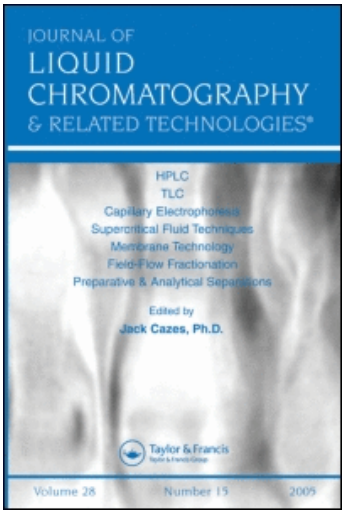


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# Characterization of Intravenous Fat Emulsion by Sedimentation Field-Flow Fractionation and Photon Correlation Spectroscopy

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# CHARACTERIZATION OF INTRAVENOUS FAT EMULSION BY SEDIMENTATION FIELD-FLOW FRACTIONATION AND PHOTON CORRELATION SPECTROSCOPY

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## ABSTRACT

Sedimentation field-flow fractionation (SdFFF) and photon correlation spectroscopy (PCS) were used to characterize fat emulsions. Mean diameters determined by SdFFF were in good agreement with those from PCS. Mean droplet sizes obtained from two SdFFF channels of different dimensions were also in good agreement, indicating no non-ideal phenomena such as solute-channel interaction occur during SdFFF fractionation. Flocculation of emulsion was affected by concentration of salt and the charge of cation. While freezing-thawing was one of the significant factors to the flocculation, storing emulsions at 60±5°C for 2- 3 days made no significant difference to the sample flocculation.

## INTRODUCTION

Sedimentation field-flow fractionation (SdFFF) is capable of separating and characterizing colloidal and particulate materials.[1-4] Samples are separated

in a thin ribbon-like channel on the basis of their effective masses. The flow through the channel has a parabolic profile whose flow velocity approaches zero at both walls and reaches a maximum at the midpoint between the walls. When the centrifugal force is applied across the channel, particles are driven toward the bottom wall, and an equilibrium distribution is established between the external force and particles' diffusion.[5] Particles positioned close to the wall are displaced slowly because of the low flow velocity near the wall. Particles positioned further away from the wall are displaced more rapidly. Since the force exerted on sample particles are related to their effective masses, the equilibrium distance from the wall depends on the particles' mass or size.[6]

The relationship between the applied force and the particle mass in SdFFF is well understood. It allows accurate prediction of particle retention. It also allows calculation of particle size, length, density, and thickness of coated materials at the particle surface, etc.[7-13] In addition to the separation and characterization of particles, fractions can be collected and further analyzed using electron microscopy or light scattering.[14,15]



For particles subjected to normal mode of FFF, retention volume $V_r$ is related to a retention parameter $\lambda$ as[1,3]

$$\frac{V^0}{V_r} = 6\lambda\left[\coth(1/2\lambda) - 2\lambda\right] \tag{1}$$

where $V^0$ is the void volume of the channel. When $\lambda$ is sufficiently small ($\lambda << 1$), eqn. (1) reduces to

$$\frac{V^0}{V_r} = 6\lambda \tag{2}$$

where $\lambda$ is the ratio of particles' diffusion coefficient to the field-induced particle velocity. In SdFFF, $\lambda$ is given by

$$\lambda = \frac{kT}{mG(\Delta\rho/\rho_s)w} \tag{3}$$

where k is the Boltzmann constant, T temperature, m particle mass, G centrifugal acceleration force, $\rho_s$ particle density, and $\Delta\rho$ density different between carrier and sample. If particles are spherical, particle diameter d is expressed by[3]

$$d = \left(\frac{6kT}{\pi G \lambda \Delta \rho w}\right)^{1/3} \quad (4)$$

By combining eqn. (2) and (4), particle size can be determined from the measured elution volume of the particle. For samples having broad size distributions, particle size can be determined for each slice of their fractograms, and the size distributions can be obtained for the samples.

