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Characterization of submicron MCT o/w emulsions using sedimentation field-flow fractionation (FFF) with power field programming

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Abstract: Sedimentation field-flow fractionation (SdFFF) operated with power-based field programming, was shown to be effective in the characterization of submicron investigational pharmaceutical emulsions. Field programming, in which the decrease of field strength with time gradually decreases the retention of sample components, extends the capabilities of sedimentation field flow fractionation in handling polydisperse and multicomponent samples. The emulsions were made of medium chain triglycerides (MCT) oil in water emulsified by phospholipids. They were analysed by different rates of field decay and different flow rates. Identical size distribution profiles were obtained under all circumstances, using the appropriate stop-flow times. Fractions were collected from the SdFFF eluting bands, and diameters were analysed by photon correlation spectroscopy, showing good agreement with values given by the FFF instrument at high flow rates and low rates of field decay. Accurate and highly reproducible size distribution profiles were obtained under various conditions. The detector response was shown to consist mostly of light scattering and was linear with concentration.

Keywords: *Sedimentation field-flow fractionation; field programming; fat emulsions; submicron; MCT; medium-chain triglyceride; particle size distribution.*

Introduction

Sedimentation field-flow fractionation (SdFFF) has proven capable of separation and characterization of emulsions made of vegetable oil emulsified by phospholipids [1–4]. The technique has been described as a one-phase analogue of chromatography [5], in which the sample components are partitioned into regions of different mobile fluid velocity in an open ultra-thin channel. The SdFFF channel is integrated into the perimeter of a centrifuge rotor, thus a sedimentation field is constantly applied across the channel faces, perpendicular to the flow axis. The field forces the sample's components towards one wall, the accumulation wall. If the particles' density is higher than the mobile fluid, they accumulate at the outer wall of the channel, whereas if their density is lower, such as oil in water (o/w) fat emulsions, the particles accumulate at the inner channel wall. The retention parameter is related to the property of the particle interacting with the field. In the case of sedimentation FFF this property is the effective mass, or particle diameter and density. Therefore, particle size distribution can be measured from retention data of the sample components by the sedimentation field-flow fractionation system.

Field programming, in which the decrease of field strength with time gradually increases the average velocity of the sample components, extends the capabilities of sedimentation field-flow fractionation in handling such polydisperse samples. The function of field decay in the present work is power-based programming [4, 6–8].

Emulsions are inherently unstable systems, which tend to cream (settle) and coalesce. Both processes depend on the size distribution of the oil droplets, as well as on other physical properties, such as surface tension, ζ potential, density and viscosity of the two liquid phases [9–12]. The emulsions studied here were made of MCT oil emulsified by phospholipids (fat emulsions), which were developed for the solubilization of highly lipophilic drugs [13–15]. The population of oil droplets in emulsions can be rather diverse in size, thus,

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in such cases it is advantageous to use field programming. The gradient operation extends the sensitivity of detection when relatively large particles are present in the sample in addition to small particles. Although SdFFF is a separative technique, the system, fluid carrier and channel, comprise a relatively gentle environment that does not inflict extreme risks of shear or stress and no noticeable changes of the original sample components were observed.

The few previous works, which established and validated the feasibility of SdFFF for the characterization of emulsions, were implemented on (mostly) commercial samples, whose long-term stability was established [1–4]. The purpose of this work is to further explore the consistency of SdFFF in giving identical profiles of size distribution under various conditions of field decay when investigational emulsions, whose long-term stability was not established, are concerned. The short term stability of such samples needs to be sufficient enough so that a practical operation of the SdFFF system under various conditions would not affect the results.

Experimental

Materials

The mobile fluid in the SdFFF system was made up to 2.25% (w/v) glycerin in double distilled water with 0.0125% (w/v) sodium azide added as bactericide (refractive index 1.33 — close to water's). It was filtered through a 0.2 μm filter before use. Density of the solution was determined by pycnometer as 1.005 g ml^{-1}.

