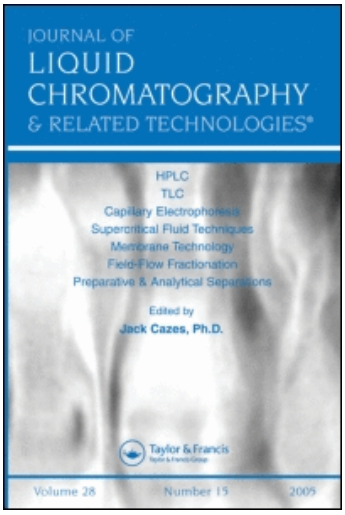

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FIELD-FLOW FRACTIONATION IN THE DETERMINATION OF RATES OF SURFACTANT ADSORPTION TO COLLOIDAL SUBSTRATES

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ABSTRACT

The biomedically interesting process of coating surfaces with poly(ethylene oxide)-containing surfactants has prompted a study of the kinetics of adsorbing Pluronic® F108 on model substrates consisting of polystyrene nanospheres. The sedimentation field-flow fractionation method (sdFFF) is found to offer an accurate and precise way to quantify the mass uptake without the need for potentially perturbing labeling reactions. The pseudo-irreversible adsorption from a 4% solution of the polymeric surfactant is found to be 80% complete within the first hour, with the remaining surface population proceeding to completion during an additional nine hour period. These findings are verified by a more conventional method based on chemical analysis. The potential problems of basing the rate analysis on measured levels of depletion, as opposed to the direct assessment of uptake provided by sdFFF, are discussed.

INTRODUCTION

Non-ionic block copolymeric surfactants of the general composition PEO_m-PPO_n-PEO_m, where PEO and PPO represent poly(ethylene oxide) and poly(propylene oxide), respectively, and m and n represent the number of monomer units in each block, have been used extensively to provide steric stabilization of colloids in suspension.[1-3] Due to their non-toxic nature, these substances have also been employed as emulsifiers and stabilizers in pharmacological preparations intended for intravenous use.[4,5] In recent years, surfactants of this type, commercially available under the trade name of Pluronic®, have been applied as biomaterial surface coatings for the purpose of reducing protein adsorption and bacterial colonization.[6-10] Once coated, the surfaces have proven to retain substantial amounts of their applied protection even under in vivo conditions, i.e., in the presence of high concentrations of electrolytes, proteins and whole cells.[11-13]

Many investigators have found the repulsive effect of these surfactants to be optimal at a molar mass of each block PEO of around 5000 Da. In our own work, the surfactant found to most effectively reduce the adsorption of the clotting protein fibrinogen, a key feature of any biomaterial, is the compound known as Pluronic F108, which is characterized by values for the m and n parameters of 129 and 56, respectively. Thus, polystyrene latex particles pre-coated with this compound have been found to adsorb two orders of magnitude less of fibrinogen per unit area from a solution in physiological saline than do their uncoated counterparts.[11]

The practical importance of these coatings has, therefore, added impetus to investigations of the rate at which surfactants of this type adsorb in a quasi-irreversible manner to hydrophobic surfaces of different chemical composition.

Rates of adsorption to a given substrate can be followed either *indirectly* by quantifying the time course of depletion of solute from a solution in contact with the substrate, or *directly* by determining the actual amounts of solute that adhere to a surface after a given exposure time. While indirect methods, as a rule, are relatively easy to carry out and typically involve the analysis of a solution by spectroscopic or analogous means after removal of the substrate, the direct methods are often more involved, as they require quantitation of the adsorbed solute in the presence of the solid substrate. However, if the substrate is to be used in the absence of its supernatant as is, for example, the case when a coated drug-release vehicle is injected intravenously, the only relevant quantity is that amount of adsorbed solute which remains on the surface after extensive wash with solute-free medium.

