A Method for the Early Evaluation of the Effects of Storage and Additives on the Stability of Parenteral Fat Emulsions

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The combination of sedimentation field-flow fractionation (SedFFF) and photon correlation spectroscopy (PCS) is shown to provide a detailed record of the droplet sizes present in fat emulsions commonly used in parenteral nutrition. The technique presented has been used to record size distribution data for a particular emulsion (Liposyn-II), demonstrating its high stability and lot-to-lot uniformity. The technique is also able to demonstrate how additions of small amounts of electrolytes [0.45% (w/v) NaCl, 0.05% (w/v) $CaCl_2$] tend to destabilize the emulsion, suggesting some caution in the use of total parenteral nutrition (TPN) mixtures. In contrast, a 1:1 mixture with human serum caused no sign of instability in the Liposyn-II. Using the emulsion as a carrier for lipophilic drugs necessitates adding solutions of the drug in nonaqueous solvents, such as DMSO (dimethyl sulfoxide). This solvent's destabilizing effect results in a droplet coalescence that becomes severe after 3–5 days following a 10% (v/v) addition, while a 5% (v/v) addition reaches the same level of coalescence in 10 days.

KEY WORDS: emulsions; stability; field-flow fractionation; size distribution.

INTRODUCTION

The widespread clinical use of fat emulsions, both as intravenous nutritional supplements (1) and, recently, as vehicles for the administration of lipophilic drugs (2–5), requires a thorough examination of the stability of the product. Emulsions are inherently unstable liquid–liquid two-phase systems, and even slight shifts in their composition or storage temperature may result in droplet aggregation, coalescence, or even phase separation. Such "creaming" has been observed both *in vitro* (6–8) and *in vivo* (9,10), where the creaming process appears to result in the formation of fat microemboli and possibly also in the occlusion of blood vessels (11). In view of the potentially harmful effects of phase segregation, it is of utmost importance to develop criteria for evaluating an emulsion's droplet size distribution prior to infusion. While traditional methods such as visual inspection or the determination of average droplet size by means of dynamic light scattering are capable of indicating drastic shifts in emulsion stability (ES), they are less informative in demonstrating the subtle shifts in droplet size distribution which may lead to creaming over a longer period of time.

Although unstable from a thermodynamic point of view, the many emulsions in clinical use are deemed "stable" because of the slow rate at which they disproportionate. The time evolution of their droplet size distribution is therefore the logical basis for assessing their stability. Even when a fat emulsion is deemed to be extremely stable, it could potentially undergo changes upon admixing with glucose, amino acids, and electrolytes, as is frequently done to produce formulations for total parenteral nutrition (TPN) (7,12). Another possible source of change which should be carefully monitored is the addition of lipophilic drugs to the emulsion prior to infusion. Because of their well-documented clinical use, the Liposyn and Intralipid fat emulsions are often considered as ideal vehicles for administration of nonpolar, water-insoluble drugs, which may be added to the emulsion in the form of a solution in a dipolar aprotic solvent (13,14) or other physiologically acceptable solvent miscible with water. Following addition, the lipophilic drug partitions into the oil phase of the emulsion, and in the absence of obvious destabilization the adduct is, in principle, ready for infusion.

Although an assessment of physical stability must be based on observed variations in emulsion particle size with time, there exist very few analytical techniques which can accurately and reproducibly determine the detailed size distribution for emulsions and other highly polydisperse, fragile colloids (15). Because of its easy and rapid data collection, dynamic light scattering, or photon correlation spectroscopy (PCS) at a fixed angle, is frequently used as the basis for stability evaluations (16,17). Unfortunately, this technique, which is extremely accurate in its size assignments for samples of uniform particle diameter, is capable only of determining the average size and an index of polydispersity for the type of broadly distributed samples of interest here.

In recent years, we have explored a combination of PCS and the single phase separation technique termed sedimentation field-flow fractionation (SedFFF) as a means for characterizing the droplet size distribution in emulsions (18). In this approach, the separation provides a detailed record of the size distribution without permitting an exact evaluation of the sizes present since the density of the droplets is unknown. However, the fractions collected during the separation contain particles which are highly uniform in size and, thus, well suited for analysis by PCS. Even without the PCS evaluation of size, the distribution curve provided by the separator represents a fingerprint of the sample at the time of analysis. A series of such fingerprints collected at different times, or after different treatments, therefore provides a clear record of emulsion stability.