Medium chain triglycerides (MCT) ranging from 8 to 10 carbons were obtained from the Societe des Oleagineux (Saint Blangy, France). Density of the oil, reported by the manufacturer, was 0.940–0.950. A value of 0.945 was reported in the literature [11]. Lecithin was of intravenous grade (Lipoid E-80) from Lipoid KG Ludwigshafen (Germany) containing mainly phosphatidylcholine and phosphatidylethanolamine in a ratio of 10:1. All other ingredients used were of pharmaceutical grade.

Instrumentation

A basic unit of particle and colloid fractionator, SedFFF™ model S101, equipped with a data station and control of RPM, capable of data acquisition and processing from FFFractionation Inc. (Salt-Lake City, Utah), was used for the fractionation. A 880-PU HPLC pump (Jasco, Japan) and a UV detector model LC-85B from Perkin–Elmer (Norwalk, CT, USA), operated at 260 nm, completed the fully operating sedimentation field-flow fractionation system. Channel dimensions were 2 cm in breadth, 0.0254 cm in thickness and 90 cm in length. The radius of the rotor was 15.1 cm. Void volume, measured using various small molecular weight substances, was 4.6 ml. Stop-flow was 40 min in all the experiments, apart from the operation with a constant field.

Fractions were collected by a Pharmacia Frac-100 fraction collector (Bromma, Sweden). Size analysis of the fractions collected from the SedFFF™ instrument was done using the Submicron particle analyser Coulter model N4SD. Details of the operation of the SdFFF system as well as the measurements by photon correlation spectrometer are given in a previous publication [4] and by Cyr *et al.* [16].

Methods

Preparation of the emulsions. The emulsion was composed of (% w/w) MCT 10, Lipoid E-80 1.2, α-tocopherol 0.02, glycerine 2.25 and water for injection made up to 100. The aqueous and oily phases were separately prepared and were filtered and heated to 70°C, combined and stirred with a magnetic stirrer. The mixture was further heated to 85°C. At this temperautre a coarse emulsion was obtained, using a high shear mixer, Polytron (Kinematic, Lucern, Switzerland) and rapidly cooled. It was then passed through an homogenizer (APV, Gaulin, Hilversum, Holland) at a pressure of 8000 psi for 4 min, with a final pH of 7.4 using 0.1 N NaOH solution. The emulsion was filtered through a 0.45 μm membrane filter, packed and sterilized at 121°C for 15 min. The complete manufacturing process was done under nitrogen atmosphere.

Results and Discussion

The basic mechanism of the normal mode of retention in SdFFF has been described numerous times [1–3, 5], therefore, only a short explanation will be given here for clarification.

The clouds of droplets, which are formed under the influence of the sedimentation field,

move downstream at an average velocity proportional to their thickness. The more compressed to the wall, the slower they move. Droplets of different sizes form clouds of different thickness, which move downstream at different velocities, thus separation takes place. The experimental data appears in the form of the detector response as a function of time, i.e. the fractogram. The currently available SdFFF instruments manipulate the raw data automatically to obtain the size distribution profile. Diameters of the sample components are calculated from their retention data, and the relative mass for each diameter is calculated according to the procedure described by Yang *et al.* [12]. The experimental retention times are converted to respective retention parameters, λ, whose exact relationship is described in most of the earlier FFF publications [1–3, 17]. Diameters of the components are then calculated from λ by the system at any time, using the following equation:

$$d = \left(\frac{6kT}{\lambda\pi\,\Delta\rho\,Gw}\right)^{1/3} \qquad (1)$$

given the parameters Δρ, the difference in density between the suspending fluid and the sample components, w the channel thickness, T the temperature and Boltzmann constant k. The sedimentation field G is calculated automatically from the spin rate (rpm), measured by a sensor in the centrifuge at any particular moment in time.

Rates of field decay and fractionating power

The details of operation under power-field decay is described in refs 6–8, therefore, only a short explanation will be given here. In the power-based field programming the initial field strength S_0 (calculated from the rotor rpm as detailed in ref. 4) is held constant for a period of time t_1 (time-lag). Subsequently, field strength is decreased over a period of time until it reaches a pre-chosen constant value (1 g in this study). After t_1 has elapsed ($t > t_1 > t_a$) the field decays according to the expression:

$$S(t) = S_0\left(\frac{t_1 - t_a}{t - t_a}\right)^p \qquad (2)$$

where $S(t)$ is the field strength at time t, p is the variable of the power program and $t_a = -p\,t_1$ for a constant fractionating power [6–8].

