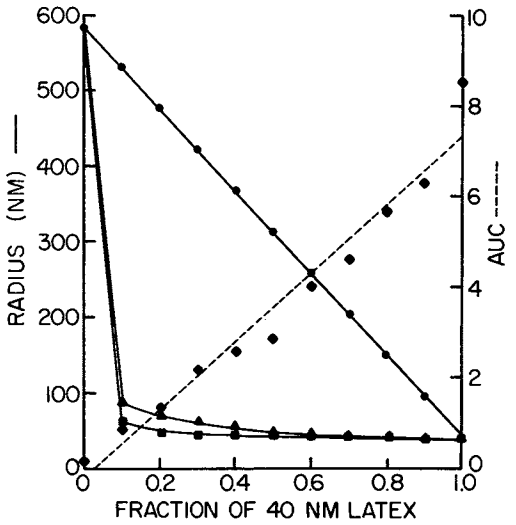

Fig. 2. Mean radius (■) and area under the curve (AUC) (◆) determined by FODA for a series of mixtures of 40- and 584-nm-radius latex dispersions. The total latex concentration for each mixture is 1% (v/v). Also shown are the number-calculated radius (▲) and the volume-calculated radius (●) for each mixture.

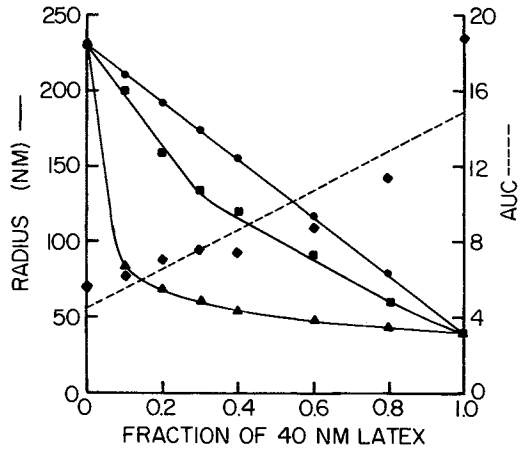

Fig. 3. Mean radius (■) and area under the curve (AUC) (◆) determined by FODA for a series of mixtures of 40- and 230-nm-radius latex dispersions. The total latex concentration for each mixture is 1% (v/v). Also shown are the number-calculated radius (▲) and the volume-calculated radius (●) for each mixture.

mixture contains no 40-nm-radius latex particles, the AUC is low, indicating a relatively small number of particles in the sample. As the fraction of the smaller latex in the binary mixture is increased, the AUC also increases. The increase in the AUC indicates that the number of particles in the sample is increasing, as expected on the basis of particle number calculations.

Figure 3 shows the mean radius determined by FODA for a series of mixtures of 40- and 230-nm-radius latexes, in addition to the number-calculated and volume-calculated radius. The mean radius determined by FODA falls midway between the number-calculated and the volume-calculated radius. In this series of mixtures there is nearly a sixfold difference in the size of the two latexes and the results in Fig. 4 indicate that neither latex predominates in this series of mixtures.

The AUC results for the mixture containing the 40- and 230-nm-radius latexes are shown in Fig. 3. The AUC increased as the fraction of 40-nm-radius latex was increased (R^2 = 0.92). The theoretical number of particles for a 1% (v/v) concentration of the 230-nm-radius latex is 1.96×10^{11} particles/ml, compared to 2.53×10^{13} particles/ml for the same concentration of the 40-nm-radius latex. The smaller number of particles per volume of the 230-nm-radius latex is reflected in the relatively low AUC when no 40-nm-radius latex was present. The maximum AUC occurred when the sample contained only the 40-nm-radius latex. Mixtures of the two latexes had intermediate AUC values, indicating intermediate particle numbers.

The ability of FODA to analyze the two binary mixtures of latexes indicates that FODA will be useful for the analysis of emulsions which usually exhibit a normal distribution. It is important to remember that the mean radius determined by FODA is a reflection of the number of particles of each size present in the sample. Thus, the mean radius determined by FODA will approach the number-calculated mean, especially as the range of droplet sizes in the emulsion increases.

FODA analysis of emulsion A gave a mean radius of 271 nm. A typical scanning electron photomicrograph of this emulsion is shown in Fig. 4 (left). The polydispersity of the emulsion is very evident in the photomicrograph; several large droplets are present which are surrounded by many smaller droplets. When 100 droplets were randomly selected and measured, the mean radius was 318 nm, a value which agrees well with the FODA determined mean.

The HLB and insonation time were varied to produce emulsion B. FODA analysis indicated that the mean radius was 375 nm. Figure 4 (right) is a scanning electron micrograph of a typical field for this emulsion. Polydispersity is evident, with even larger droplets present than seen in emulsion A. The average radius determined by measuring 100 random droplets was 416 nm, again agreeing with the FODA analysis.

The droplet size distribution of emulsion A determined by Coulter counter analysis is shown in Fig. 5 (bottom). The average droplet radius is 1200 nm. However, the differential distribution does not approach 0 at the lower limit of the droplet size range. This suggests the presence of a large number of droplets that are below the limit of detection by the Coulter counter. However, these small droplets are seen in the scanning electron photomicrograph (Fig. 4) and are included when the mean droplet size is determined by FODA.

