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Experimental verification of the steric-entropic mode of retention in centrifugal field-flow fractionation using illite clay plates

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ABSTRACT

The commonly used theory to describe the normal Brownian mode of field-flow fractionation (FFF) assumes the particles to be point masses and hence the shape is ignored. Beckett and Giddings extended this theory to include the effect of thin rods and discs being forced very close to the accumulation wall. By including the decrease in the entropy this causes, they derived new expressions for the retention of such nonspherical particles in FFF. The steric-entropic theory predicts that when the sample cloud thickness is less than the major dimension of the rods or discs then particles elute earlier than predicted by the Brownian mode theory. This leads to an underestimation of the buoyant mass and equivalent spherical diameter calculated from FFF data. In this paper we report for the first time experimental data for the retention of thin illite particles in centrifugal FFF that agrees well with these steric-entropic predictions. Not only do the size distributions calculated using the Brownian mode theory shift to lower size when the field is increased but the shift in the retention ratio of the peak maxima of the FFF fractograms could be predicted fairly accurately by the steric-entropic equations.

1. Introduction

Field-flow fractionation (FFF) is now an established separation method with high selectivity and fractionating power [1]. It is particularly useful for the separation and characterization of large macromolecules down to a diameter of about 1 nm and small particles up to about 50 μm where many other techniques experience difficulties.

In 1966 the FFF concept in thin flow channels was introduced by J Calvin Giddings [2]. This initial paper outlined what has become known as the normal mode of retention, although the term Brownian mode is probably a more appropriate description. The concept was soon validated first using a thermal field to separate dissolved polymer molecules [3] and later latex particle suspensions using centrifugal settling [4]. Berg and Purcell [5] independently demonstrated the separation of *E. coli* bacteria using a thin-channel centrifuge device. However, It was the Giddings' group at the University of Utah that dominated the development of FFF over the next 20 years or so.

In the Brownian mode of retention the sample is introduced into a thin channel (typically 100–500 μm thick and 0.5–4 cm wide) and a field is applied across the thin dimension so that sample particles or molecules are forced towards the accumulation wall. For small particles (e.g. <1 μm) Brownian diffusion sets up a counter flux opposing the field flux and an equilibrium cloud of particles is established. When the channel flow is turned on, the sample cloud is swept along the channel at a velocity approximately equal to that of the fluid velocity vector at the center of mass of the sample cloud. Since this is purely a physical process, unlike adsorption chromatography, a theory could be derived that successfully predicted the retention time of eluted components. A major assumption in this derivation was that the particles were point masses, in that they occupied negligible volume in the sample cloud, and could approach infinitely close to the accumulation wall [6]. Of course the particles are still assumed to have a finite size in terms of their Brownian diffusion away from the accumulation wall due to the concentration gradient that is established in the cloud. This results in sample components forming an equilibrium cloud near the accumulation wall which has a concentration profile which is greatest at the wall and decreases exponentially with distance from the wall (see Fig. 1a). Samples with the thickest cloud will be pushed along by the parabolic flow profile faster than components with thinner clouds. Thus for colloidal particles less than about 1 μm in diame-

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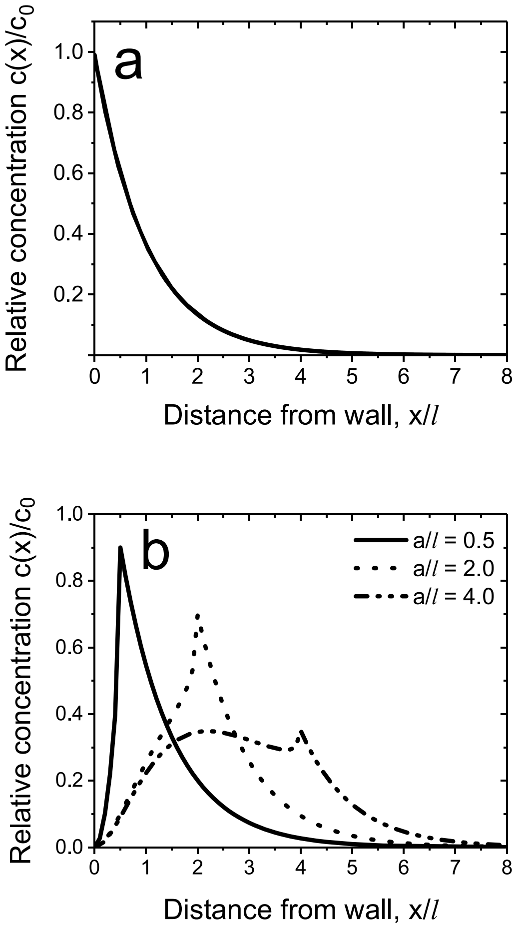

Fig. 1. Sample cloud concentration profiles ($c(x)$ versus x/l) for (a) Brownian mode (point mass) (Eq. (1)) and (b) steric-entropic mode for discs with several a/l values (Eq. (11)).

ter small particles usually elute before larger ones according to the Brownian mode theory.

