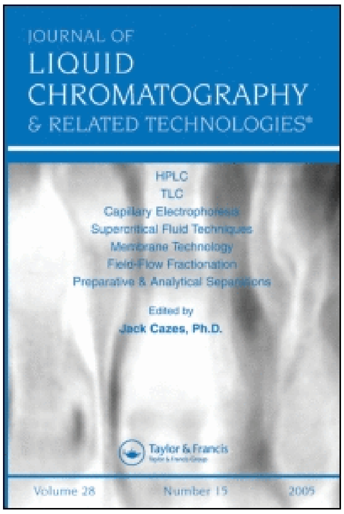

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OPTIMIZATION OF EXPERIMENTAL CONDITIONS FOR CHARACTERIZING COLLOIDS AND MACROMOLECULES BY FIELD-FLOW FRACTIONATION

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OPTIMIZATION OF EXPERIMENTAL CONDITIONS FOR CHARACTERIZING COLLOIDS AND MACROMOLECULES BY FIELD-FLOW FRACTIONATION

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ABSTRACT

Field-Flow Fractionation (FFF) is a relatively new technique for characterizing colloidal materials and macromolecules. It is an elution technique and the retention volume under selected experimental conditions is immediately related to the number and weight average particle diameter or to the particle mass. The accuracy in the determination of the above parameters for a given colloid or macromolecule is dependent on a number of experimental parameters among which the surface potential of the particles, the nature, pH and ionic strength of the suspending medium, the effective Hamaker constant, the concentration and pretreatment of the sample, as well as, the relaxation time and the applied field strength.

In the present work the influence of the experimental parameters mentioned previously on the interaction energy between various colloidal particles and the FFF channel wall is discussed and a number of useful applications verifying these interactions are presented. The applications include the characterization of inorganic and organic colloids (ca. Hydroxyapatite, sulphides, and starch granules), as well as, macromolecules (polymethylmethacrylate beads).

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INTRODUCTION

Field-flow fractionation (FFF) is a technique ideally suited to the analytical scale separation and characterization of colloidal materials and macromolecules.[1-5] One of the analytical strengths of FFF is that separation occurs in a channel of such simple geometry, within which there is flow of such uniform profile, that exact equations can be developed relating the component's retention to underlying physicochemical parameters. Different FFF methods were distinguished according to the nature of the external field used.

Sedimentation FFF (SdFFF) is the FFF subtechnique which utilizes a centrifugal or a gravitational (GFFF) field to sediment component particles into the characteristic steady-state layer against the channel wall. The thickness of the layer is an explicit function of the applied field, particle size, and the density of both the particles and the carrier solution. The largest particles are forced most vigorously toward the channel wall where flow velocity is lowest and, hence, these particles become the most highly retained. The fractogram, which is a graph of the detector response versus the volume of carrier solution eluted, becomes a linear mass spectrum for well retained particles[6] until steric effects dominate, at which transition point there is a fold-back in elution order.[7-9] The largest particles tend to move more quickly than the smaller ones in steric FFF (SFFF), and the separation mechanism is based more on inherent particle size than on the ability of a particle to undergo Brownian diffusion. When the Earth's gravitational field is utilized, the steric FFF technique is termed gravitational FFF (GFFF).

Generally, the measurement of a solute's behavior in an FFF channel can be used to deduce fundamental physicochemical parameters, thus helping characterize or identify the solute material. It is therefore important to determine the closeness of the agreement between theory and experiment in FFF to assess the potential accuracy of this procedure. As a consequence, a number of studies of retention and zone spreading have been made by using various subtechniques of FFF.[2,6] Generally, the retention equations have been well verified, often accurate to within a few percent, when the interactions between the solutes and the channel wall are absent, and the potential energy of a spherical particle depends mainly on the particle diameter and the density difference between the particle and the medium, while the zone spreading measurements are subject to larger relative uncertainties than retention measurements.

In potential-barrier field-flow fractionation (PBFFF), where the colloidal particles interact with the FFF channel wall,[3,9-11] the total potential energy of a spherical particle depends on the particle diameter, the surface potentials of the particle and the wall, the Hamaker constant, and the ionic strength of the carrier solution.[3,12,13] Variation of one or more of these parameters can lead to total or partial adhesion/detachment of the colloidal particles on/from the FFF channel wall.