Three rates of field decay were tested for the analysis of the MCT emulsion at 1.5 and 2 ml min^{-1}: Method A: $t_1 = 8$ min ($t_a = -64$); Method B: $t_1 = 10$ min ($t_a = -80$); Method C: $t_1 = 12$ min ($t_a = -96$). The rest of the experimental parameters, such as the initial (380*g*) and final (1*g*) field strengths, stop-flow time, and rate of data acquisition, were identical in all three cases. The value of Δρ used for the calculation of these profiles was 0.06 (see experimental section). The three superimposed size distribution profiles are shown in Fig. 1 I (1.5 ml min^{-1}) and 1 II (2 ml min^{-1}). In spite of the differences in the rates of field decay, the three profiles nearly overlapped. Similar results were reported earlier using a commercial emulsion [4].

Comparison to photon correlation spectroscopy

A size distribution profile of the non-fractionated sample of MCT fat emulsion has been measured by photon correlation spectroscopy periodically. The average diameter was

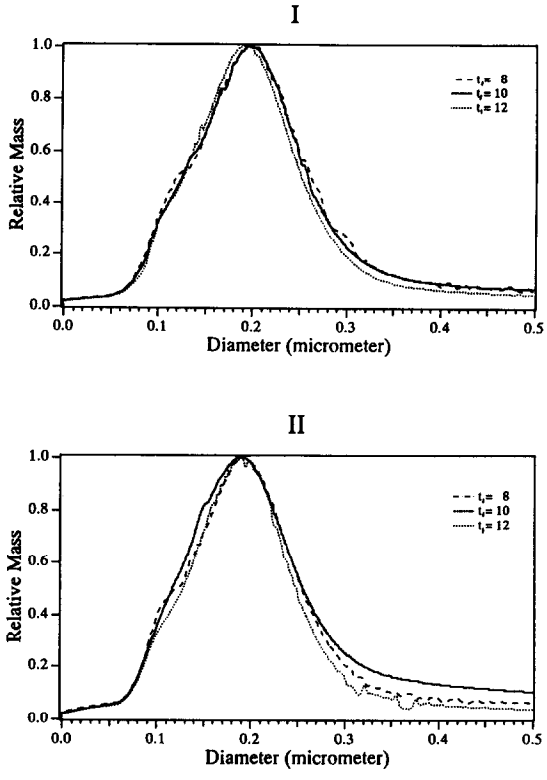

Figure 1
Characterization of MCT fat emulsion using three different rates of field decay. Method A: $t_1 = 8$ min ($t_a = -64$ min); Method B: $t_1 = 10$ min ($t_a = -80$ min); Method C: $t_1 = 12$ min ($t_a = -104$ min). Initial field was 380*g* and final field was approximately 1*g*, flow rate = (I) 1.5 ml min^{-1}, (II) 2 ml min^{-1}.

0.193 μm with coefficient of variation σ/d_{mean} = 0.22 according to the PCS (average of 10 repeated measurements). The results from the SdFFF instrument after a fit to Gaussian distribution were d_{mean} = 0.196 μm, and σ/d_{mean} = 0.24.

The instrument measures intensity-weighted size distribution, which is displayed as an intensity histogram (the relative intensity of scattered light for each size). The intensity distribution can be converted by the instrument to weight distribution (the relative weight of droplets of each size in the sample) knowing the refractive index of the particles and the medium, and using the Mie equation. The histograms are analysed for the mean size and standard deviation which are reported by the instrument. The standard deviations are converted to coefficients of variation, and are shown as error bars in the following graphs presenting results from PCS measurements.