In the present study, we have employed the SedFFF/PCS strategy to evaluate the stability of a 20% fat emulsion (20% Liposyn-II), both upon long-term storage and following the addition of either electrolytes or DMSO. In a separate communication we compare the differences in size distribution observed with the same technique for two products (Liposyn-II and Intralipid) (19).

EXPERIMENTAL

Sizing Methods

Sedimentation FFF

The sedimentation field-flow fractionation (SedFFF) technique has been described in detail elsewhere (20–22),

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and in this context we will only recapitulate the essential theoretical features which permit translating the fractionation pattern into a size distribution curve. The fractionation takes place in a thin (dimension w), liquid-filled channel of rectangular cross section which is fit into a centrifugal rotor capable of spinning with velocities of up to 3000 rpm (1600g). This rotor is designed to allow for the continuous flow of liquid from the stationary pump, via the spinning channel, through the stationary detector, and on to a fraction collector. The ability to separate complex colloids according to size, and to utilize the resulting fractions for further analysis of composition and activity, or for a verification of the size by means of an independent method, is one of the most important advantages of the FFF techniques.

Under the influence of the centrifugal field, samples injected into the SedFFF channel migrate radially and accumulate at one of the walls perpendicular to the field direction. Thus, particles denser than the carrier fluid end up settling at the outer wall, while those that are less dense will float to the inner wall of the channel. At all times there is a competition between concentration and diffusion, and as this migration process reaches equilibrium, each particle size in a broadly distributed colloid will be found in a concentration profile $c(x)$ that decays exponentially with distance x from the wall in a channel with thickness w,

$$c(x) = c(0) \exp(-x/w\lambda) \quad (1)$$

This distribution is characterized by the dimensionless parameter λ, which is uniquely determined by the "effective mass" of the particle, i.e., the product of its mass m and buoyancy factor $\Delta\rho/\rho_p$, where $\Delta\rho$ is the density difference between the particle and the surrounding fluid, and ρ_p is the density of the particle,

$$\lambda = kT/m\ (\Delta\rho/\rho_p)Gw \quad (2)$$

Here G is the applied gravitational acceleration, and kT is the thermal energy of the system. Since the mass can be expressed as the product of density and volume, which for spherical particles easily relates to their diameter d, Eq. (2) can be slightly rearranged to give

$$\lambda = 6kT/d^3\ \Delta\rho\pi Gw \quad (3)$$

The sample's equilibration under the field is allowed to take place in the absence of flow, and only when equilibrium is established is flow resumed through the thin channel. Its parabolic velocity profile will transport the various particle zones downstream at rates which will reflect the thickness of each particular sample cloud. The net result is a separation of the clouds, such that the least compressed will emerge from the separator ahead of those moving closer to the wall where flow is more sluggish. Since an observed elution volume, V_e, directly reflects the thickness of the particle cloud, as specified by parameter λ, the retention ratio R (the ratio of the column void volume, V^o, to V_e) is the basis for a determination of particle diameter:

$$R = V^o/V_e = 6\lambda[\coth(1/2\lambda) - 2\lambda] \quad (4)$$

The larger the elution volume, the more closely the bracketed function of Eq. (4) approaches unity, making $R = 6\lambda$ a good approximation for this relationship for retentions in excess of 10 column vol. By combining Eqs. (3) and (4) it is easily realized that, as elution progresses, the elution volumes become directly proportional to the droplet mass, i.e., to the third power of the diameter.

As evidenced from Eq. (3), the conversion of retention data into values for the corresponding particle diameters requires knowledge of the density difference between particle and carrier. While exact determinations of the carrier density pose no problems, the densities of composite materials such as emulsions, liposomes, and subcellular organelles depend on their actual composition and is as such frequently unknown. In these instances, it is convenient to rely on the SedFFF technique to give fractions of a high size purity (due to the proportionality between V_e and d^3) and size the particles of these fractions by means of an independent method. For composite samples of uniform density, diameters determined in this way may then be inserted in Eq. (3), which, in combination with Eq. (4), will give the appropriate value for $\Delta\rho$ (18).