A commercial fat emulsion such as intralipid which have been emulsified in water with soybean oil is widely used for parenteral nutrition. The stability of the emulsion is limited to 18 months at about 4 °C. For rapid and efficient medication to patients, the intravenously fed patient required a number of nutrients such as amino-acid, electrolytes, carbohydrates, and trace elements as a total parenteral mixture. Such emulsion has some problems in its stability. The emulsion is rapidly flocculated by electrolytes and this makes it difficult to modify total parenteral nutrition. These flocculated emulsions can block the blood capillary and cause impaired circulation. It is, thus, important to determine the droplet size and size distribution of fat emulsions.[16-18]



SdFFF has been used for characterization of emulsion materials. A theoretical and experimental validation was provided for the measurement of the droplet size distribution of emulsion samples.[19] The size distributions of polydisperse emulsion samples were determined by using SdFFF and PCS.[20] Recently, SdFFF was established for emulsion characterization at various experimental conditions.[21,22]

Fat emulsions are polydisperse, and thus, it is difficult to measure their mean droplet sizes and size distributions accurately. SdFFF has merits for the characterization of fat emulsions. It provides good separating power and flexibility. Sample degradation is minimized owing to the open geometry of the channel. In this work, SdFFF was used, in combination with PCS, to measure the mean droplet sizes of fat emulsions. Narrow fractions were collected from SdFFF runs, and subjected to PCS measurements. Results obtained from SdFFF were compared with those from PCS. Stability of fat emulsions was examined against salt species, salt concentration, and temperature.

## MATERIALS AND METHODS

Sedimentation FFF used in this study is the model S101 Colloid/Particle Fractionator from FFFractionation, LLC. (Salt Lake City, UT). The channel

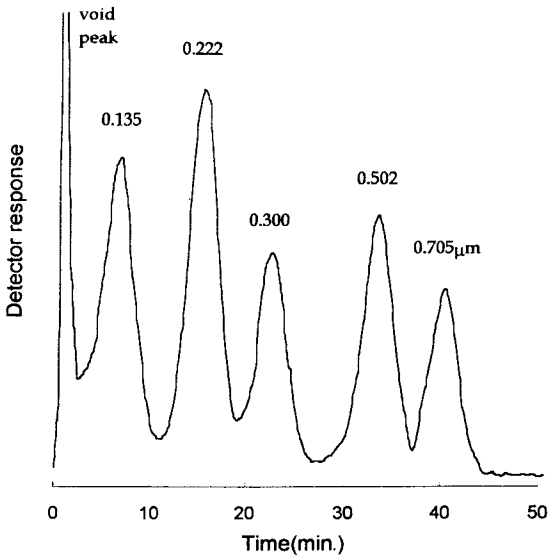



**Figure 1**. Fractogram of a five component-mixture of polystyrene latex beads obtained by a power programmed SdFFF. Experimental conditions are: initial field strength = 1950 rpm, final field strength = 75 rpm, pre-decay time = 6.0 min, $t_a$ = - 48 min, and flow rate = 4.16 mL/min.



surface was a polished Hastelloy-C alloy. Two channels having different dimensions were used in this work. One ("channel-1") has the length of 89.1 cm, breadth of 1.0 cm, and thickness of 0.0127 cm. The void volume of the channel-1, measured as the elution volume of sodium nitrite minus the volumes between the injector and channel and between the channel and detector, was 1.19 mL.

Another channel ("channel-2") has the breadth of 2.0 cm and a thickness of 0.0254 cm and the same length. The void volume of the channel-2, measured by the same method used for the channel-1, was 4.30 mL. The radius of the rotor is 15.1 cm.

The intralipid 10% was obtained from the Green Cross Inc. (Seoul, Korea) and was injected into SdFFF without dilution. Injection volume was 1.0 to 3.0μL. Reported density of the sample is 0.917 g/cm$^3$. The carrier liquid was doubly distilled and deionized water containing 2.25% glycerol (Sigma, St. Louis, MO) and 0.02% sodium azide (Merck, Darmstadt, F. R. Germany) as a bactericide. Polystyrene latex standards were obtained from Duke Scientific

(Palo Alto, CA). The standards were diluted about 100 times with water containing 0.1% of FL-70 (Fisher Scientific, Pittsburgh, PA) and 0.02% of sodium azide.