Various fractions were collected from the eluted bands and were analysed immediately by photon correlation spectroscopy (PCS), to verify the droplets' diameters. The values given by the PCS were compared to the corresponding FFF diameters, given by the data system of the instrument, for the par-

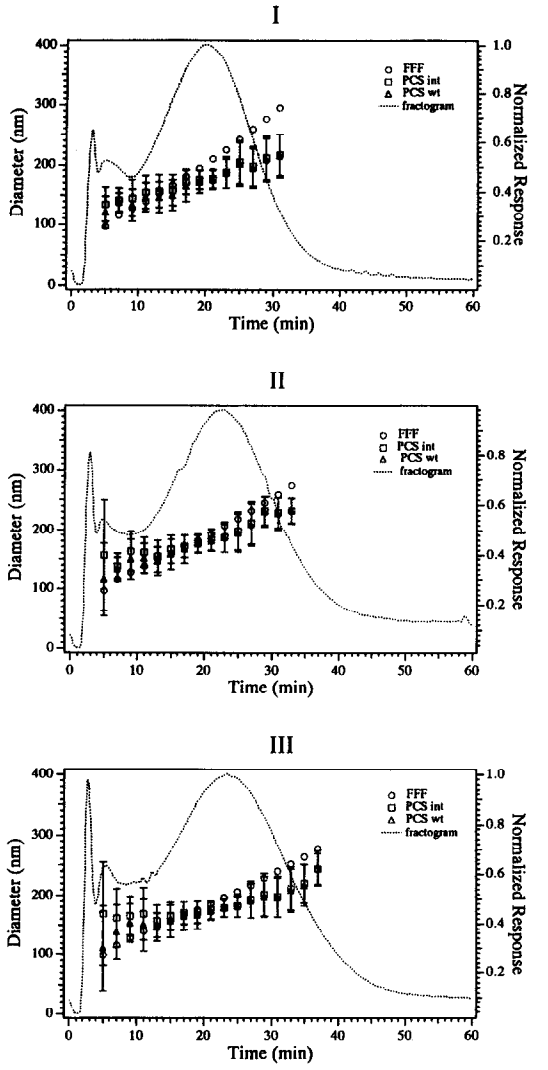

Figure 2
Average diameter of droplets in the fractions, collected from the SdFFF instrument during elution, measured by SdFFF and by PCS, Int. — from the intensity distribution and wt. — from the weight distribution. (I) Method A; (II) Method B; (III) Method C, as specified in Fig. 1, flow rate was 1.5 ml min^{-1}.

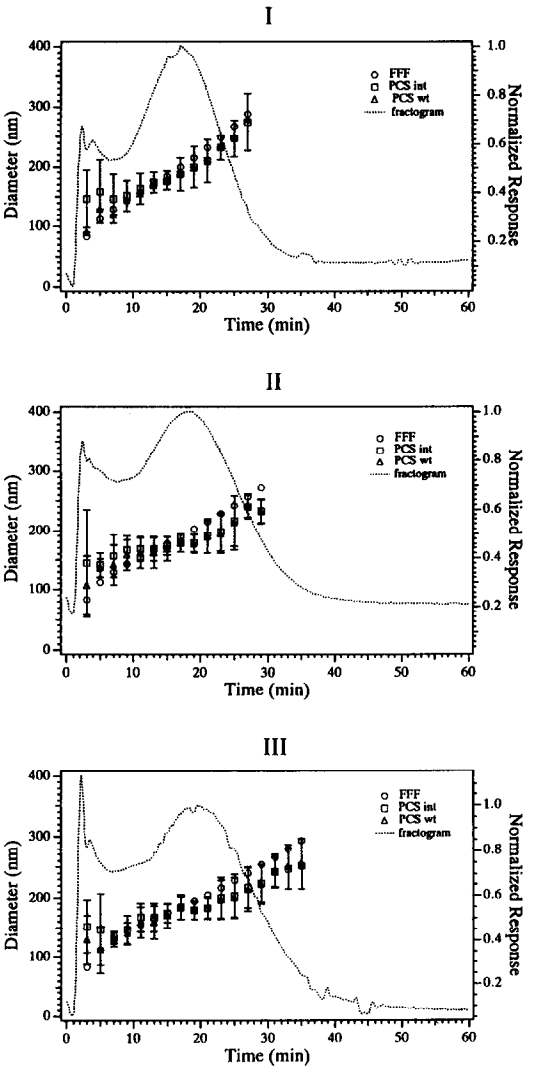

Figure 3
Average diameter of droplets in the fractions, collected from the SdFFF instrument during elution, measured by SdFFF and by PCS, Int. — from the intensity distribution and wt. — from the weight distribution. (I) Method A; (II) Method B; (III) Method C, as specified in Fig. 1, flow rate was 2 ml min^{-1}.