The ability of SedFFF to fractionate and size colloidal particles has been amply verified in previous studies from this and other laboratories (18,23). As a routine measure, the performance of each separator is generally checked by the fractionation and size assignment of monodisperse latex standards whose sizes and densities are well-known. A typical standard run of polystyrene particles is illustrated in Fig. 1. From the elution volumes associated with each peak maximum, one calculates the size of particles appearing in the peak by application of Eqs. (3) and (4). These sizes, labeled d_F in Fig. 1, are in good agreement with those determined by PCS for the collected peak fractions.

In using optical detection with a light source whose wavelength is comparable to the size of the sample particles, the extinction coefficient which relates detector response and concentration will be a strong function of particle diam-

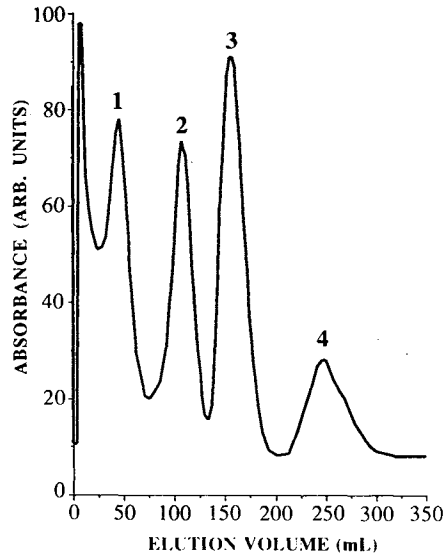

Fig. 1. A typical fractogram of a polystyrene mixture under a field of 1000 rpm and a flow rate of 2.7 mL/min. The peak numbers refer to samples whose nominal and SedFFF derived diameters (d_N and d_F, respectively) have the following values (nm): (1) $d_N = 272$, $d_F = 274$; (2) $d_N = 394$, $d_F = 391$; (3) $d_N = 482$, $d_F = 477$; and (4) $d_N = 627$, $d_F = 607$.

eter. Nevertheless, fractograms of the type examined in Fig. 1 are, as a rule, easily converted into size distribution curves, since extinction coefficients for each component are readily determined in separate experiments. The situation is entirely different when samples are broadly distributed, as in the case of emulsions. Here, extinction coefficients would have to be determined for each separate fraction, either by dry weight measurements or by breaking the emulsion and spectrophotometrically quantifying its components (24); the actual distribution curve would then be deconvolved out of the fractogram by division with the scattering correction function. This complex process is not needed in cases where the fractogram is to be used as a "fingerprint" of the sample for comparative purposes.

Photon Correlation Spectroscopy

The PCS technique is frequently used to determine the average diameter of suspended particulates in the submicron size range (17). In this measurement, light from a coherent laser source impinges on the sample's particles and is scattered, more or less uniformly depending on their size, in all directions. This light is collected at one or more angles, and its intensity recorded as a function of time. The scattered light from neighboring particles will interfere as a function of the distance between the scatterers, and from the time evolution of the scattering intensity, one calculates the relative motion of the particles, i.e., their diffusivity D. For spherical particles, D is related to particle diameter d through the Stokes–Einstein formula:

$$D = kT/3\pi\eta d \quad (5)$$

where kT has the same meaning as above and η represents the viscosity of the medium.

While uniform collections of particles are readily and accurately sized by this method, broad distributions can not be characterized in detail; for these samples the method gives only an average size and an index of polydispersity (14). Such samples are, however, well described by the combination of a separation technique, such as the SedFFF, which generates fractions of uniform size, and the PCS, which unambiguously assigns a size to each fraction whose concentration is above the detection limit (18).

Equipment and Operation

The SedFFF unit was custom built, essentially according to descriptions in Ref. 22; the dimensions of the flow channel are as follows: length, 94.5 cm (including the tapered ends); breadth, 2.0 cm; and thickness (w), 0.0254 cm for a measured void volume of 4.76 mL. Its rotor radius is 15.5 cm. The effluent from the separator was monitored as the absorbance of UV light at 254 nm, using a Linear detector (LINEAR UV-106). All fractograms represent the relationship between the absorbance in arbitrary units and the eluant volume. The carrier (DI water) was delivered by means of a Minipuls 3 peristaltic pump (Gilson). Following a manual sample injection, the entire analysis, including the interruption of flow for the purpose of relaxing the sample into its equilibrium distribution, the acceleration of the rotor to a specified spin rate, the resumption of flow, and the collection of detector response and effluent weight pairs of data, was under computer control (Leading Edge PC/AT), as described elsewhere (25). Injection volumes were 1 μL, unless otherwise specified.