In the absence of some easily quantifyable indigenous chromophore, direct determinations of solute uptake are often requiring the introduction of a readable label prior to the adsorption/wash step. Although, many times, this is a viable route, there is always a concern that the labeling reaction may have altered the behavior of the solute molecule and that a determined adsorption rate, therefore, may be an artifact of the system. In order to quantify such uptakes with high accuracy, the substrate must present a large enough surface area to ensure the adsorption of measurable amounts. Whenever possible, it is, therefore, desirable to carry out the adsorption to colloidal substrates, which present a favorable surface-to-volume ratio. In the present communication, we wish to illustrate three ways in which to gain insight into the adsorption of the Pluronic F108 surfactant onto polystyrene latex particles. Two of these are of the direct type, while the third describes the indirect assessment of the amounts associated with the substrate after selected reaction times. Of these, one of the direct methods is based on a sdFFF strategy previously developed in our laboratory.[14] Results from this method, which reports mass uptake on a *per particle* basis, are compared with results from the more conventional quantitations of an introduced chromophor. All three methods are inappropriately slow for an exact analysis of the products of short reaction times, i.e., times of the order of a few seconds. For insight into this domain, the newly developed electrical FFF[15] shows great promise, as will be reported elsewhere.

METHODS

Sedimentation FFF (sdFFF) has long been known to accurately determine the mass, or size, of colloidal particles of uniform composition, while *Flow FFF* (flFFF) determines the diffusion coefficient or hydrodynamic diameter of a retained sample.[16-18] All FFF techniques, operating under "normal mode" in channels of the infinite parallel plate geometry, share a common relationship between the observed retention ratio, R, and the reduced layer thickness λ of the solute cloud that forms when an injected sample is allowed to equilibrate under the influence of an externally applied field:

$$R = V^{\circ}/V_r = 6\lambda[\coth(1/2\lambda) - 2\lambda] \quad (1)$$

Here, V° and V_r represent the channel's void volume and the observed retention volume, respectively. The reduced layer thickness λ is inversely related to both the channel thickness w and the magnitude of the applied field. For sdFFF under a gravitational acceleration G, a particle of mass m, size d,

density ρ_p and a density difference with respect to the suspension medium amounting to $\Delta\rho$, parameter λ_{sd} has the following forms:

$$\lambda_{sd} = kT/\ m(\Delta\rho/\rho_p)Gw \tag{2a}$$

$$\lambda_{sd} = 6kT/d^3\pi\Delta\rho Gw \tag{2b}$$

The latter expression only holds for particles of uniform density. In the case of core-shell particles, or adsorption complexes such as the ones of interest here, the size depends entirely on the surface arrangement of the adsorbed molecule which, in all likelihood, is unknown. Also equation 2a needs to be modified in work with adsorption complexes, to account for the different densities which characterize the core (ρ_c) and the shell (ρ_s), respectively. In a suspension medium of density ρ_m, the expression for the reduced layer thickness is as follows:[14,19]

$$\lambda_{sd} = kT/\{m_c(1-\rho_m/\rho_c) + m_s(1-\rho_m/\rho_s\} \tag{3}$$

with m_c and m_s representing the mass of the core particle and the shell, respectively. The mass of the core, or bare, particle can be determined in a separate analysis. From this analysis, one also arrives at a value for the diameter d, by applying eqs. 1 and 2b. This value, in turn, yields the area per particle, πd^2; by dividing the shell-mass per particle, m_s, with this area one directly determines the surface concentration of adsorbed material. It should be stressed that this determination avoids the otherwise common errors associated with assessing the amount of surface area (particle concentration) in contact with a given aliquot of solute during the adsorption experiment.