As the physicochemical measurements by SdFFF and GFFF are subject to relatively large uncertainties, when interactions between colloidal particles and the channel wall are present, in this study we will try to reconfirm the retention studies and then to optimize the experimental conditions for the accurate characterization of various colloidal materials and macromolecules with industrial, environmental, and medical applications.

EXPERIMENTAL

Materials

The samples used in the present work were the following:

i) Polymethylmethacrylate (PMMA) monodisperse particles from Polysciences, Inc. with nominal diameter 0.350 μm.

ii) Polydisperse, irregular colloidal particles of hydroxyapatite (HAP) [$Ca_5(PO_4)_3OH$] provided by Dr. Kapolos with number average diameter 0.261 ± 0.046 μm.

iii) Polydisperse, irregular colloidal particles of the mixed sulphides $Cu_{0.2}Zn_{0.8}S$ and $Cu_{0.5}Zn_{0.5}S$ provided by Dr. Dalas with number average diameters (5.74 ± 1.59) μm, and (6.02 ± 1.91) μm, respectively.

iv) Barley starch granules with a triple size distribution provided by Dr. Chmelik (Institute of Analytical Chemistry, Brno, Chech.).

The suspending medium was triply distilled water containing 0.5% v/v of a low foaming, low alkalinity, phosphate-, chromate-, and silicate-free detergent FL-70 (Fisher Scientific Co.) with a pH ranging between 9.0 and 10.5 and 0.02% w/w sodium azide (Fluka AG) as bacteriocide. The FL-70 solution is an uncharacterized mixture of ionic and non-ionic surfactants.

The electrolytes added to this carrier solution to adjust its ionic strength were: (i) Potassium nitrate from Riedel-De Haen A.G., and (ii) Barium nitrate from Riedel-De Haen A.G.

Apparatus and Procedure

In the present work two field-flow fractionation systems were used: The sedimentation FFF system and the gravitational FFF system. Both systems have been described in detail elsewhere.[3,4,5] The dimensions of the SdFFF system were 38.4 cm × 2.35 cm × 0.0197 cm with a channel void volume of 1.68 cm^3, measured by the elution of the non-retained peak of sodium benzoate. The outside wall of the channel, which was placed 6.85 cm from the centre of rotation, was bare, polished Hastelloy C alloy, which is principally Ni (56%) with 17% Mo, 15% Cr, 5% Fe, 4% W, and traces of Mn and Si.[14]

The dimensions of the GFFF system were 50.3 × 2.0 cm × 0.028 cm, with a channel void volume of 2.80 cm^3.

Detection in both systems was accomplished by means of a Gilson model 111 Holochrome UV detector (operating at 254 nm), while a Gilson Minipuls peristaltic pump was used to pump the carrier solution and the sample into the channel. All experiments were performed at 25°C.

A defined amount (20-100 μL) of the used sample was injected with a syringe into the SdFFF and the GFFF channels. The relaxation time varied between 5 and 20 min, while the carrier flow rate varied between 35 and 180 cm^3h^{-1}. The field strength in the SdFFF system was kept constant during a defined experiment, but varied between 350 and 1800 RPM in all the experiments performed in the present work.

RESULTS AND DISCUSSION

It is known that the total potential energy of interaction *V*, between a spherical particle and the channel wall in SdFFF and GFFF is given by the relation:[3,12]

$$V = \frac{4}{3}\pi\left(\frac{d}{2}\right)^3(\rho_s - \rho)Gx + 16\varepsilon\frac{d}{2}(kT/e)^2\tanh(e\psi_1/4kT)\tanh(e\psi_2/4kT)e^{-\kappa h} - \frac{A}{6}\left[\frac{d(d+2h)}{2h(d+h)} - \ln\left(\frac{d+h}{h}\right)\right] \quad (1)$$

where *d* is the diameter of the spherical particle or the Stokes diameter for a non-spherical particle, ρ_s is the density of the particle, ρ is the density of the dispersing medium, *G* the sedimentation field strength expressed as acceleration, *x* is the coordinate position of the center of particle mass, *M* is the dielectric constant of the liquid phase, *e* is the electronic charge, ψ_1 and ψ_2 are the surface potentials of the particle and the channel wall, respectively, κ is the reciprocal double-layer thickness [$\kappa^{-1} = (MkT/2e^2I)^{1/2}$], *I* is the ionic strength of the suspending medium, *A* is the effective Hamaker constant which depends on the molecular properties not only of the particle sphere and channel wall material but also of the suspending medium, h is the gap width between the planar wall and the closest surface element of the spherical particle, k is Boltzmann's constant and T is the absolute temperature.

