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Discoveries Concerning the Transport of Colloids and New Forms of Chromatography

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Matter of colloidal dimensions takes many forms and impinges in countless ways on our daily lives, so it is not surprising that knowing the size of colloidal materials is fundamental to properly understanding their behavior or successfully adapting them to their manifold uses. This became of immediate concern to me several years ago when I was asked to develop an alternative to the then existing methods for determining the size of a commercial polymer latex whose particles were around 1 μm in diameter. At that time electron microscopy and various light-scattering procedures were the principal techniques for particle size measurement, and each method had its limitations. Microscopy was accurate, but it was slow and required complex instrumentation and skilled operators to make the measurements. Light-scattering techniques of that time were very fast and used relatively inexpensive instruments but often gave meaningless results if the colloid was polydisperse, and many of the materials of interest were just that.

It was in response to this challenge that I considered extending chromatography beyond the molecular region and into this supermolecular or colloidal region. The result of that research was a technique that I called hydrodynamic chromatography (HDC).[1] Though it is conventional from an operating point of view, HDC is unusual in at least a couple of ways. In the first place it breaks with one of the often cited prerequisites for successful chromatography, namely, that the species of analytical interest be soluble in the mobile phase. The "solutes" of HDC are in many cases quite insoluble in the mobile phase; they are in fact in suspension and are often large enough to be visible in a light microscope.

But it is in the manner of its separation and how the stationary phase participates that HDC is most unusual. The heart of the HDC device is a column packed with solid spherical particles whose interior is usually inaccessible to the colloidal "solutes" being separated. As a result, any separation that does take place is brought about by phenomena operating exclusively within the space between the particles of the packing, the stationary phase acting mainly as a provider of this vital void space. This separation within a single phase, the mobile phase, has led to the assertion by some that HDC does not fit the common definitions of chromatography and is therefore a misnomer. I hope to show that a somewhat broadened definition of chromatography allows for the comfortable accommodation of HDC.

As a starting point I will describe what led to the idea that is central to the HDC invention, namely, that particles can be fractionated simply by passing them through a bed packed with solid particles. Then I will show some of the many ways in which HDC is being applied to colloid problems. To complete the Account I will show how our research illuminated the intriguing phenomena of particle transport in packed beds and exploited subtle surface chemical effects to give some remarkable separations based not only on size but also

(1) Small, H. *J. Colloid Interface Sci.* **1974**, *48*, 147.

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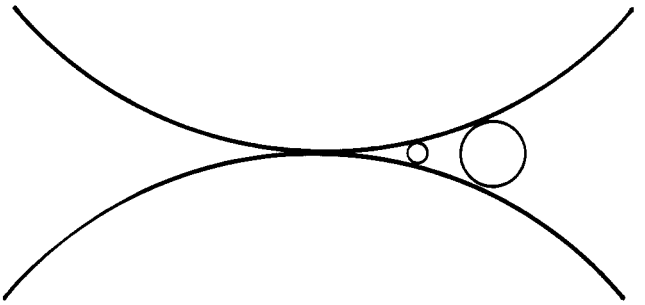

Figure 1. The "crevice" model. The region of contact between two spherical particles is more accessible to smaller particles than it is to larger particles, and it was envisioned as offering a means of separating particles.

on the chemical composition of the colloids.

How HDC Got Started

I believe it was Maxwell who said, "Theory guides, experiment decides." The evolution of HDC is an excellent example of that dictum.

Initially I considered three different approaches to separating colloidal particles chromatographically. The first would extend gel permeation chromatography (GPC) to these supermolecules by using an aqueous mobile phase along with a porous stationary phase; the greater accessibility of the internal pore space of the packing to colloids of smaller size would provide the basis for separation with the expectation that larger particles would elute ahead of smaller particles. But this approach had problems. In the first place, packings with pores that could admit 1-μm colloids—for that was the size of my immediate interest—were not available; but even given packings of the requisite porosity, I argued that the low diffusivity of colloids of this size would so impair mass transfer into and out of the packing that poor chromatographic efficiency would be an inevitable result. So this approach was abandoned.

The second approach proposed filling the column with solid, that is, nonporous, particles and relying on the crevice regions at the points of particle contact to provide the size discriminating regions as suggested in the simple model of Figure 1. If this were the operating mechanism, then larger particles could be expected to elute ahead of smaller ones since they would effectively see a smaller accessible volume.

The third proposal involved an entirely different separation mechanism. In this case I postulated that flocculation–deflocculation interactions between the colloid and the packing would delay transit of particles through the column analogously to the effect of adsorption–desorption at the molecular level. But in this case, from what was known of colloid interactions, larger particles would be expected to be more retained than smaller particles.