Experiments were carried out using an SLC-100 pump (Samsung Electron Devices, Suwon, Korea), Durex metering pump cc-100-s-4 (Eldex Laboratories, Inc. Napa, CA), and a Linear UVIS 200 (Reno, NV) UV detector fixed at 254 nm of wavelength. Detector response was transferred to a IBM-compatible PC and processed using the Field-Flow Fractionation Data Analysis Software 2.0. A power programming[23] was used to avoid the steric transition and excess retention of fat emulsions. The programming parameter, p was set at 8, and the time constant $t_a$ was set to be equal to - $pt_1$ to achieve constant fractionating power throughout the entire elution range.

Fractions of fat emulsions were collected from SdFFF runs and analyzed using photon correlation spectroscopy (PCS). Measurements were made at 25°C. The PCS system was 4700C from Malvern Instruments Ltd (Worcestershire UK). The light source of the PCS was He-Ne laser at 632.8 nm, and measurements were made at a 90 degree fixed angle.



## RESULTS AND DISCUSSION

The performance of SdFFF system was tested using polystyrene latex standard particles. Figure 1 shows a separation of five standards in the diameter range of 0.135 to 0.705μm, obtained from the channel-2 (having 0.0254 cm thickness). The field strength was power-programmed with the initial field of 1950 rpm, final field of 75 rpm, pre-decay time ($t_1$) of 6 min, $t_a$ of -48 min and flowrate of 4.16 mL/min. The carrier solution contained 0.1% (v/v) FL-70 and 0.02% sodium azide. Under these experimental conditions, five standard particles were separated within 50 min with a good resolution.

External field was varied for initial evaluation of the retention of fat emulsions. Figure 2a shows fractograms obtained using the channel-1 at three different initial field strengths, 380 G (1500rpm), 547 G (1800rpm), and 745 G (2100rpm). Field programming was employed to avoid steric transition. As previously explained, size distribution can be obtained from the SdFFF fractogram.

Figure 2b shows size distributions obtained for the fractograms shown in Figure 2a. Size distributions are broad with the high end reaching up to about 0.9 μm. No significant difference was found among size distributions obtained at different filed strengths. The mean droplet diameters were determined from

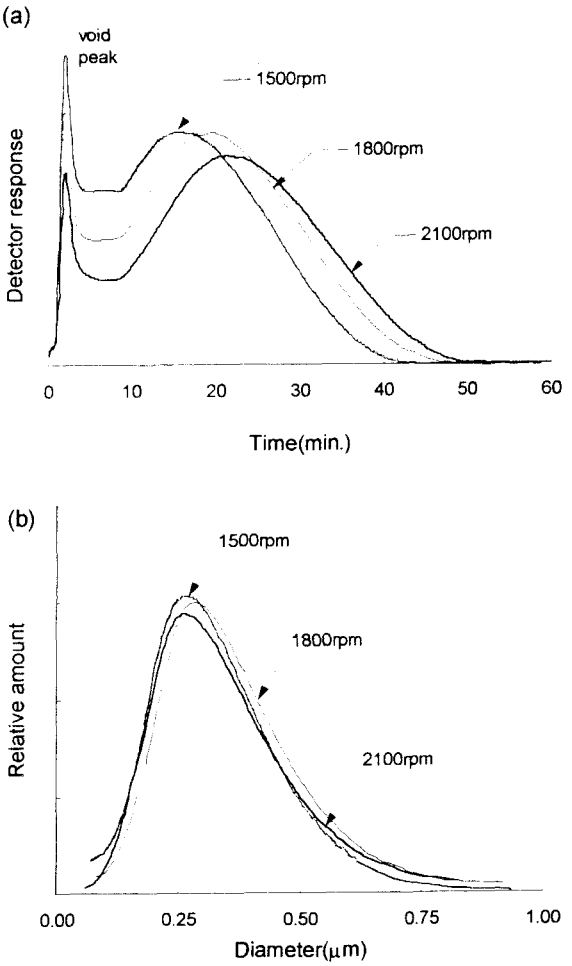



**Figure 2**. Fractograms (a) and size distributions (b) of fat emulsions obtained using the channel-1 (thickness of 0.0127 cm) at different initial field strengths, 380, 547, and 745 G. Experimental conditions are: pre-decay time = 8.0 min, $t_a$ = - 64 min, and flow rate = 0.90 mL/min.

the first moment of the size distributions. Mean diameters obtained at three different field strengths were in good agreement with the relative different of less than ±5%. Measured mean diameters were 263 μm at 380 G, 280 μm at 547 G and 268 μm at 745 G.