The first term of the right side of Equation (1) expresses the potential energy of the spherical particle under the influence of an external field, when the interactions between the particle and the channel wall are negligible. The second term of the same Equation describes the repulsive interaction between the particle and the wall, while the third term the corresponding attractive forces of the London-van der Waals type.

The concentration profile, c, near the channel wall is given by the Boltzmann expression:

$$c = c_o \exp(-V/kT) \qquad (2)$$

where the total potential energy V is given by the Equation (1).

The complex potential energy term given by Equation (1) and the associated concentration profile given by Equation (2) will depend upon a variety of properties of the particle, the suspending medium and the channel wall, including particle diameter, particle and medium densities, particle and wall surface potentials, temperature, ionic strength, and Hamaker constant. At a given SdFFF system, where the surface potential, of the wall ψ_2 is constant, the total potential energy of interaction, V, and hence the retention volume of a particle with a defined size and density in SdFFF, is a function of the surface potential of the particle, ψ_1, of the effective Hamaker constant, A, and of the ionic strength of the carrier solution, I, supposing that the medium density and the temperature are kept constant.

Ionic Strength of the Medium

As it was pointed out earlier, the ionic strength of the medium influences the reciprocal double-layer thickness and hence the repulsive interaction energy between the particle and the channel wall. The ionic strength, I, depends on both the concentration, c, and the cationic or anionic charge, z, of the indifferent electrolyte added to the carrier solution. According to the theory at high ionic strengths the attractive forces play an increasing role, eventually leading to a decrease in the retention ratio value and subsequently to an increase in the d value. In order to select the optimum values of the medium ionic strength for minimizing the interactions between the SdFFF channel wall and the colloid used, experiments were performed at different ionic strengths by using as model sample the polymethylmethacrylate (PMMA) beads with nominal diameter of 0.350 μm.