The light-scattering measurements were made either with a fixed 90°-angle PCS instrument of Type BI-90 or with a variable-angle system of Model BI 1020, both from Brookhaven Instruments.

Fractionation and Protein Analysis

During the separation of a 10-μL Liposyn-plasma mixture, fractions of 1.5 mL were collected from the outlet of the SedFFF channel; fractions collected after more than 20 mL contained droplets which were highly monodisperse in size. After PCS assessment of the droplet size in each fraction, its protein content was determined using the BCA protein analysis kit purchased from PIERCE. The principle of this assay has been described elsewhere (26), and for the purpose of this account it suffices to mention that the samples were added to the BCA reagent prior to a 1-hr incubation at 60°C. Bovine serum albumin (BSA) was used as the standard in calculations of the total amount of protein in a given fraction with a detection limit of 0.5 μg/mL. The presence of the emulsion caused a size-dependent scattering contribution to the absorbency at 562 nm (the protein detection wavelength). This contribution was corrected for by subtraction of the pre-incubation extinction at the same wavelength.

The total amount of lipid in each fraction was determined by a spectrophotometric (Perkin Elmer, Lambda 6/PECSS system) method developed in this laboratory. In this procedure, the 20% Liposyn-II product, which was assumed to contain exactly 20% of lipid, was diluted with deionized water to different concentrations and dissolved in acetonitrile (HPLC grade, Fisher Chemical Co.) at a ratio of diluted Liposyn-II:acetonitrile = 1:9 (by volume). Standard curves were established by relating absorbances at 190 nm to the corresponding lipid concentrations, and these curves were used to determine the total amount of lipid in each fraction.

The total surface area in each fraction was calculated based on information obtained from the above measurements, i.e., droplet size from PCS and total amount of lipid from the spectroscopic assessment.

Materials

Polystyrene latex was purchased from Seradyn Inc. (10% by weight). The density of the latex is 1.05 g/mL. Liposyn-II, both 10 and 20%, were obtained through the Medical Center, University of Utah, Salt Lake City (manufacturer: Abbott Laboratories, North Chicago, IL 60064). Total parenteral nutrition (TPN) mixtures were obtained as gifts from the Pharmacy of the University Hospital, University of Utah. Fresh human serum was obtained from the Blood Bank, University Hospital, University of Utah.

RESULTS AND DISCUSSION

As judged by SedFFF, the Liposyn-II emulsion shows remarkable batch-to-batch reproducibility. Figure 2 compiles fractograms collected on fresh samples over a period of

3 years (18). The earliest analysis was undertaken to demonstrate the option of collecting fractions during the SedFFF run and submitting them to particle size analysis by PCS for the purpose of determining the density difference between carrier and emulsion. Once determined, this density difference could be used [in conjunction with Eqs. (3) and (4)] to convert the elution volume scale into a size scale, as indicated in Fig. 2. The three fractograms shown in the figure all display the same features of a minor void peak, containing unretained small droplets, and the major peak, which indicates the presence of droplets in the 200- to 500-nm size range. In each case, the peak fraction gave a PCS reading of 272 ± 5 nm. The stability upon storage at room temperature was likewise established through the series of fractograms shown in Fig. 3; these were collected on one batch of Liposyn-II over a period of 5 months. Again, there is a clear coincidence, both between the size range present at different times and the droplet diameters associated with the peak fraction.

Since the phospholipid emulsifier confers a certain surface charge to the droplets, one might assume that the addition of electrolytes would alter the stability of the emulsion, as has indeed been observed by visual inspection (27). The effects of electrolytes and amino acids on the stability of fat emulsions have been treated extensively by other authors (12,28,29). In terms of the SedFFF analysis, such changes should be clearly visible as shifts in the distribution curves displayed by the fractograms. In this case, a shift toward larger sizes would be an indication of either droplet coalescence or the formation of aggregates or flocs. If the flocs could be redispersed upon dilution, the collected fractions, which are heavily diluted as they exit from the channel, would change their size either immediately or over a period of time. A PCS reading of such fractions would then show a diameter significantly smaller than that indicated by the FFF elution position.