So with these ideas as a point of departure we attempted to separate mixtures of polystyrene latex particles of different sizes using columns filled with cation exchange resin particles which, though they were readily permeable to water and small molecules, were quite impermeable to materials of colloidal dimensions; in other words, they were solid particles from the colloid's point of view.

The first attempts used rather short columns of relatively coarse packing, and the results were discouraging in that little if any separation was observed

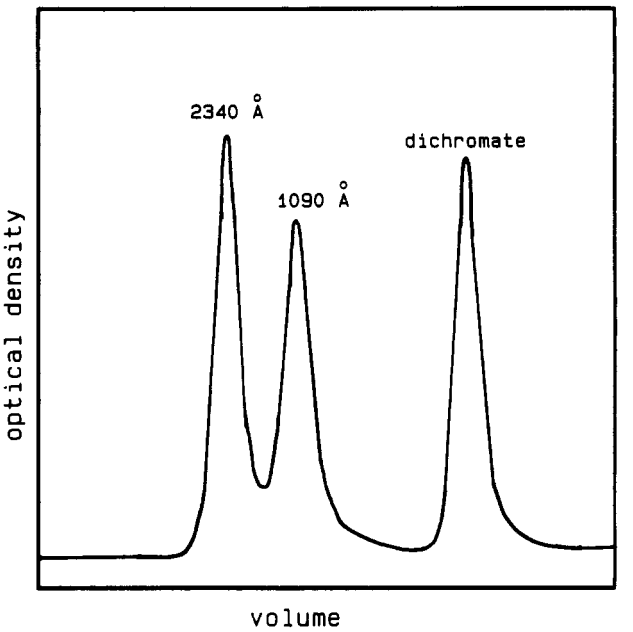

Figure 2. The separation of particles by hydrodynamic chromatography. When colloidal particles, in this case polystyrene latex particles, are eluted from packed beds of solid nonporous particles, larger particles elute ahead of smaller particles and both ahead of a small "marker" species, sodium dichromate. This separation was achieved on 3 m of column packed with cation exchange resin particles roughly 20 μm in diameter (ref 1).

even when we tried to separate a mixture of two particle sizes whose diameters differed by as much as 20-fold. We persisted with longer columns, finer packings, and lowered flow rates, and separations such as shown in Figure 2 eventually became routine. At an early stage of the research it became evident that, under the conditions chosen, large particles eluted ahead of smaller particles, giving some plausibility to the crevice region mechanism. But more on that later.

At this point it is appropriate to show how the success represented by Figure 2 led to our solving a number of challenging problems in colloid particle size analysis. First some details on a typical HDC experiment.

The hardware of HDC has much in common with conventional liquid chromatography, namely, a train of components comprising the following: a pump capable of very steady and preferably pulseless delivery at moderate pressures, a sample injection device, a column, a colloid detector (usually a spectrophotometer measuring turbidity), and various means of data collection and processing. The column, the heart of the HDC instrument, is packed with usually spherical particles; ion-exchange resins, nonfunctionalized polymer beads, and glass spheres have been used, but for a number of practical reasons the resin beads perform best. The resolving power of the packed columns is critically dependent on the size of the spherical packing and on the manner in which it is packed. Beads of 15–20 μm in diameter are commonly used: the smaller the packing, the better the separation. But a compromise must always be made between better separation and the tendency of the bed to trap particles, a feature that is aggravated as one uses smaller packings. Early HDC instruments used 3–5 m of column, but with improvements in packing procedures augmented with computerized data processing, columns as short as 0.5 m are adequate for solving many particle size problems.[2]

Although separating power diminishes proportionately to length of column, the shorter columns have the advantage over longer columns of a much lesser tendency to capture and retain colloid particles. A commonly used eluent for HDC is a dilute solution of sodium phosphate (typically 0.01 M) and an anionic or nonionic surfactant (1–2 g/L).

All HDC applications start with properly calibrated columns. Polystyrene latexes of known particle size have been extensively employed as model colloids both in exploring HDC and as a means of calibrating an HDC instrument. In a typical experiment a small marker (e.g., sodium dichromate) is injected along with the latex to act as an internal monitor of flow variations. Figure 2 is a chromatogram from a mixture of two monodisperse latexes and a simultaneously injected marker. The elution behavior of the latex for a particular packing may be characterized either by the difference in elution volume of the marker and the latex or by the R_f, which is simply the ratio of the elution rate of the latex to that of the marker. Determinations of R_f for particles of known size provide the calibration data necessary for characterizing particles of unknown size.