In the case of flFFF, the separation chamber has semi-permeable walls which allow the application of a pressure differential across the channel, as well as the one applied along the length dimension of the channel which drives the carrier fluid from injection port to channel exit. This transverse pressure drop results in a cross-flow, V_c, of carrier medium which transports injected particles to the accumulation wall. The resulting diffuse particle cloud has a reduced layer thickness, λ_{fl} whose magnitude depends on the sample's diffusion coefficient, D, in addition to the applied cross-flow:

$$\lambda_{fl} = DV^o/V_cw^2 = kTV^o/3\pi\eta w^2V_cd \tag{4}$$

In retention analyses using either method, the zones will become increasingly broader with increasing channel flow rate. However, this broadening will decrease with increased retention of the sample, i.e., with

increased field strength. In FFF, as in other zonal separation methods, the separation efficiency is a function of the zone broadening suffered during the separation process. The customary measure of this broadening is the Plate Height, H, which is defined as the generation of zonal variance per unit distance migrated.[20] In FFF, the plate height is normally considered to be composed of the following terms:[21]

$$H = H_n + H_p + H_i \tag{5}$$

where the polydispersity contribution H_p is kept near zero in work with uniform standard particles, the instrumental band broadening H_i is kept minimal with a careful system design and good operating procedures, while the non-equilibrium contribution H_n dominates the process. The magnitude of this term depends strongly on the level of retention expressed by λ and less so on the linear flow velocity $\langle v \rangle$, the thickness w of the FFF channel, and the diffusion coefficient D of the sample. At high retention, i.e., low values of parameter λ, this plate height contribution is well approximated by:[21]

$$H_n = 24\,\lambda^3 w^2 \langle v \rangle / D \tag{6}$$

The resolution of particles of different mass increases with increasing field strength and decreases with increased flow velocity. An optimal separation program for a kinetics analysis, such as the one of interest here, will seek to accommodate a maximum number of analyses with high mass resolution in a given period of time. In seeking such an optimum, it is important to recall the relationship between resolution (Rs) on the one hand and selectivity (retention difference) and efficiency (plate height) postulated by Giddings:[22]

$$Rs = \Delta t / 4\sigma_t = \frac{1}{4}\, S_d\, (\Delta d/d)\, \sqrt{L / H} \tag{7}$$

Here, an Rs value of unity or above symbolizes complete resolution, implying that the difference Δt in elution time between two components of different diameters exceeds four standard deviations in peak width (time units). The right hand expression in eq. 7 casts Rs in terms of the size selectivity, S_d of the separation (S_d = 3 for sdFFF at high retention), the relative size difference between the two components ($\Delta d/d$), and the square root of the ratio between column length L and plate height H. Inserting the expression for the dominant plate height term of eq. 6, and grouping terms that are constant for a given system and sample pair, makes clear the impact on resolution of the experimental variables field strength (G) and average flow velocity ($\langle v \rangle$):

$$Rs = \text{const.} \sqrt{G^3 / \langle v \rangle} \quad (8)$$

The instrumentation used for the sdFFF analysis has been described in principle elsewhere.[23] The present "column" consists of two highly polished pieces of Hastelloy metal, sandwiched around a stainless steel spacer which specifies the channel dimensions as follows: thickness w = 254 μm, breadth b = 2 cm, and length L = 94 cm. The ends are tapered with 60 degree angles at each apex, and the void volume V°, determined experimentally from injections of acetone and corrected for dead volume, is 4.83 mL.

The column is coiled to fit inside a rotor basket whose dimensions are such that the channel itself is positioned 15.5 cm from the axis of rotation. Hence, the spin rate of the centrifuge in revolutions per minute (RPM) is converted into channel gravitational acceleration, G, in the following manner:

$$G = [(1/60)\text{xRPMx}2\pi]^2 \text{ x } 15.5 \text{ cm s}^{-2} \quad (9)$$

Sample injections of 1-4 mL were made directly onto the channel while at stand still. Immediately following injection, the channel flow was stopped and the centrifuge accelerated to its set spin rate where the sample was allowed to relax into its equilibrium distribution (typically 16-25 minutes) before the flow was reinitiated.