The three panels in Fig. 4 illustrate the time course of disproportionation which takes place upon the addition of 9 m*M* $CaCl_2$ to the Liposyn-II. Immediately after mixing with the divalent salt, the emulsion was injected into the FFF channel, relaxed at the chosen spin rate of 1500 rpm for 30 min, and separated during the course of 45 min. The distribution was visibly altered, compared to the unadulterated Liposyn-II. Two hours after mixing, the sample was again injected. This time there were no droplets, either small or large, eluting while the field was still applied. However, as the field was turned off, there emerged a large peak of presumably massive particles or flocs (middle panel in Fig. 4). The peak fraction now contained droplets with a PCS determined diameter of 492 nm, which remained stable for several hours. Immediately after this run, the sample was again fractionated, this time at a weaker field chosen to resolve the aggregates seen upon removal of the field in the last run. The third panel in Fig. 4 shows the presence at 500 rpm of a barely resolved doublet whose two FFF-based sizes correspond to 630 and 683 nm, respectively. In these determinations the density difference between particle and suspension medium was assumed to be the same as for the original emulsion. PCS, in turn, showed sizes for these fractions corresponding to 671 nm. Although the results in Fig. 4 appear to indicate that some rather substantial changes take place in the Liposyn-II emulsion upon the addition of $CaCl_2$, the av-

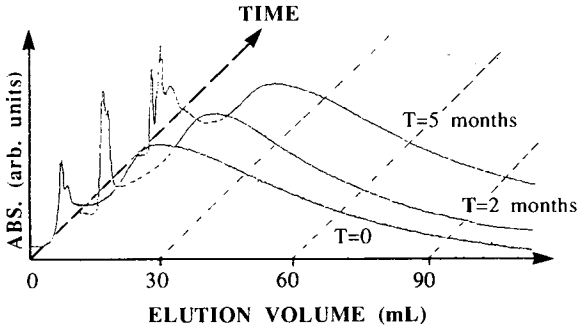

Fig. 3. Results of SedFFF analysis of Liposyn-II emulsions after storage at room temperature for periods of up to 5 months. Run parameters: field strength, 1000 rpm; and flow rate, 2.92 mL/min.

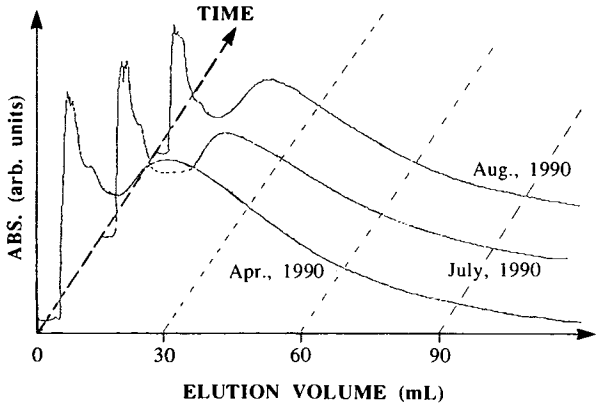

Fig. 2. Fractograms collected from fresh or aged Liposyn-II emulsions. The fractograms show that Liposyn-II exhibits excellent batch-to-batch reproducibility. Run parameters: field strength, 1000 rpm; flow rate, 2.92 mL/min.

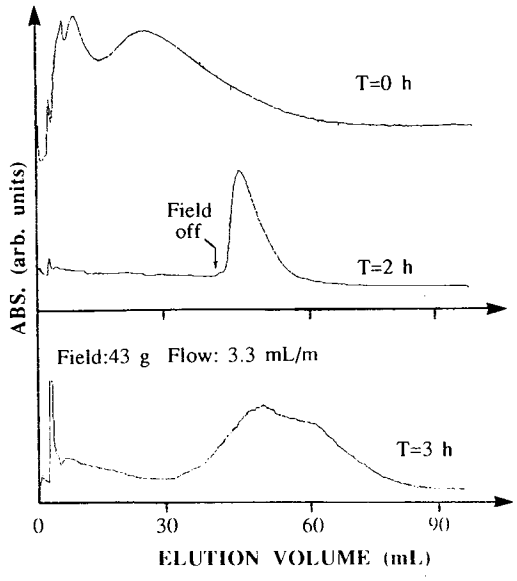

Fig. 4. Illustration of the time course of disproportionation upon a 1:1 (v/v) addition of 9 m*M* $CaCl_2$ (final concentration, 4.5 m*M*) to Liposyn-II. Run parameters: field strength, 1500 rpm; flow rate, 3.5 mL/min.

erage droplet size reflected by PCS remains relatively constant over several hours (ranging from 270 to about 300 nm).