Although this Brownian mode retention theory has proven to be very robust several perturbations have been introduced to deal with effects such as concentration overloading of the sample cloud [7], particle wall repulsion effects [8], wall exclusion of particles very close to the wall [9] and secondary relaxation in field programmed runs [10,11]. The most significant of these modifications occurs when the sample cloud is pressed very close to the wall [12] and the particle center of mass is prevented from approaching the wall by at least one particle radius [13]. This eventually results in a reversal of the elution order when larger micron sized particles are used and referred to as the steric mode of FFF. More detailed analysis of this retention mode showed that hydrodynamic lift forces contribute to push particles away from the accumulation wall resulting in a dependence of the retention time on the channel fluid flow rate, which could not be explained by the simple steric concept [14]. Thus we now prefer to label this the lift-hyperlayer mode of FFF separation. The simplified criteria for the lift-hyperlayer mode to operate is when the particle radius (a) is greater than the mean cloud thickness calculated for point mass particles (l), although there is a transition region where both FFF mechanisms contribute.

The point mass theory for the Brownian mode of FFF can often be used with good accuracy as submicron particles are usually much smaller than the sample cloud thickness, hence the physical exclusion effect is negligible. Naturally the theory is most simple if the particles are assumed to be spherical and simple equations apply

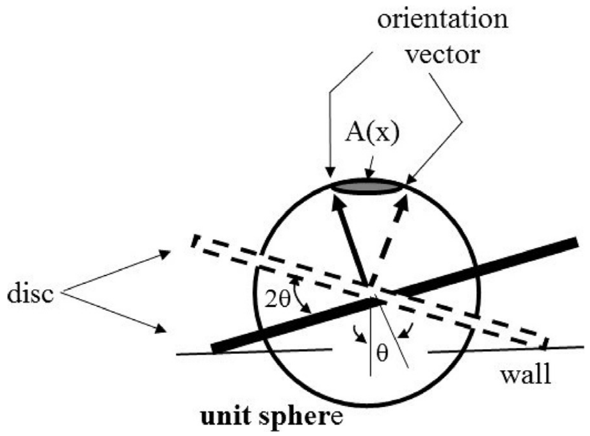

Fig. 2. Schematic diagram of a disc close to the accumulation wall. The orientation vector defines the particle orientation and the area on the surface of the unit sphere (A(x)) gives an indication of the relative freedom of the particles to move.

with regard to frictional drag and diffusion. The equations can be modified provided the relationship between the friction coefficient and particle shape is known. In any case FFF results are usually reported as equivalent spherical values. Thus in the case of diameter being determined by flow FFF it will be the diameter of a sphere that has the same diffusion coefficient as the actual nonspherical particles. However, for Centrifugal FFF (CeFFF) it is the diameter of a sphere with the same buoyant mass.

In 1997 Beckett and Giddings [15] introduced a third mode of FFF, referred to as the steric-entropic mechanism, which is applicable for particles deviating greatly from a spherical shape and are pushed very close to the accumulation wall. They considered the case of long rods and thin discs and derived expressions to account for deviations to the Brownian theory applicable to spheres. Previously it was assumed that as long as $a_{es} < l$ (where a_{es} is the equivalent spherical radius) then the point mass Brownian mode equations could be used. However, when particles have one major physical dimension that is greater than the sample cloud thickness (l) then they spend a considerable amount of time in a situation where they are restricted in their normal rotation. Note the cloud thickness l here refers to the value calculated for the exponential concentration distribution of point masses and not the actual distribution of the discs. Fig. 2 represents this situation for circular disc particles where their center of mass is closer than the radius of the disc. This restriction in the motion of the disc causes them to have a lower entropy and contributes to an push away from the wall. This would be expected to increase the thickness of the cloud and result in earlier elution times and is the basis of the steric-entropic effect.

In this paper we outline an experimental procedure to measure the contribution of steric-entropic effect for disc-shaped clay particles. The experimental evidence presented in this paper is the first set of data that validates the existence of the steric-entropic effect in FFF. Thin platey clay samples with a fairly narrow size distribution (a_{es} based on centrifugal settling measurements was about 100–200 nm) were separated by CeFFF using different field strengths. In addition the standard deviation of the particle size distribution obtained from the CeFFF fractogram at 300 rpm was only 0.12. Particles from the peak maximum were collected and the plate area and the equivalent circular plate radius based on the plate area (a_{ec}) were determined by scanning electron microscopy (SEM). Increasing the field strength decreases the cloud thickness and this should increase the contribution of the steric-entropic effect and hence any deviation from the elution time predicted from the point mass Brownian mode theory should be accentuated.

This testing of the steric-entropic theory was made possible by the availability of relatively monodisperse plate shaped particles.