The flFFF measurements, in turn, were carried out in a Universal Fractionator from FFFractionation, Inc. (Salt Lake City, UT); the dimensions of its flow channel are: thickness w = 184 μm, breadth b = 2 cm, and length L = 39 cm, for a void volume of 1.30 mL. The actual channel thickness is less than that of the PTFE spacer (254 μm) due to a compression of the YM10 membrane (Amicon) which constitutes the accumulation wall of this channel. The "field," in this case, was a cross-flow of 1 mL/min, while the longitudinal flow was 4 mL/min. As in the case of the sedimentation analog, sample injections were made directly into the channel with a microsyringe. In both cases, effluents were monitored by means of a Linear UV-106 detector.

Photon Correlation Spectroscopy (PCS) was performed on a Brookhaven Model BI-200 instrument with a 72 channel BI-2030 correlator, essentially as described by Weiner.[24] In this manner, the diffusion coefficients of the particulate samples were determined, either by a direct exponential fit of the correlation function or by a polynomial fit whereby the second moment value was collected. In both cases, the diffusion coefficients were converted to the

corresponding hydrodynamic particle diameters by application of the Stokes-Einstein relationship. Each sample was measured 10 times to provide the desired confidence in the measurement.

Adsorption Kinetics by Chemical Assessment was carried out to verify the sdFFF analysis.[14] In this approach, the 261 nm PS latex was allowed to adsorb an end-group labeled Pluronic F108.[25] Previous studies had indicated that the labeled and unlabeled surfactants adsorb to PS latex to the same degree, and that the labeling reaction, therefore, had left the system unperturbed. The label consisted of a pyridyl disulfide group that was easily cleaved off by reduction with 25 mM dithiothreitol (DTT). The released thiopyridone was readily quantified spectrophotometrically from its absorbance at 343 nm. Its extinction coefficient at this wavelength is 8080 $M^{-1}cm^{-1}$.

For the analysis, several 0.4 mL aliquots of 10% PS 261 nm (unwashed) were incubated in solutions of Pluronic F108 in phosphate buffered saline (PBS - pH 7.4, I = 0.15 M). The concentrations at the start of adsorption were: surfactant = 4%, PS latex = 2.5%. These aliquots were rotated end-over-end at room temperature for various lengths of time (5 min, 30 min, 1 h, 2 h, 5 h, 10 h, and 24 h). A Millipore filter was then used to separate the supernatant from the particles. The removed supernatants were examined for their residual concentrations of labeled Pluronic, as described above, while the coated particles were extensively washed on this filter using PBS. After a final suspension in 1 mL of this buffer, reductive cleavage for 30 minutes, and a centrifugation step (14,000 RPM in an Eppendorf Centrifuge for 20 minutes), the released thiopyridone was quantified by a Lambda-6 spectrophotometer from Perkin-Elmer. The remaining PS particles were dried and weighed for estimation of their surface area, and the surface concentration at each time point was finally computed.

MATERIALS

The polystyrene latex particles were supplied by Seradyn, Inc. in suspensions whose concentrations were 10% by weight. They were coated without prior washings. These particles had a density of 1.05 g mL^{-1}.

The bare particles were sized in aqueous solutions of the FL-70 detergent (0.1 %) from Fisher Scientific. For purposes of evaluating particle size and mass by the various analytical techniques, these solutions were assumed to have the same properties as water, i.e., a density of 1.00 g mL^{-1} and a viscosity of 0.89 cp at the ambient temperature of 25 °C.

The Pluronic F108 surfactant (molar mass 14,600 Da) was a generous gift from the BASF Corporation; the density of this (unhydrated) surfactant is 1.16 g mL^{-1}. The pyridyl disulfide activated analog (F108-PDS) was prepared in our laboratory, exactly as described in ref. (25). For each prepared batch, the degree of substitution (average value 86 mole% PDS per mole F108) was determined spectrophotometrically after reductive cleavage with DTT from the Boid Co.