Applications of HDC

HDC can be applied to a variety of colloid problems, but most of my examples will be drawn from the area of polymer latexes since that is the area with which I have most experience.

Particle Size Measurement. The determination of particle size is one of the most common applications of HDC. Many polymer latexes are close to being monodisperse, and a particle size measurement is a simple matter of determining the R_f of the latex and using the calibration curve to determine the particle size. When HDC is calibrated with well-characterized standards, it is compellingly precise and accurate; an uncertainty of less than 1% in establishing the particle size of monodisperse latexes is common. On several occasions where I found differences between the diameters of latexes as determined by electron microscopy and HDC, careful remeasurement by microscopy showed the initial microscopy values to be in error.

Emulsion Polymerization Kinetics. In emulsion polymerization research and development, much work is devoted to determining the particle size of the final emulsion, its polydispersity, and the rates of particle growth. A common experiment in emulsion polymerization kinetics involves loading a reactor with two crops of small "seed" particles, catalyst, surfactant, etc., feeding the reactor with monomer(s), and observing the competitive growth rate of the two sets of particles. HDC is uniquely capable of providing this information, essentially on the fly. This is a particular boon in the routine operation of large-scale reactors especially where adjustment of feed or reaction conditions must be made at a particular stage of particle growth. The rapid response of modern HDC instruments enables almost instantaneous monitoring of particle size with concomitant benefits for reactor control.

Particle Aggregation. Interactions between latex particles can cause them to aggregate; this is sometimes inadvertent and usually undesirable while at other times it is intentional and beneficial.

In one case we needed to learn if shearing of a particular latex would cause it to aggregate. Electron microscopic examination of the latex before and after shearing was inconclusive since the unsheared latex showed aggregates which might have been a result of the method of sample preparation. Chromatograms of the sheared and unsheared latex, however, clearly showed the presence of an extra peak in the former that could be attributed to aggregates. In the unsheared latex this peak was absent.[1]

In another instance the chromatogram of a supposedly monodisperse latex showed pronounced skewing toward higher particle size, indicating that aggregates were present. When a suspension of the latex was "ultrasonicated", the skewing disappeared, giving the chromatogram that was characteristic of a monodisperse latex and leading us to the conclusion that aggregates were present and that they were dispersed by the ultrasonic treatment. These examples illustrate how in some cases one can reach important qualitative conclusions through HDC simply by examining a chromatogram without resorting to a complete particle size analysis.

There are a number of latex-based products wherein controlled aggregation of the latex component is a desirable and in some cases necessary condition for it to function properly. Thixotropic, aqueous-based paints that are "thick" and dripless on the applicator but freeflowing when brushed or rolled is one such example. Water-soluble polymers are often added as a component of the paint to impart this desirable rheology to the final product. It is believed that they perform their function by forming weak bridges between the latex particles that survive mild shearing forces, giving the thick rheology, but break down under the more extreme stress of brushing or rolling to give the necessary thin rheology. HDC offered a possible means of throwing some light on this. Chromatograms run on unmodified latexes when compared to chromatograms of latexes to which polymeric thickeners were added showed a shift to faster elution times as more thickener was added, indicating that particle bridging was indeed occurring. Furthermore, the efficacy of various polymeric thickeners correlated nicely with their effect on the R_f of the latexes to which they had been added.[3]

Swelling Effects. HDC has a unique ability to reveal particle swelling effects in polymer latexes. Swelling can be an extremely important factor since it alters the volume fraction occupied by the particles, which is a major factor in controlling rheology. When the polymer phase is of a nonpolar type such as styrene or styrene–butadiene, then the volume of the particles will not be affected by altering the pH of the aqueous environment or its ionic strength or the type or amount of surfactant it contains. On the other hand, if the latex contains a sufficient level of an ionic comonomer such as acrylic acid, then the particle size can be profoundly affected by changes in these factors. Figure 3 illustrates the observations for a series of styrene/butadiene/acrylic acid latexes of varying acid content wherein the environment was changed by altering the pH and the surfactant in the HDC eluent.[3] It is evident how HDC