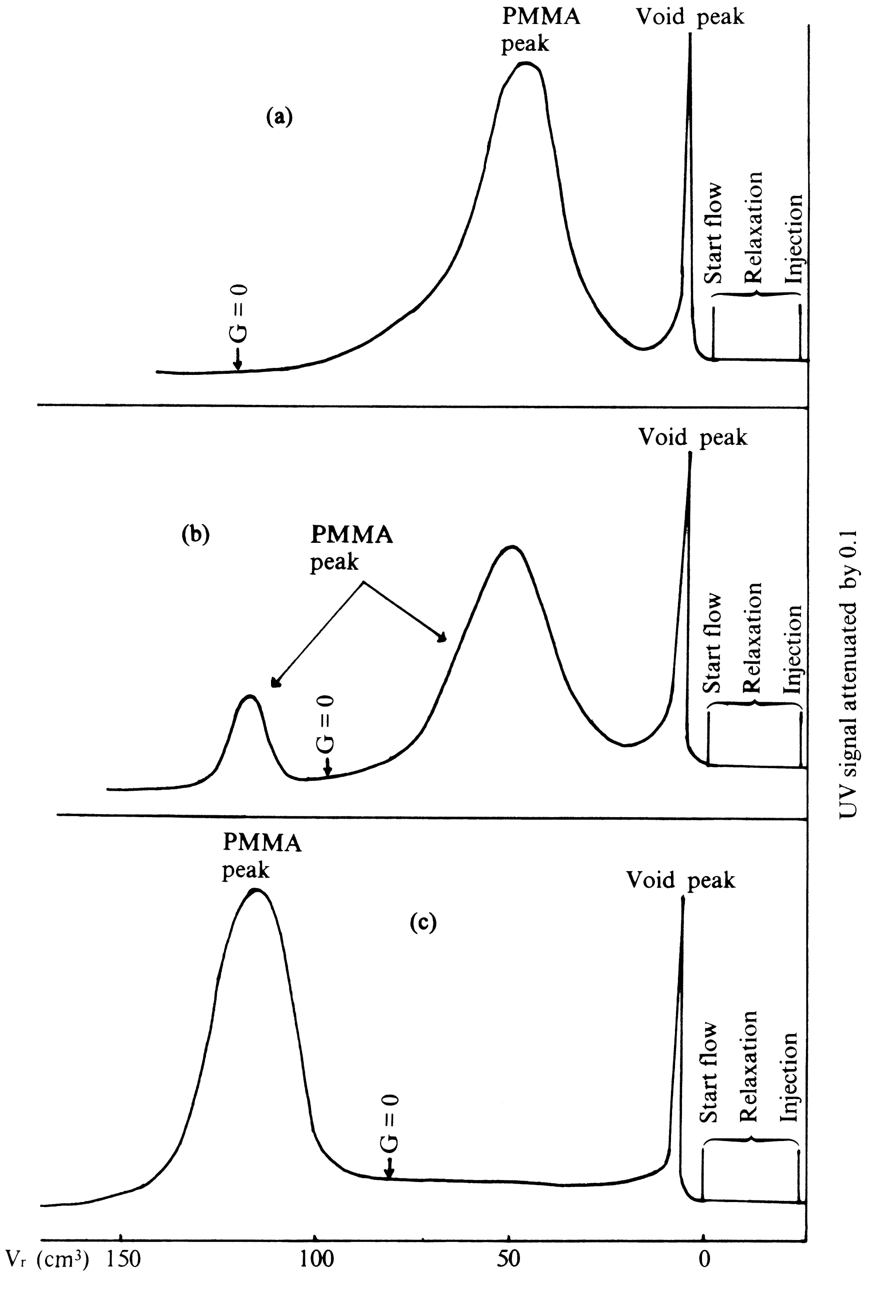
PMMA peak
Void peak
(a)
G = 0
Start flow
Relaxation
Injection
Void peak
(b)
PMMA peak
G = 0
Start flow
Relaxation
Injection
UV signal attenuated by 0.1
PMMA peak
Void peak
(c)
G = 0
Start flow
Relaxation
Injection
V_r (cm³) 150
100
50
0

Table 1

Particle Diameters of the Monodisperse PMMA Particles Obtained by SdFFF with a Carrier Solution Containing Three Different Barium Nitrate Concentrations, and Corresponding Percentages of the Adherent PMMA Sample Mass on the SdFFF Channel Wall

$C_{BA(NO_3)_2}$ (M)	d (μm)	Percentage of the Adherent Particle Mass
9×10^{-6}	0.369	0
3×10^{-5}	0.378	20
3×10^{-4}	0.360	100

While the presence of the potassium nitrate (KNO_3) elelctrolyte in the suspending medium consisted of 0.5% v/v FL-70 + 0.02% w/w NaN_3 did not influence the retention ratio of the PMMA particles in the SdFFF system, the presence of the barium nitrate [$Ba(NO_3)_2$] electrolyte in the same medium strongly influenced the interaction forces of the PMMA particles with the Hastelloy C channel. The obtained fractograms at three different barium nitrate concentrations, which are given in Figure 1, show that at the lower electrolyte concentration ($c = 9 \times 10^{-6}$M) the interactions between the PMMA particles and the channel wall are absent and no adhesion of the particles occurs at the SdFFF channel (cf. Figure 1a). On the other hand at higher electrolyte concentration, there is a limiting critical concentration [$c = 3\times10^{-4}$M $Ba(NO_3)_2$] at which adhesion of all of the PMMA particles occurs at the beginning of the column.

Variation of the carrier solution to one containing a lower electrolyte concentration [ca. 9×10^{-6}M $Ba(NO_3)_2$] released the total number of adherent PMMA particles (cf. Figure 1c). At an intermediate electrolyte concentration [ca. 3×10^{-5}M $Ba(NO_3)_2$] a partial adhesion of about 20% of the injected mass of the PMMA sample on the Hastelloy C occured (cf. Figure 1b), and the adherent particles were released by the variation of the carrier solution to one containing 9×10^{-6}M $Ba(NO_3)_2$.