RESULTS

Validation of the Sizing Technique

Our ability to use the protocol described earlier for sdFFF determination of amounts of adsorbed surfactant[14] requires that instrument parameters such as void volume (V^0) and channel thickness (w) are well characterized. Technique validation is therefore essential, and is best carried out as a comparison of sizes determined by several different methods. Table 1 summarizes the sizes determined for five different latex particles (uncoated) using the sdFFF, flFFF, and PCS techniques. The agreement between techniques is seen to be good, and the precision in the data set as a whole is acceptable.

Selection of Optimal Conditions for sdFFF

As discussed in the Methods section, the ability to determine slight shifts in particle size or mass requires careful selection of operating conditions, such that the resolution offered by the system is high. In FFF, a high resolution is usually achieved by operating at high field strength and slow channel flow, as indicated by eq. 8. This, however, implies long analysis times that are particularly impractical for a kinetic study, such as the present one, in which multiple samples need to be processed within a limited time. Somewhat arbitrarily, it was decided to aim for a run time of around 30 minutes. With a 25 minutes' relaxation time, this meant that one sample could be analyzed per hour. In order to build up the rate curve, several consecutive incubations had to be initiated in order to generate the necessary number of points to describe the time evolution of the adsorption process.

The substrate to be used in the adsorption reaction was a PS latex with a nominal diameter of 261 nm. As this particle took up the Pluronic F108 surfactant, its mass did increase to cause a shift in the sdFFF elution pattern in the direction of higher retention, i.e., longer retention times. At maximum

Table 1

Comparison of Particle Sizing Measurements for Polystyrene Particles by Different Methods

Nominal	SdFFF	FlFFF	PCS
212	219.0±1.7	214.0±1.0	216.0±6.0
261	260.0±0.5	257.0±10.0	261.0±3.2
272	269.0±3.5	271.0±8.0	270.0±5.4
314	333.0±1.4	336.0±7.0	335.0±13.3
394	389.0±3.0	394.0±18.0	397.0±18.7

Each sample was injected 3 times in FFF measurement and 10 times in PCS measurement, respectively.

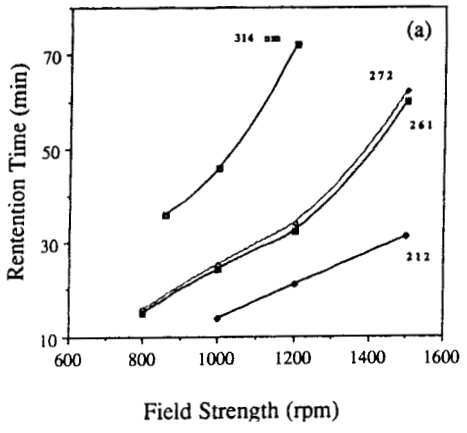
surface coverage, the new mass may well be simulated by a 272 nm latex. Therefore, a series of sample separations was carried out to identify suitable conditions for differentiating PS 261 from PS 272 in a time frame of 30 minutes. The results of this study, compiled as Figures 1a-c, led to the selection of a flow rate of 2.0 mL min^{-1} ($\langle v \rangle$ = 0.656 cm s^{-1}) and a spin rate of 1200 RPM.