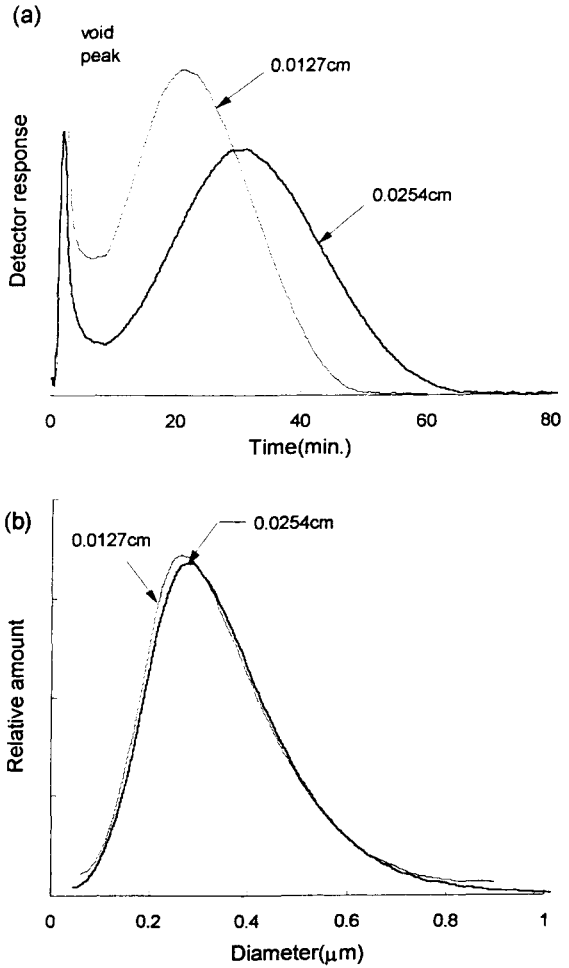



**Figure 3**. Fractograms (a) and size distributions(b) of fat emulsions obtained using channels of different thickness. Experimental conditions are: initial field strength = 745 G (2100rpm), final field strength = 1 G (75 rpm), stop-flow time = 12min, pre-decay time = 8.0 min, $t_a$ = - 64 min for both channels. Flow rate = 0.90 mL/min for the channel-1 and 3.24 mL/min for the channel-2.

The validity of the size-based fractionation and of the resulting size distribution curves obtained from SdFFF can be verified in several ways. One is to compare the size distributions obtained under different experimental conditions, or to compare the size distributions obtained from different SdFFF systems. Another way is to collect fractions from SdFFF fractograms and to

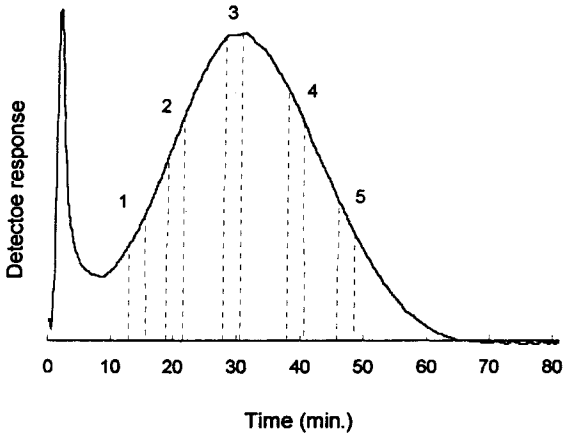
examine them using other techniques such as electron microscopy or PCS.[24] Figure 3a shows elution profiles of the same sample obtained from two different channels: channel-1 has the thickness of 0.0127 cm and the channel-2 has the thickness of 0.0254 cm. The same field-programming parameters were used for two channels: initial field strength of 745 G (2100rpm), final field strength of 1 G (75rpm), pre-decay time of 8.0 min, $t_a$ of - 64 min, and the stop-flow time of 12 min. Flow rate was set at 0.90 mL/min for the channel-1 and at 3.24 mL/min for the channel-2 to achieve the same linear flow velocity for both channels.