Figure 1 (left). Fractograms (detector response versus retention volume V_r) of monodisperse PMMA spherical particles obtained by SdFFF. The carrier solution contains 0.5% v/v FL-70 + 0.02% w/w NaN_3 with various amounts of barium nitrate: (a) $c_{Ba(NO_3)_2} = 9\times10^{-6}\,M$; (b) $c_{Ba(NO_3)_2} = 3\times10^{-5}\,M$; (c) $c_{Ba(NO_3)_2} = 3\times10^{-4}\,M$; sample = 100 μL; flow-rate = 106 cm^3h^{-1}, relaxation time = 10 min.

While the electrolyte concentration influences the interaction forces between the PMMA particles and the column the obtained particle diameters for the PMMA particles at the three $Ba(NO_3)_2$ concentrations, which are given in Table 1, are quite consistent and in good agreement with that obtained by TEM ($d = 0.350$ μm).

Surface Potential of the Particle

The surface potential, ψ_1, of a colloidal particle is immediately related to the pH of the suspending medium. There are several equations relating both these quantities, among which, especially for oxides in water, is the following:[3]

$$\psi_1 = \frac{kT}{2.303e}\left(pH_{zpc} - pH\right) \tag{3}$$

where pH_{zpc} is the pH at which the net charge on the surface is zero.

The influence of the suspension pH on the retention ratio in SdFFF was investigated by using as model sample the PMMA with two different carrier solutions having as pH the values of 9.0 and 6.5. In the first case where pH = 9.0 no adhesion of the PMMA particles on the Hastelloy C was observed, as the whole number of injected PMMA particles was eluted from the column (cf. Figure 2a). Changing the suspension pH to a lower value of 6.5 led to a partial adhesion of about 10% of the injected PMMA mass on the channel wall (cf. Figure 2b). The above experimental findings, which are consistent with the predictions of Equation (1) show that those diameters determined by SdFFF in order to be accurate, must be selected as the proper values of the pH suspensions, so that the surface potentials of the particles will be increased. As the pH_{zpc} value varies with the nature of the colloid, the optimum value of the suspension pH, for minimizing the interactions between the colloidal particle and the channel wall, is a characteristic quantity of the colloidal material under study.

Effective Hamaker Constant

The effective Hamaker constant, A, can be varied by several ways among which the surface tension of the solution, γ, as A and γ are related by the formula:[15]

$$A \cong 2.1 \times 10^{-21}\, \gamma\ J \tag{4}$$

where γ is in mJ m^{-2}. The variation of the suspension surface tension can be easily succeeded by several ways among which is the presence or not of a surfactant in the carrier solution.