ticular time of collection. The values of the FFF diameters were taken from the mid-point of the 2-min fractions. Each fraction contained 3 ml effluent (at 1.5 ml min^{-1}), or 4 ml effluent (at 2 ml min^{-1}). Figures 2 I–III and 3 I–III show the superposition of diameters obtained by the two methods, photon correlation spectroscopy and sedimentation field-flow fractionation at 1.5 and 2 ml min^{-1}, respectively.

It was assumed in this approach that the density of the oil droplets was constant over the entire range of diameters. This assumption was based on the notion that the surface active components of the oil droplet, the phospholipids, do not contribute significantly to its density. It is well known that liposomes (phospholipid vesicles) are neutral buoyancy colloids [18]. Moreover, even if they affect the density of the oil droplet, the change in surface area between 100 and 200 nanometer spheres is not significant enough to change the droplets' density (approximately 2% [19]). It is usually assumed by the pharmaceutical technologists that the density of oil droplets is homogeneous [11].

The agreement between diameters calculated by the SdFFF instrument and those measured by PCS was better when a flow rate of 2 ml min^{-1} was used for the lower decay rates (t_1 = 10, 12 min). Less agreement between the SdFFF and PCS diameters was observed, using the higher rate of field decay (t_1 = 8 min) at the lower flow rate (1.5 ml min^{-1}) in the fractions containing large particles. The initial field was relatively high for this type of field decay, and secondary equilibrium effects probably interfered [20]. As suggested also by the theory [6, 7], it is preferable to operate the system at the higher flow rate (2 ml min^{-1}) and to use lower field decay ($t_1 > 10$ min) in order to obtain more accurate results.

Operation under a constant field

Once it is established that the MCT emulsion does not contain additional populations of oil droplets of larger sizes, it is preferable to use a constant field [1–3]. The sample was analysed using 169*g* (1000 rpm) as the sedimentation field and flow rate of 2 ml min^{-1}. Since the size of the oil droplets was relatively small, a long time was needed for relaxation, at least 60 min, (preferably 80 min). The size distribution profile that was obtained using relaxation times of 60 and 80 min is shown in Fig. 4. A profile of

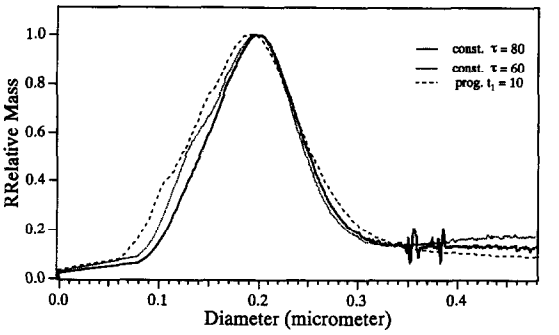

Figure 4
Size distribution profile of 10 μl of 10% (diluted) MCT fat emulsion using a constant field with relaxation times 60 and 80 min. Field strength was 169*g* and flow rate was 2 ml min^{-1}. The profile obtained by field programming was taken from Fig. 1 (II), Method B.

size distribution from the operation under field programming (Method B flow rate 2 ml min^{-1}) was superimposed on the figure for comparison. The profiles nearly overlapped, indicating that field programming does not introduce significant setbacks in the characterization of emulsions. The range of small particles shows less than perfect agreement between the two modes of operation, because this is a measurement at the limit of applicability of SdFFF at the field strength used. The smaller sizes could be more accurately characterized at higher field strengths.

Band broadening due to polydispersity

When the shape of the profile of droplets reflects the real polydispersity of the sample, it remains invariant at various flow rates [4]. The domination of polydispersity on the profile of size distribution was tested using a constant field at four different flow rates, 0.5, 1, 1.5 and 2 ml min^{-1}. All four profiles overlapped each other, and a fit to Gaussian gave rise to ±2% difference between their σ values.