The addition of NaCl at physiological ionic strength also results in a destabilization, as judged from the fractograms in Fig. 5, although the process appears to be significantly slower than in the case of the divalent ions calcium or magnesium (not shown).

Since the clinical use of parenteral emulsions often involves the formulation of total parenteral nutrition (TPN) mixtures by adding electrolytes, amino acids, and glucose to the basic emulsion (12), we wished to examine the stability of the resulting product using the SedFFF technique. Figure 6 compares a fractogram collected for the original Liposyn-II preparation with that for the corresponding TPN mixture; the mixture was injected into the fractionator about 1 hr after it was prepared in the Pharmacy of the University Hospital at the University of Utah. Clearly, the sedFFF result indicates that the properties of the emulsion change upon addition of the other nutrients, as evidenced by the significant shift seen in the distribution curve. Gone is the main droplet peak that normally elutes around 43 mL at the applied field of 1500 rpm (389 g), and instead one observes a smaller peak (peak 1 in Fig. 6) which emerges at a shorter retention time (or reduced retention volume). The slightly elevated baseline after this peak is an indication that some material is constantly being released from the separation channel. After several runs, a visual inspection was made of the interior of the channel, supporting the notion that an oil film had coalesced on the accumulation wall. Phase segregation was also indicated by a visual inspection of the TPN sample. Although formation of the product leads to a visible change in the color of the emulsion, from the intensely white and opaque original Liposyn-II to the slightly yellowish and somewhat more translucent TPN, the routinely performed, fixed-angle PCS measurement did not indicate the mixing to

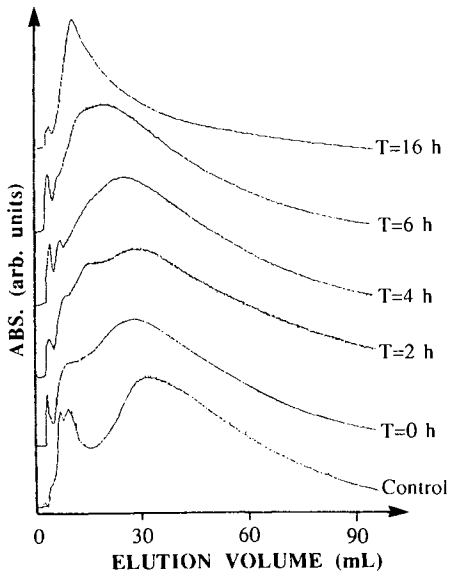

Fig. 5. SedFFF analysis of Liposyn-II after a 1:1 dilution with NaCl at physiological ionic strength [final concentration, 0.45% (w/v)]. The relative size distributions were checked at 2, 4, 6, and 16 hr after mixing. Run parameters: field strength, 1000 rpm; flow rate, 2.92 mL/min.

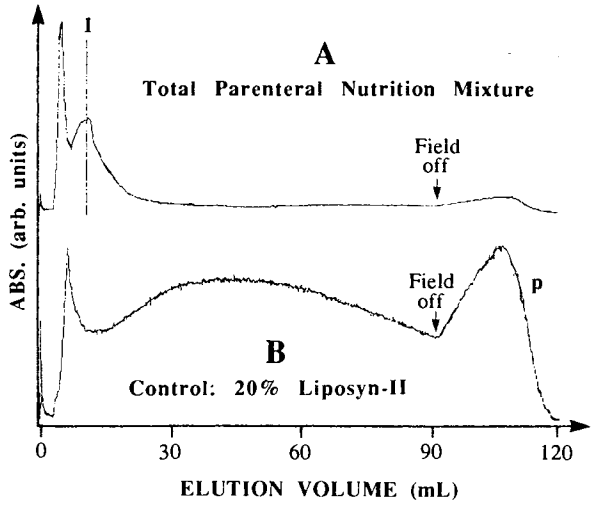

Fig. 6. Fractograms of (A) a clinically used total parenteral nutrition (TPN) mixture, formulated through the addition of electrolytes, sugar, and amino acids to the Liposyn-II emulsion and (B) the Liposyn-II emulsion prior to mixing. Run parameters: field strength, 1500 rpm; flow rate, 2.90 mL/min.

be associated with drastic changes in the droplet size. Indeed, the average diameter appears to have been reduced by no more than 33 nm, from the original d_p of 275 nm to the new level of 245 nm. This is, in all likelihood, due to the addition of electrolytes.