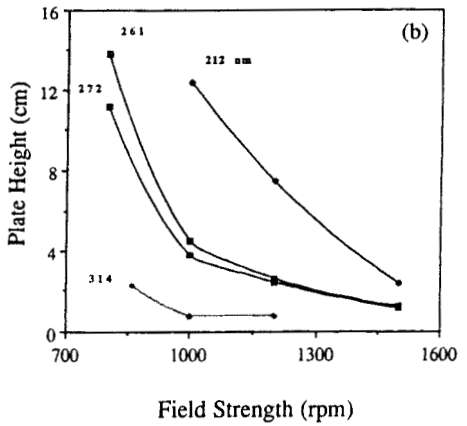
Rate Analyses

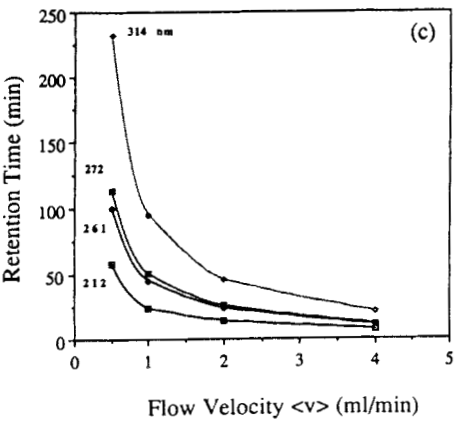
Under the selected conditions, it was possible to differentiate between different levels of uptake, as seen in Figure 2. Particles subjected to adsorption times ranging from 5 min to 24 h were sampled and analyzed. Since the injected samples were still surrounded by their surfactant-containing supernatant during the stop-flow relaxation, the short reaction times are uncertain. Once the entire data set was collected, the determined surface concentrations G were fitted to an expression of the form:

$$d\Gamma_t/dt = k_s (\Gamma_\infty - \Gamma_t)^q, \quad (10)$$

where Γ_∞ and Γ_t are the surface concentrations at infinite time and time t, respectively, q is the apparent order of the adsorption reaction, and k_s is the rate constant associated with the reaction, according to the sdFFF experiment. As seen in Figure 3, the latter two parameters are found to be: q = 3.32 and k_s = 0.611.







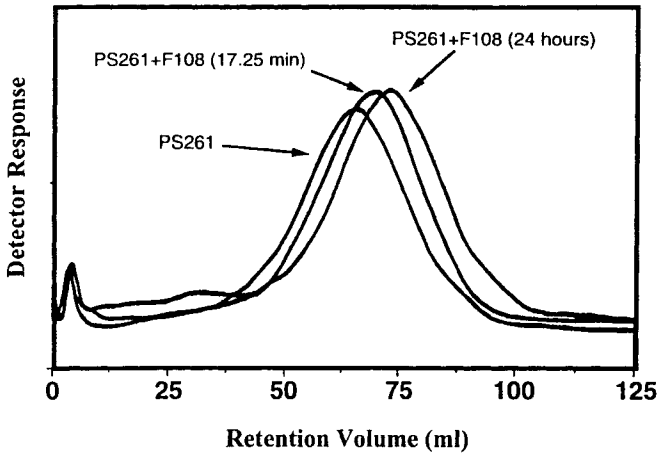


Figure 2. Comparison of fractograms of bare and surfactant coated particles. The shift of the peaks of coated particles to larger retention volumes is due to the increase in mass caused by the surfactant adsorption. Experimental conditions: flow = 2.0 mL/min, field = 1200rpm, relaxation time = 16 minutes.

In order to verify the accuracy of this analysis, the adsorption kinetics was also followed by an alternate direct method, in this case based on quantitation of an introduced, spectroscopically identifiable label, as described in the Methods section above.

The rate curve determined by this approach is likewise included in Figure 3 and is labeled "Chemical." The two parameters q and k_c, identifying order and rate constant, respectively, are for this curve: q = 3.68 and k_c = 0.635, in remarkably good agreement with the characteristics determined by sdFFF.

The labeled Pluronic F108 did also serve as a suitable marker for an indirect analysis of the adsorption kinetics. In this case, the uptake of surfactant by the particles was determined from the reduction in supernatant concentration with time.

Figure 1 (left). Optimization of Resolution in SdFFF: (a) Plot of field strength versus retention time, (b) The effect of field strength on plate height, (c) Plot of velocity versus retention time (1000 rpm). Flow velocity = 2 mL/min in (a) and (b).

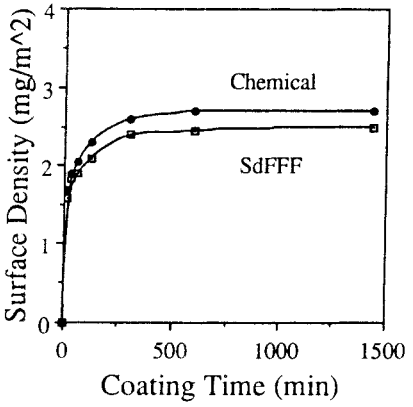


Figure 3. Adsorption rate curves recorded with the SdFFF and Chemical methods.