Figure 3b shows size distributions obtained from fractograms shown in Figure 3a. If there were non-ideal phenomena such as interaction between sample and the accumulation wall, or disruption of the sample distribution in the flow stream, or steric effect, then the size distributions obtained from two different channels will be different. The size distributions obtained from two different channels are similar with the mean droplet diameter of 0.27μm, suggesting no such non-ideal phenomena occurs. SdFFF is thus an useful tool for the determination of the size distribution of fat emulsions. It is noted that the thinner channel, channel-1 may not be useful for flocculated samples as steric inversion may occur during SdFFF run.



In order to confirm the accuracy of the mean diameter of fat emulsion measured by SdFFF, fractions were collected at 5 different positions of the fractograms and were analyzed using PCS. Each PCS measurement was repeated 20 times, and they showed good reproducibility with the relative error of less than ±4%. As shown in Figure 4, the mean diameters obtained from PCS are in good agreement with those obtained from SdFFF with relative difference of 4 - 11 %.

To study the effect of the salt concentration on the sample flocculation, various kinds of salts were added to the original sample and injected directly into the SdFFF without dilution. It is known that addition of electrolyte gradually reduces the energy barrier preventing aggregation until a point is reached where no barrier remains. The height of the energy barrier depends on the electrolyte concentration and counter-ion valence. The flocculation is dominated by the valence of the ion of the added electrolyte of charge opposite to that of the colloidal particles.[25,26] Elution profiles and size distributions of emulsions obtained at different concentrations of sodium chloride are shown in Figures 5a and 5b. At low salt concentrations, droplet size distributions do not change significantly from that of the original sample. When the concentration of sodium chloride is further increased, size distribution starts changing due to flocculation.




| Fraction No. | PCS(nm) | SdFFF(nm) | rel difference(%) |
|---|---|---|---|
| 1 | 164 | 156 | -5.1 |
| 2 | 188 | 196 | 4.1 |
| 3 | 239 | 258 | 7.4 |
| 4 | 326 | 367 | 11.2 |
| 5 | 436 | 465 | 6.2 |

**Figure 4**. Fractogram and measured droplet size of fat emulsion obtained from the channel-2 (thickness of 0.0254 cm). Experimental conditions are: initial field strength = 745 G (2100rpm), final field strength = 1 G (75 rpm), pre-decay time = 8.0 min, $t_a$ = - 64 min, stop-flow time = 12min, and flow rate = 3.24 mL/min.



In case of calcium and magnesium ions, as shown in Figures 6a and 6b, the change in the elution profile is more noticeable than in the case of sodium chloride, showing broadening of the profile and even a symptom of steric transition. The addition of magnesium ion made a slight change at the end of the elution profile compared to that of calcium ion. When the concentration of salt is further increased, the samples rapidly become creamy or oiled.

The cumulative mass distribution, Figure 6b, shows that droplets larger than 0.3 μm are produced by flocculation. It was impossible to determine accurate size distributions of flocculated samples due to the steric transition occurring at the later eluting region. It seems that divalent cations are more effective than monovalent cations for flocculation. These results indicate that both the type of electrolyte and electrolyte concentration are important factors to the flocculation of the fat emulsions during their storage.