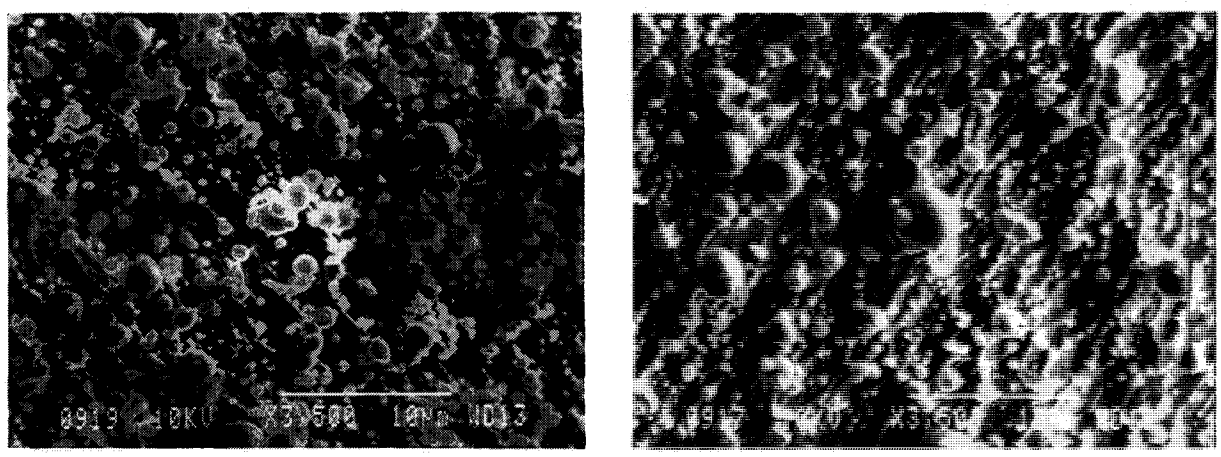

Fig. 4. Scanning electron photomicrograph of emulsion A (left) and B (right) at a magnification of 3500. The bar represents 10 μm.

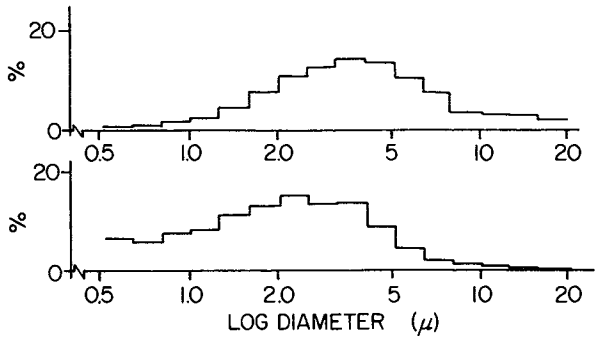

Fig. 5. Differential droplet size distribution of emulsion A (bottom) and B (top) by Coulter counter analysis.

Figure 5 (top) shows the droplet size distribution of emulsion B as determined by the Coulter counter. The average droplet radius is 1800 nm. This value is five times larger than that obtained by scanning electron microscopy or FODA. The ability of FODA to include the smallest droplets in its analysis is important, as it is likely that the physical properties of an emulsion are strongly affected by the smallest droplets.

To investigate the ability of FODA to analyze commercial emulsion products, an intravenous 20% lipid emulsion was examined. Analysis of a series of dilutions (Fig. 6) reveals that the lipid concentration is directly related to the AUC (R^2 = 0.99). This indicates that all the droplets are freely diffusing and that no droplet interactions are occurring in the commercial emulsion which contains 20% lipid. The absence of droplet interactions may contribute to the physical stability of the emulsion, as droplet interactions may be an early step in the coalescence process.

The average droplet radius determined by FODA for the dilutions of the intravenous 20% lipid emulsion (Fig. 6) ranged from 100 to 123 nm, with an average of 111.3 ± 7.0 nm. This is slightly smaller than the 150- to 250-nm average radius claimed for this product (11) and may reflect FODA's ability to detect the smallest droplets in the emulsion.

The droplet size of emulsions intended for intravenous administration is extremely critical both initially and during aging. The presence of very large droplets in these emulsions could cause capillary blockage. FODA's utility for monitoring these products was further illustrated by analysis of the intravenous 20% lipid emulsion after 22 months of aging at ambient room temperature. Droplet interactions were absent in the aged emulsion, as the lipid concentration was directly related to the AUC (R^2 = 0.97). However, the average droplet radius for the series of dilutions was 163.5 ± 11.0 nm. Thus, the mean droplet radius as indicated by FODA increased by approximately 50 nm during the 22-month aging period. Independent evidence, such as microscopy or Coulter counter analysis, for the absence of large droplets would complement the FODA analysis, as FODA will not detect droplets which are too large to undergo Brownian motion.

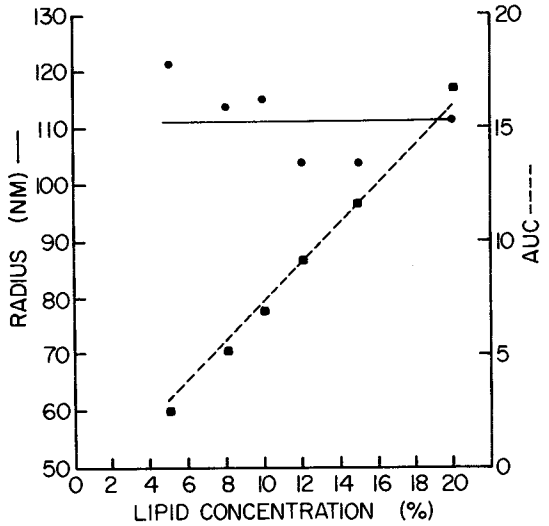

Fig. 6. Effect of oil concentration obtained by diluting a commercial intravenous 20% lipid emulsion on the mean droplet radius (●) determined by fiber-optic Doppler anemometry and the area under the curve (AUC) of the modified Lorentzian power spectrum (■).

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