When polydispersity dominates peak broadening, values of diameters of oil droplets in the fractions, collected from the elution band, would reflect the real diameters. The mean diameter of droplets in these fractions, measured by PCS, would agree with the *d* values given by SdFFF over the entire range of collection. Otherwise, too small FFF diameters would be obtained at the front and too high FFF diameters would be obtained at the rear of the profile. Such deviations from the PCS values may indicate that the sample components were driven away from the centre of gravity of the band due to non-equilibrium

and/or diffusive effects rather than due to differences in their size.

The domination of polydispersity over non-equilibrium or diffusive effects in the profile of the MCT emulsion was shown in Fig. 3 where the agreement between FFF and PCS was better. The *d* values of various fractions, collected from the eluting FFF band, analysed both by SdFFF and PCS were close on both sides of the centre of gravity of the peak in the fractogram. This closeness indicates that the sample components were driven away from the centre of gravity of the band mainly due to differences in their size rather than due to diffusive and/or non-equilibrium effects.

The detector signal

Detection of clouds of particles is based on measurements of the attenuation of the UV signal in the detector. The signal depends both on absorption as well as on light scattering, which is a size dependent property, therefore, it might distort the band shape by over- or under-weighing components in the FFF band. The contribution of absorption to the detector signal in case of emulsions, especially medicated ones, can be significant, due to the various UV absorbing additives in them. However, the emulsion that was studied here contained only minute amounts of UV absorbing agents (0.02% α-tocopherol), and attempts

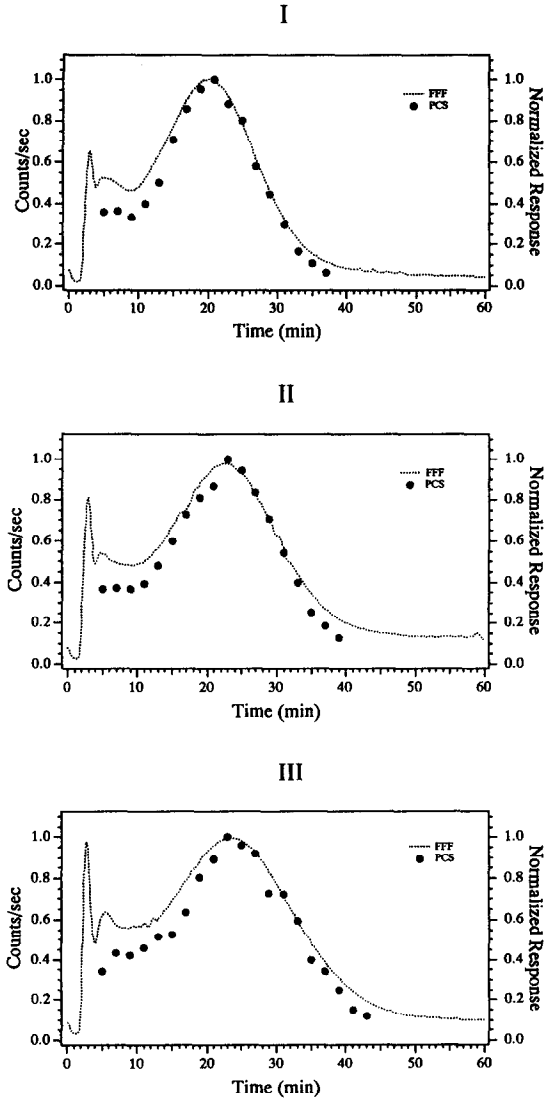

Figure 5
Normalized fractograms obtained by SdFFF compared to the normalized values of counts per second given by the PCS for the fractions collected from the effluents. (I) Method A; (II) Method B; (III) Method C, as specified in Fig. 1, flow rate was 1.5 ml min^{-1}.