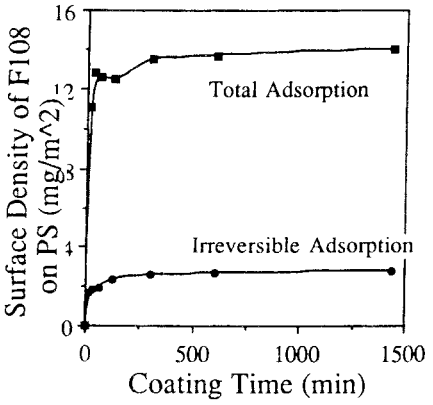


Figure 4. Adsorption kinetics for Pluronic F108 on polystyrene latex, 261 nm, determined by the indirect (depletion) chemical method.

The data show some scatter, as seen from the curve labeled "Total adsorption" in Figure 4. Nevertheless, it appears from this figure that the labeled surfactant formed a multi-layered coat on the particles, since the surface densities determined by depletion far exceeded those determined by direct assessment of the amount of surfactant that was pseudo-irreversibly adsorbed to the particles and remained on the surface even after extensive wash.

DISCUSSION

Adsorption studies, whether of equilibrium uptakes or the rates of attachment, are often done in an indirect manner, i.e., by following the depletion out of a liquid phase of the adsorbing entity. Although the resulting isotherm or rate process may be correctly characterized, the developed adsorption complex may well have little resemblance with a modified surface that is practically useful. This is due to the fact that the modified surface, as a rule, is to serve in an environment free from soluble adsorbate, as is the case when sterically stabilized and drug loaded liposomes are introduced into the human circulatory system, or when antibody coated latex particles are utilized as agglutinating indicators in immunodiagnostic products. For the latter type of complexes, the only relevant parameter is the amount of adsorbate that remains associated with the solid substrate under practical working conditions.

Direct determinations of adsorbed solutes are often difficult to carry out due to a lack of an indigenous label that can be assayed reproducibly, even in the presence of the substrate to which it is adsorbed. However, the deliberate introduction of an extraneous label can, at times, so perturb the behavior of the solute that such physical characteristics as binding and rate constants have little in common with their counterparts which describe the behavior of the unadulterated solute. It is therefore often a desirable goal to be able to perform direct characterizations of adsorption complexes without the need for labeling. The use of sedimentation field-flow fractionation to determine mass uptakes on colloidal substrates may consequently be of significant value.

The adsorption of PEO containing block copolymers to surfaces, for the purpose of suppressing the adhesion of biological macromolecules or particles, has considerable practical appeal. For this reason, as well as for the purpose of gaining basic scientific knowledge, we have set out to investigate the kinetics of forming pseudo-irreversible adsorption complexes between the potent adhesion suppressor Pluronic F108 and surfaces of hydrophobic composition, exemplified by colloidal latex standards of polystyrene.

In this work, the variance in the sdFFF size measurement is significantly less than 1%, implying that the variance in the corresponding mass analysis is well below 3%. Since the problem at hand is to determine subtle differences in particle mass, come about as a result of the adsorption of surfactant, it is relevant to note that the difference in buoyant mass between a bare and a fully Pluronic F108-coated PS particle with a core diameter of 261 nm, as used here, is 16% or in comfortable excess of the random error in the measurement.

Due to the need for relaxation in the sdFFF analysis, and for an exhaustive wash step in case of the "chemical" analog, both of which require around 20 minutes, the early time points are highly uncertain in each case. Yet, from Figure 3 one can unambiguously conclude that around 80% of the surface population takes place during the first hour. The remaining 20% are taken up much more slowly, presumably after extensive rearrangement of the already adsorbed molecules, and the process appears to be complete after about 10 hours.