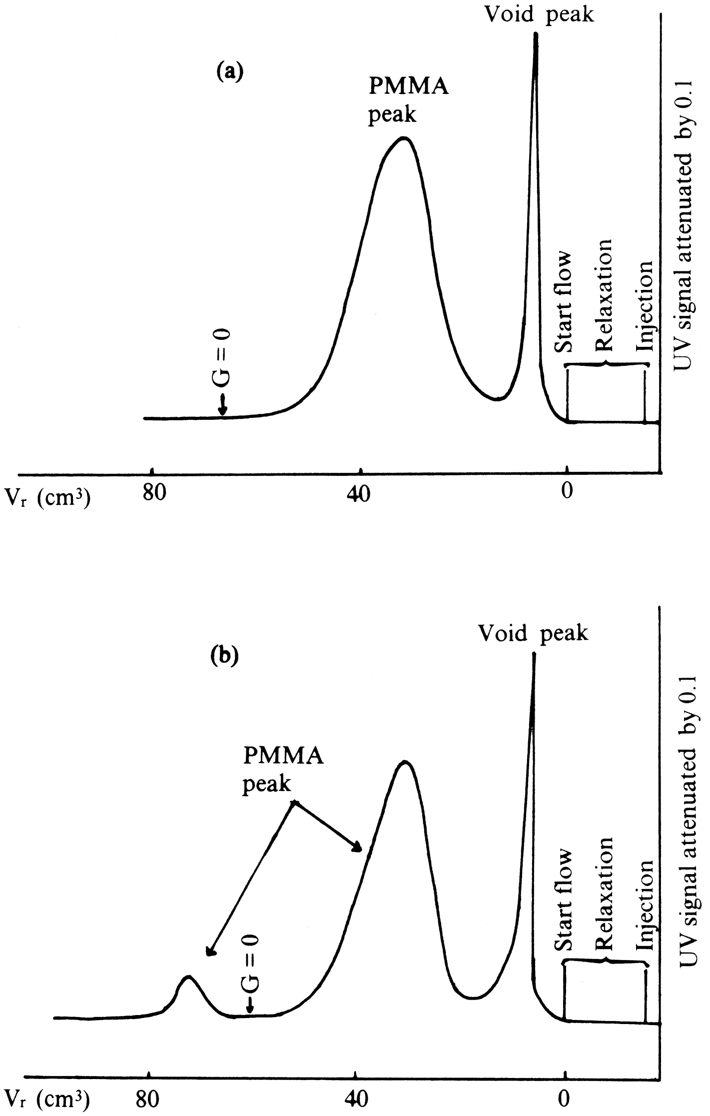

Figure 2. Fractograms of monodisperse PMMA spherical particles obtained by SdFFF by using two carrier solutions with different pH values: (a) pH = 9.0; (b) pH = 6.5.

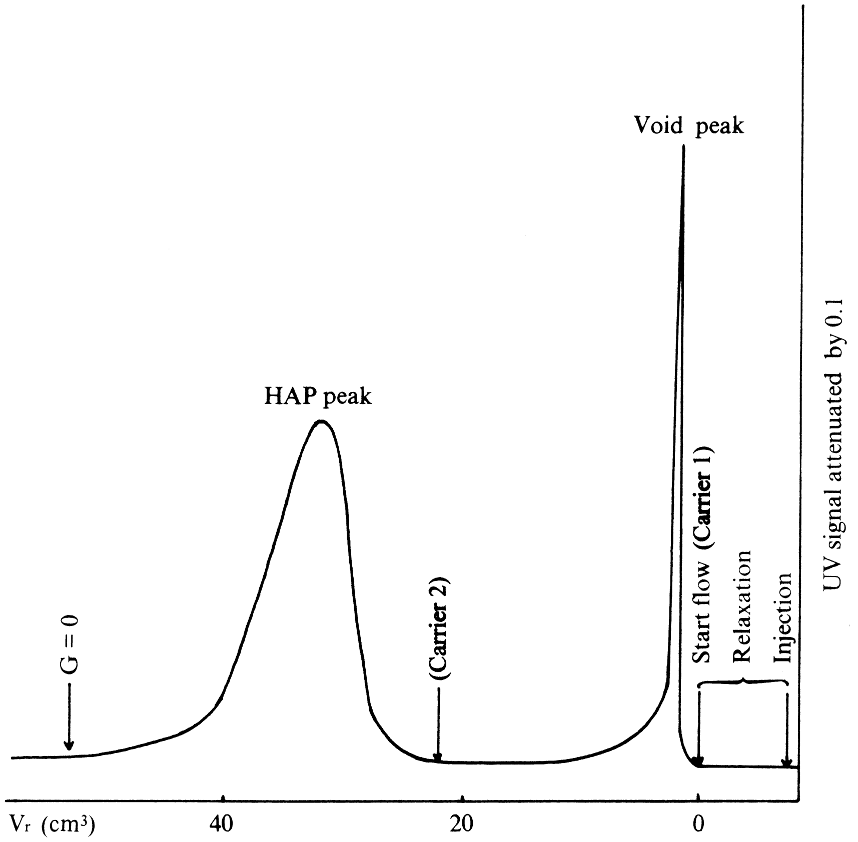

Figure 3. Fractogram of hydroxyapatite (HAP) particles obtained by SdFFF which shows the adhesion and detachment of these particles on and from the Hastelloy C surface: Carrier 1 = 10^{-3}M KNO_3 (pH = 7); carrier 2 = 0.5% v/v FL-70 + 0.02% w/w NaN_3 (pH = 9.4); sample = 100 μL; flow-rate = 140 cm^3h^{-1}; relaxation time = 10 min.