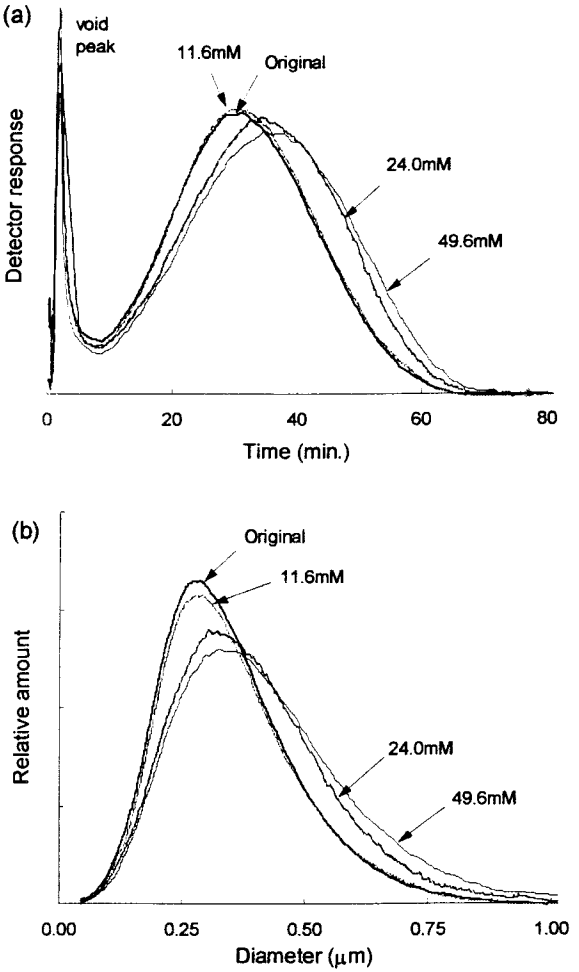



**Figure 5**. Fractograms (a) and droplet size distributions (b) of fat emulsion after the addition of sodium chloride. Experimental conditions are same as those of Fig. 4.

SdFFF is one of the methods that can be used to study critical concentration for flocculation. It is noted, however, that the samples (and also the salt) are diluted in the SdFFF channel, and this may affect the sample flocculation. Figure 7 shows fractogram of a sample flocculated by freezing. Freezing induces phase change, and causes sample flocculation. Eventually, emulsions become oiled.

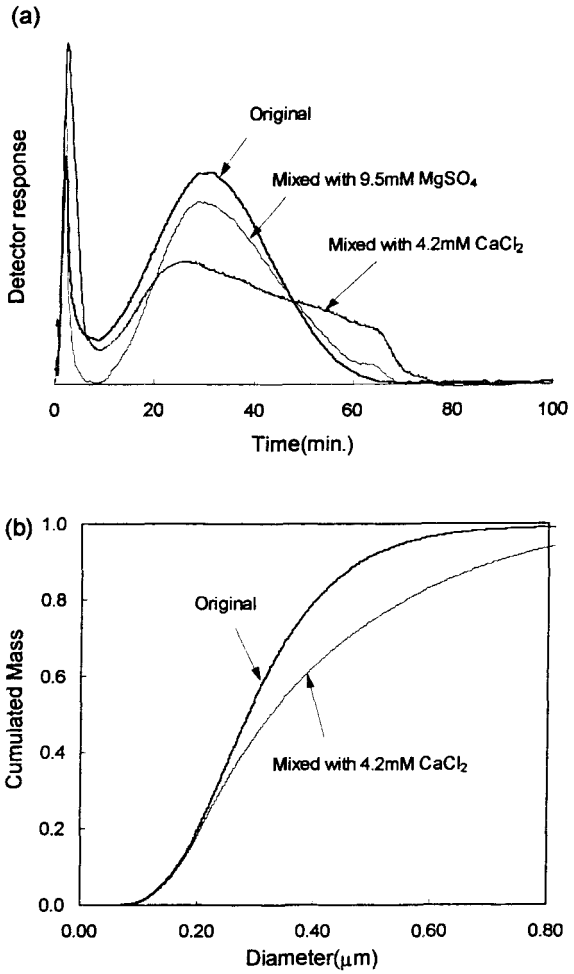



**Figure 6**. Fractograms (a) and cumulated mass distribution curves (b) of fat emulsion after the addition of magnesium sulfate and calcium chloride. Experimental conditions are same as those of Fig. 4.

To re-disperse the emulsion, the frozen sample was thawed and then homogenized at 10,000 rpm 3 times for 3 min each. The fractogram labeled "redispersion" is for the homogenized sample. It shows a slight change at the end of the fractogram. The size distribution, however, did not change. This indicates that, once intralipid is frozen (and thus flocculated), it can not be re-dispersed, even with the means such as shear-mixing homogenization. While

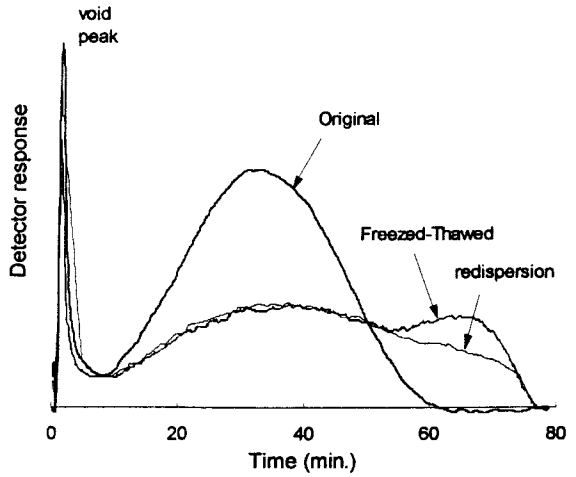



**Figure 7**. Fractograms of original and freezed-thawed fat emulsion. Experimental conditions are same as those of Fig. 4.