Such drastic changes in the apparent properties of the emulsion as those illustrated in Figs. 4 to 6 may lead one to question the fate of the emulsion as it enters the vascular system and mixes with the blood plasma. In an *in vitro* simulation of this situation, Liposyn-II (without additives) was mixed with freshly collected human serum at a 1:1 volume ratio and stored at room temperature. As above, the mixture was sampled at regular intervals and fractionated to yield the apparent distribution curves displayed in Fig. 7. Surprisingly, these curves indicate that the contact with serum leaves the emulsion unaltered with respect to its droplet size distribution for a period of several days. In fact, the "instability" which eventually appeared after about a week was likely due to bacterial contamination of the sample, rather than a disproportionation of the emulsion.

The lack of a deleterious effect in serum, with its significant ionic strength, is in stark contrast to the destabilization of the emulsion caused by the addition of saline of physiological ionic strength (I = 0.15 M). In an effort to examine whether the stability in serum is due to the formation of a protein adsorption layer on the surface of the emulsion droplets, the Liposyn–plasma mixture was fractionated by means of SedFFF. Selected fractions were collected and their droplet size was determined by PCS, while the concentration of lipid was determined spectrophotometrically, as described under Sizing Methods. From the combined knowledge of size and concentration, it is possible to estimate the amount of interface present in each respective fraction. Assuming the cross-sectional area of albumin (HSA), the most abundant of the serum proteins, to be 140 × 40 Å^2 (30), one can estimate the amount of protein which would be found in any given fraction under conditions of monolayer coverage

by HSA. By way of an example, the peak fraction in Fig. 7 would contain about 5 μg/mL of HSA in a monolayer configuration; this is well above the detection limit (0.5 μg/mL) of the method. Since no protein was detected in this fraction, one must conclude that any protein irreversibly bound to the droplets would represent significantly less than a monolayer.

Due to their well-documented suitability for intravenous administration, the parenteral fat emulsions are currently being evaluated as vehicles for the delivery of lipophilic drugs. In a protocol developed by one of us (13,14), the nonpolar drug is dissolved in dimethyl sulfoxide (DMSO) and added to the emulsion under gentle agitation. The miscibility of DMSO and water drives the drug to rapidly partition into the oil phase, and the loading process, for certain compounds, is completed within a few minutes. Given the ability of DMSO to associate with both water and nonpolar substances, one might ask whether or not the addition of DMSO causes a destabilization of the Liposyn-II emulsion. In order to examine this question, two DMSO samples (250 and 500 μL, respectively) were each mixed with a 5-mL sample of Liposyn-II. These mixtures were stored in the laboratory under ambient temperature and were sampled occasionally for a period of several days. The samples were examined by SedFFF, and the elution volume of the peak fraction was recorded as a function of time from the moment of mixing. Figure 8A exemplifies the observed steady shift toward higher retention (larger elution volumes) with time. These shifts, which are an indication of droplet coalescence, are quite significant for the larger (10%) DMSO addition, as illustrated in Fig. 8B; they should be compared with the stable behavior of the control, which remained invariant with time, as discussed in conjunction with Fig. 2. Curiously, no such shifts were indicated in a PCS analysis of the emulsions. Instead, the average diameters determined by this technique remained at 292 ± 12 for both mixtures during the full 10-day evaluation period.