The verification of the influence of the presence of the surfactant FL-70 on the retention ratio in SdFFF was carried out by using as model sample the submicron polydisperse particles of hydroxyapatite (HAP) with nominal diameter of (0.261 ± 0.045) μm. Using as carrier solution triple distilled water with sodium azide alone, the HAP particles were totally adhered at the beginning of the SdFFF Hastelloy C wall. When the carrier solution was changed to one containing triple distilled water with 0.5% v/v detergent FL-70 and 0.02% w/w sodium azide, a sample peak appeared (cf. Figure 3) as a consequence of the release of the HAP particles. The detachment process can be attributed either to the decrease in the surface tension, due to the presence of the surfactant, which leads to a decrease in Hamaker constant, and consequently in the potential energy of attraction, or to the increase in the suspension pH (the pH varies from 7.0 to 9.4 by the addition of the surfactant) which influences the particle's surface potential. The variation in the suspension ionic strength does not seem to play any role in these adhesion/detachment processes as the ionic strength is kept approximately constant (~10^{-3}M) in both processes.

In order to show whether the effect of Hamaker constant alone is responsible for the variation of the retention ratio in FFF, experiments were performed in the GFFF system with the sulphide $Cu_{0.2}Zn_{0.8}S$ with nominal diameter of 5.46 ± 1.78 µm. Injection of a small amount (25 µL) of the sulphide into the GFFF glass channel wall with a carrier solution consisted of triple distilled water with sodium azide alone (carrier 1) gave no peak due to the total adsorption of the particles at the beginning of the channel wall. Changing the carrier solution to one containing triple distilled water with sodium adize and 0.5% v/v surfactant FL-70 (carrier 2) released all the adhered particles.

The adhesion of the sulphide particles on the glass channel wall with carrier 1 (pH=7), as well as the detachment or the same particles with carrier 2 (pH = 9.4) can be attributed to the effects of Hamaker constant (due to the presence of the surfactant), and the surface potential of the sulphide particles, which influence the potential energy of interaction between the particles and the wall. The electrophoretic mobilities of the sulphide particles were found to be almost identical in both carrier solutions and thus the effect of surface potential must be excluded. Therefore, the only factor affecting the interaction between the particles and the glass wall and, hence the retention volume of the sulphide particles in GFFF must be the Hamaker constant.

Except for the particle's surface potential, the ionic strength of the suspending medium, and the effective Hamaker constant, the sample pretreatment and overloading and the relaxation time must be investigated in order for the measurements by FFF to be accurate.

Sample Pretreatment and Overloading

There are several colloidal systems with a high degree of stability after a certain time from the preparation of the sample, the time depending on both the nature of the colloid and the dispersing medium. The variation of the retention volume, and hence of the number average diameter, with the time from the preparation of the sample was carried out in GFFF with the sulphide $Cu_{0.5}Zn_{0.5}S$, and is shown is Figure 4. The stability of the sulphide particle size at times greater than about 140 h indicates that, before use, one should agitate these particular samples for a time interval of about six days.

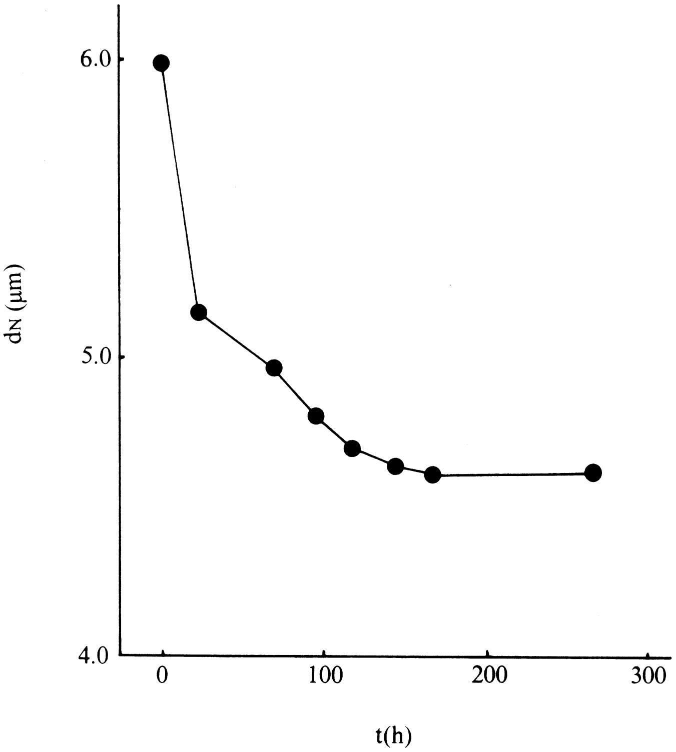

Figure 4. Variation of the number average diameter for the sample $Cu_{0.5}Zn_{0.5}S$ obtained by GFFF with the agitation time. Carrier = 0.5% v/v FL-70 + 0.02% w/w NaN_3; sample = 25 μL; flow-rate = 42 cm^3h^{-1}; relaxation time = 5 min.