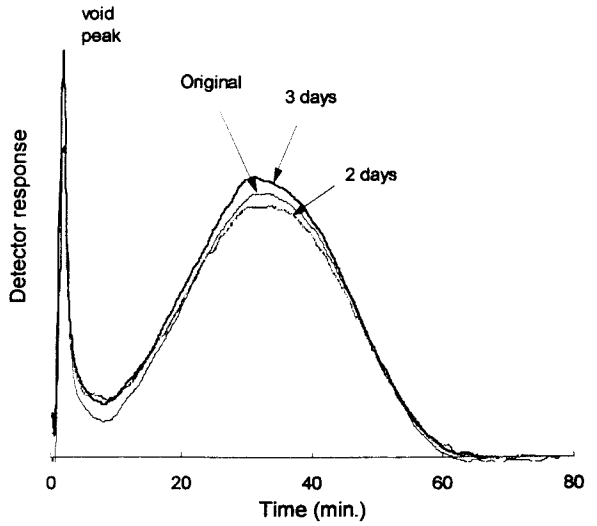



**Figure 8**. Fractograms of fat emulsion stored at 60±5 degrees. Experimental conditions are same as those of Fig. 4.

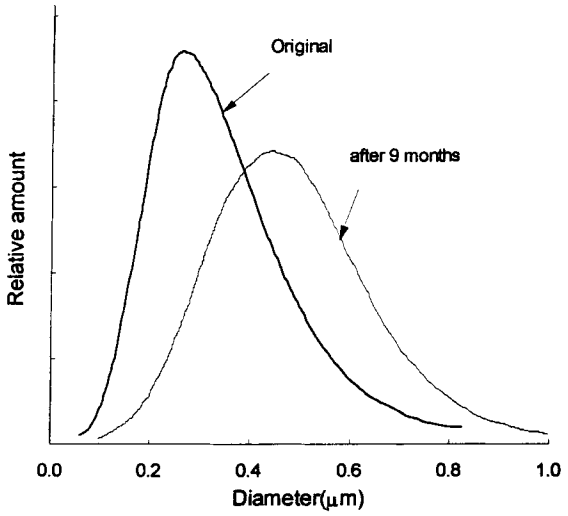



**Figure 9**. Size distribution curves of fat emulsion stored for 9 months at room temperature. Experimental conditions are same as those of Fig. 4, except the initial field strength = 1900 rpm.



storing the sample at below 0°C showed a significant difference in droplet size distribution, keeping them under high temperature did not make a significant change (see Figure 8). Samples were kept in an oven at 60±5 °C for 2 - 3 days. Increasing the temperature generally brings about changes in viscosity, interfacial tension, and adsorption at the interface. Also, potential energy and Brownian motion increase with temperature and double layer potentials change.[25] It was found that temperature, as a short term stress, was not a significant factor for emulsion stability. The stability of emulsion, however, decreased rapidly with increasing temperature, and it was impossible to obtain an SdFFF fractogram.

Aging effect was also studied. Generally, an emulsion stored at 4 - 8°C through the expired date changes droplet diameter less than about 5%. Figure 9 shows size distribution of a sample stored for 9 months at room temperature after the first sampling through a rubber septum. The mean droplet diameter increased by about 30% from that of the original sample. Small degree of temperature fluctuation during long term stress may have made the sample to be flocculated easier than when the sample is stored under 4°C.

In conclusion, SdFFF is a useful tool for determining the mean droplet diameter and the size distribution of fat emulsions. Good agreement was observed between mean diameters obtained from two different SdFFF channels. Mean diameters obtained from SdFFF and PCS were also in good agreement. SdFFF is applicable for characterization of flocculated fat emulsions, and for the determination of the critical flocculation concentration of the sample.

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