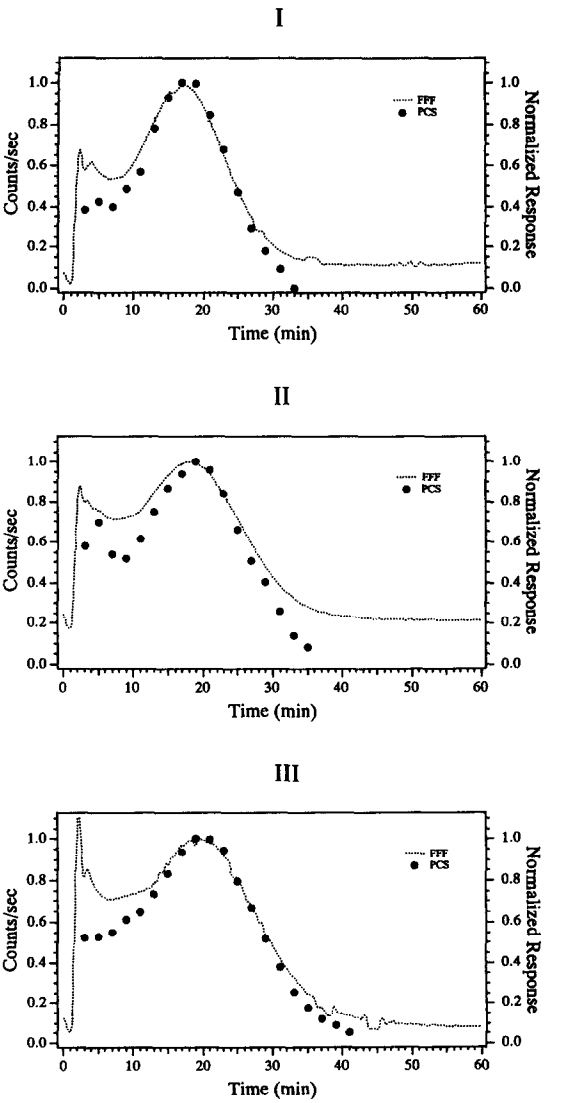

Figure 6
Normalized fractograms obtained by SdFFF compared to the normalized values of counts per second given by the PCS for the fractions collected from the effluents. (I) Method A; (II) Method B; (III) Method C, as specified in Fig. 1, flow rate was 2 ml min^{-1}.

to dissolve it and measure the UV absorption were not successful. On the other hand, light scattering of the fractions collected from the photon correlation spectrometer could be measured from the values of total counts of photon per second for a sample, given by the instrument. The normalized values of photon counts per second for the fractions were superimposed on the normalized fractograms and the results are shown in Figs 5 and 6 using 1.5 and 2 ml min^{-1}, respectively. Good agreement was obtained, confirming that the response was based mostly on light scattering.

The agreement in d_{mean} and σ/d_{mean} obtained by the PCS instrument (intensity and weight distribution) and that obtained by the SdFFF instrument indicates that, although based on light scattering, the SdFFF detector signal was not distorted significantly.

The linearity of the FFF detector's signal was examined using increasing injection volumes, 5–20 μl samples of 10% (diluted) emulsion. The response was linear with concentration (correlation coefficient = 0.988), therefore, the fractograms, from which the corresponding size distribution profiles were calculated, reflected the amount of oil droplets in the sample. Linearity of the detector response when there were more UV absorbing agents in the sample was established in previous studies of a commercial emulsion, Intralipid [4].

Conclusion

Sedimentation field-flow fractionation, operated with power-based field programming was proven feasible for the characterization of MCT fat emulsions, which can be very polydisperse in nature. The fact that the original non-fractionated sample and the fractionated sample gave the same mean diameter and that various conditions of analysis did not affect the size distribution of the emulsion significantly served as an indication to the fact that no changes in the original sample were detected.

When characterization of an unknown sample of submicron emulsion is needed, the FFF run requires previous knowledge of the difference in density between the oil droplets and the suspending solvent, and the geometrical parameters of the operating system. A relatively high initial field with slow field decay can be employed at first to cover the entire range of submicron diameters. Then a relatively low field should be used to detect any presence of large particles. The diversity and modality of sizes of the oil droplets population will be disclosed simply in the fractogram, since SdFFF is a separative technique. Once the sample contains mostly small particles with relatively low polydispersity, then it is preferable to use a constant field.

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