A colloidal system is never thermodynamically stable, as the aggregation is a common phenomenon for both natural and industrial colloids. In case the aggregation process is a kinetic process with a significant rate, in studies of the colloidal state, the kinetics of aggregation are of paramount importance. Such kinetic studies by using SdFFF and GFFF have already been published previously.[16-18]

As sample overloading in FFF is a phenomenon that distorts the measurement of particle properties, it must be avoided or minimized. It must be pointed out that a careful balance is required because when concentrations are reduced to levels too low, detector responses may become inadequate. Finding a proper balance of factors that provides an adequate detector signal without

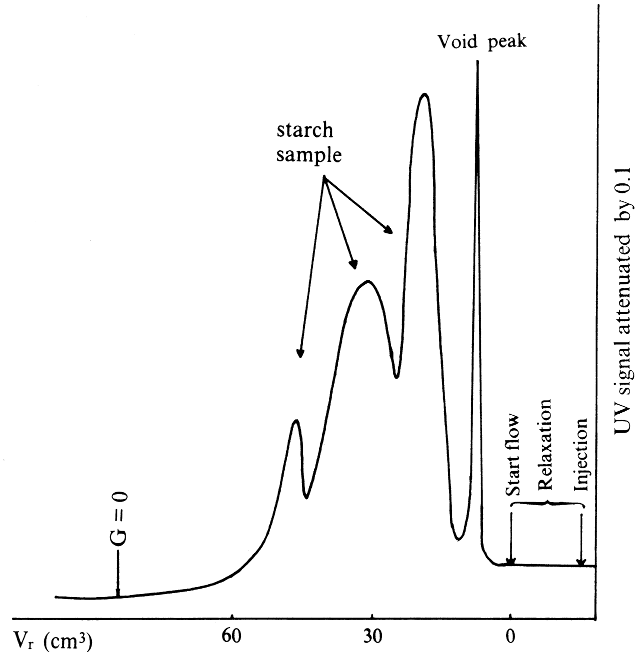

Figure 5. Fractogram of barley starch sample. Conditions are: carrier solution = triple distilled water + 0.5% v/v FL-70 + 0.02% w/w NaN_3 (pH = 9); flow-rate = 195 cm^3h^{-1}; relaxation time = 5 min; RPM = 312, T = 295 K).

observable overloading is one of the most important experimental requirements imposed on practical FFF operation. Overloading in FFF is associated with unexplained shifts in particle retention which leads to uncertainties in measured properties because concentration varies continuously along both the flow and transverse axes.

Relaxation Time

The influence of the relaxation time, t_r, on the retention ratio in SdFFF was investigated by performing experiments with barley starch granules at two different relaxation times. As Figure 5 shows a triple size distribution is obtained by SdFFF in accordance with the results found by TEM pictures. The obtained results for the three populations with mean diameters d_1, d_2, and d_3 are compiled in Table 2. As one can see from the Table the d_2 and d_3 values are independent of t_r, while the d_l value decreases as the t_r increases.

Table 2

Number Average Diameters of Barley Starch Granules d_1, d_2, and d_3, Obtained by the SdFFF Technique With Two Different Relaxation Times*

Relaxation Time (min)	d_1 (μm)	d_2 (μm)	d_3 (μm)
5	17.64	10.03	6.95
15	15.90	10.32	6.86

* Carrier flow rate = 195 cm^3h^{-1}, pH = 9, RPM =312, T = 295 K; carrier solution: triple distilled water + 0.5% v/v FL-70 + 0.02% w/w NaN_3.

As the calculated by TEM pictures d_1 values are closer to those obtained by SdFFF when t_r = 5 min, one can conclude that the relaxation time is an important parameter which must be properly selected, so that the particles form the equilibrium layer under the influence of the applied field.

As a general conclusion one could say that the optimization of the experimental conditions used in FFF, leads to the characterization of colloidal materials and macromolecules with a relatively high accuracy.

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