As noted, the very early phase of the adsorption kinetics occurs much too rapidly for accurate analysis with either of the two methods described here. It has, therefore, been gratifying to discover that the electrical FFF, currently under development in our laboratories, does not require stop-flow relaxation and can, therefore, provide insight into the very early time points. Indeed, highly stable surfactant-particle complexes have shown to form as the result of adsorption times of a mere 10 s.[26] Further examination of the early phase of the adsorption process is under way and will be the subject of a subsequent communication.

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REFERENCES

1. T. F. Tadros, B. Vincent, J. Phys. Chem., **84**, 1575-1580 (1980).

2. J. A. Baker, J. C. Berg, Langmuir, **4**, 1055-1061 (1988).

3. B. Kronberg, P. Stenius, Y. Thorssell, Colloids and Surfaces, **12**, 113-123 (1984).

4. S. S. Davis, L. Illum, Biomaterials, **9**, 111-117 (1988).

5. R. Jeppson, S. Ljungberg, Acta Pharm. Suecica, **10**, 129-140 (1973).

6. M. Amiji, K. Park, Biomaterials, **13**, 682-692 (1992).

7. J. H. Lee, P. Kopeckova, J. Kopecek, J. D. Andrade, Biomaterials, **11**, 455-463 (1990).

8. R. H. Müller, **Colloidal Carriers for Controlled Drug Delivery and Targeting**, CRC Press, Boston, 1991.

9. T. Blunk, D. F. Hochstrasser, J.-C. Sanchez, B. W. Müller, R. H. Müller, Electrophoresis, **14**, 1382-1387 (1993).

10. M. J. Bridgett, M. C. Davis, S. P. Denyer, Biomaterials, **13**, 411-416 (1992).

11. J.-T. Li, K. D. Caldwell, Colloids and Surfaces B: Biointerfaces, **7**, 9-12 (1996).

12. J. S. Tan, D. E. Butterfield, C. L. Voycheck, K. D. Caldwell, J.-T. Li, Biomaterials, **14**, 823-833 (1993).

13. L. Illum, L. O. Jacobsen, E. M. Müller, S. S. Davis, Biomaterials, **8**, 113-117 (1987).

14. J.-T. Li, K. D. Caldwell, Langmuir, **7**, 2034-2039 (1991).

15. K. D. Caldwell, Y.-S. Gao, Anal. Chem., **65**, 1764-1772 (1993).

16. J. C. Giddings, Science, **260**, 1456-1465 (1993).

17. J. C. Giddings, F. J. Yang, M. N. Myers, Science, 1244-1245 (1976).

18. J. C. Giddings, G. Karaiskakis, K. D. Caldwell, M. N. Myers, J. Coll. Interface Sci., **92**, 66-80 (1983).

19. R. Beckett, J. Ho, Y. Jiang, J. C. Giddings, Langmuir, **7**, 2040-2047 (1991).

20. J. C. Giddings, **Unified Separation Science**, John Wiley & Sons, Inc., New York, 1991.

21. G. Karaiskakis, M. N. Myers, K. D. Caldwell, J. C. Giddings, Anal. Chem., **53**, 1314-1317 (1981).

22. J. C. Giddings, Pure & Appl. Chem., **51**, 1459-1471 (1979).

23. J. C. Giddings, M. N. Myers, K. D. Caldwell, S. R. Fisher, "Analysis of Biological Macromolecules and Particles," in **Methods of Biochemical Analysis**, D. Glick, ed., John Wiley & Son, Inc., New York, 1980, pp. 79-136.

24. B. B. Weiner, in **Modern Methods of Particle Size Analysis**, H. G. Barth, ed., John Wiley & Sons, Inc., New York, 1984, pp. 93-116.

25. J.-T. Li, J. Carlsson, J.-N. Lin, K. D. Caldwell, Bioconjugate Chem., 7, 592-599 (1996).

26. Q. Chen, M. S. Thesis, University of Utah, Salt Lake City, UT, 1996.

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