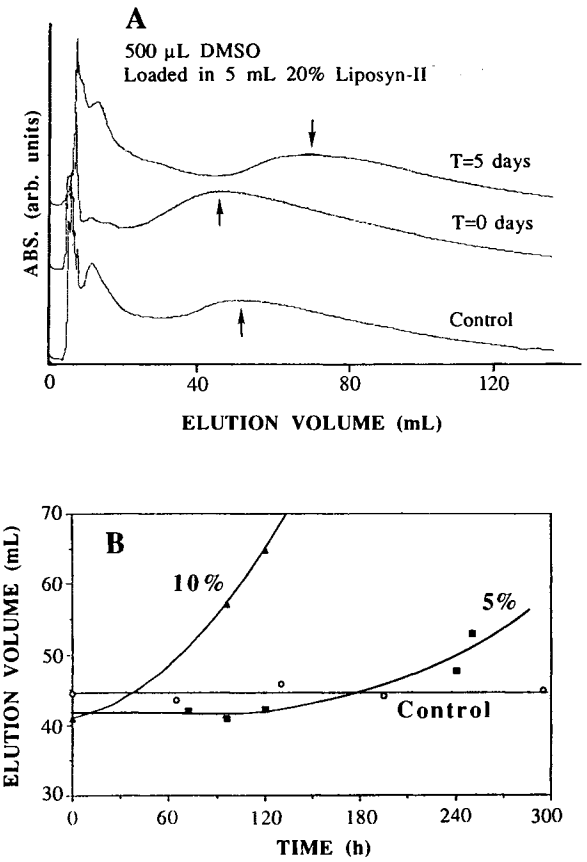

Fig. 8. Effect of DMSO addition on the stability of Liposyn-II emulsion. (A) SedFFF fractograms of Liposyn-II emulsion containing 10% (v/v) DMSO; (B) comparison of peak elution positions (indicated by arrows in A) for emulsions of different DMSO composition [5 and 10% (v/v), respectively] versus time after mixing. Run parameters: field strength, 1000 rpm; flow rate, 2.92 mL/min.

CONCLUSIONS

The ability to evaluate emulsion stability is of particular importance in the field of pharmaceutics, where emulsified products are frequently prepared for intravenous administration to the human body. Although photon correlation spectroscopic techniques are frequently used to pinpoint shifts in the average size of emulsion droplets, these techniques alone are not capable of determining the detailed shifts in droplet size distribution which are early signs of instability. However, in combination with a size-based separation method, such as sedimentation field-flow fractionation, the PCS is highly informative and consistently produces convincing evidence of the level of stability of a given product. Thus, it has been possible to demonstrate that the commercial parenteral nutrition product Liposyn-II is extraordinarily stable under ambient conditions. This is also true for the other parenteral nutrition product on the U.S. market (Intralipid), as will be discussed in a separate communication (19). Just as the droplet size distribution remains constant for a particular lot during periods of 6 months or more, there appears to be very little lot-to-lot variation among these products. In contrast, the SedFFF/PCS method has shown that additions of small quantities of electrolytes and of a dipolar aprotic solvent may destabilize the Liposyn-II emulsion in a manner that could jeopardize its suitability for intravenous administration. In particular, the formulation of so-called total paren-

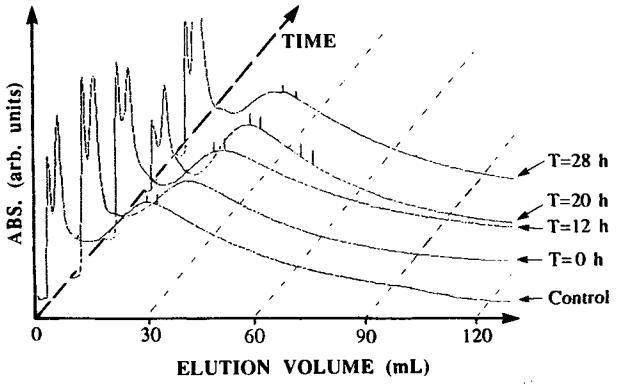

Fig. 7. After mixing with freshly collected human serum at a 1:1 volume ratio and storing at room temperature, the Liposyn-II elution pattern (droplet size distribution) remains unaltered for periods of several days. BCA protein analysis (see text for detail) revealed that this "serum stabilizing effect" is not due to irreversible protein (mainly HSA) adsorption. Run parameters: field strength, 1000 rpm; flow rate, 2.92 mL/min.

teral nutrition (TPN) mixtures from this emulsion can lead to a serious and rapid destabilization, which is readily observed from the sedFFF elution pattern. However, in the absence of destabilizing additives, the Liposyn-II emulsion appears to tolerate contact with human serum for several days without undergoing shifts in the size distribution of its fat droplets. As expected, this finding is consistent with the well-known clinical efficacy of the lecithin-stabilized fat emulsions used in parenteral nutrition.

The studies reported here appear to give strong evidence that the SedFFF/PCS method is a reliable analytical technique for surveying the effect of additives on the stability of emulsions. In view of the clinical importance of the parenteral nutrition products, it is hoped that the method can be of use in the clinical pharmacy.

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