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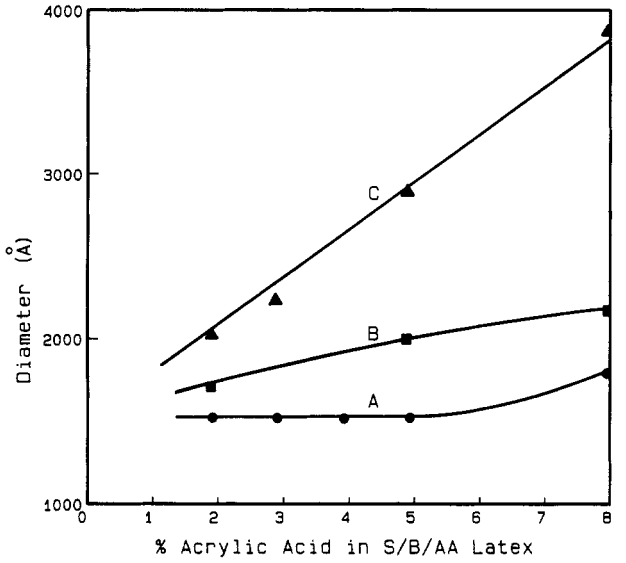

Figure 3. When polymer latexes contain a highly polar and ionizable component such as acrylic acid, then the elution behavior is markedly affected by the composition of the eluent, particularly with respect to its pH and the type of surfactant that it contains. For curves A, B, and C the eluent compositions were respectively pH 7, sodium lauryl sulfate; pH 10, sodium lauryl sulfate; and pH 10, Triton X-100 (ref 3).

revealed some profound changes in the size of the latex particles in response to changes in the aqueous environment.

Why Do Particles Separate?

While developing a means of particle size measurement was the primary goal of the research, the experiments taught us much about the transport of colloidal particles through packed beds and revealed many interesting phenomena to ponder and explain. For example, there was the most basic question, Why were the particles separating? Was the simple crevice theory the explanation, or was it more complicated than that? And what of the third proposal for particle separation, based on flocculation–deflocculation? Was the analogy with sorption–desorption naive, or were there indeed circumstances wherein such a mechanism might operate? We will now consider the mechanistic side of HDC for some answers to these intriguing questions.

In most HDC experiments the marker is constrained for various reasons to travel in the void space of the column: in the case of ion exchange resin packings, Donnan exclusion forces keep it there. So the elution rate of the marker is therefore a measure of the flow rate of the eluent through the column. An experiment such as that illustrated by Figure 2 reveals that large particles elute faster than smaller particles and that the R_f of the colloid is greater than unity, in other words, that the particles move through the bed with a higher mean velocity than the fluid carrying them. How do we accommodate these two key observations with chromatographic theory, or as some would have it, is it indeed chromatography?

Initially the crevice model (Figure 1) seemed qualitatively plausible; however, a calculation of the total volume of these tiny "donuts" of discriminating volume at the contact regions of the packing revealed it to be far too small to account for the separation obtained.

Devising an alternative theory came up against the prevailing notion that chromatography invariably requires two phases, one stationary, the other mobile. Separation takes place when the distribution of the species to be separated is unequal between these two phases. So the two absolute minimum prerequisites for chromatographic separation were, first, two contiguous phases in relative motion and, second, unequal distribution of solute between these phases. How could the separation of HDC which was taking place in a single phase, namely, the void space of the packed bed, be reconciled with these basic requirements?

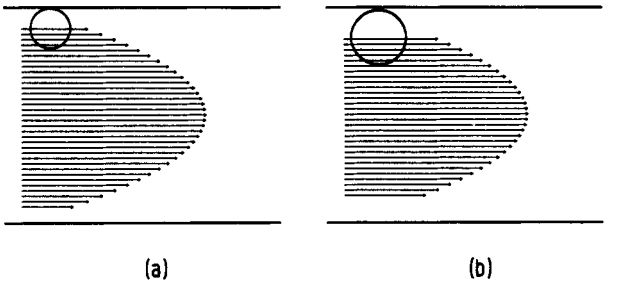

Figure 4. The capillary model of HDC. Colloidal particles are sterically prevented from enjoying the slowest velocities near the fluid wall interface. Larger particles are more excluded from this region (b) than are the smaller particles (a) and consequently move through the capillary with a higher mean velocity.

Commonly in liquid chromatography, the condition of relative motion is very obvious: one phase is stationary with respect to the column while the other, the liquid, is not. In HDC the relative motion is manifest in a more subtle manner. Because of viscous forces, the flowing eluent moves more sluggishly the closer it is to the packing–eluent interface. The liquid flow in the interstitial void space is conceptually very similar to the familiar Poiseuille flow in a capillary, and it is convenient to consider flow in the complex void space more simply as flow in a capillary (Figure 4). Here then is the source of relative flow: not relative flow between two contiguous, distinctly different phases, but instead relative flow between *regions of a single phase*!

How may this capillary model be reconciled with the other requirement for chromatographic separation, selective distribution of solutes between phases, or, as required by HDC, selectivity between regions of a single phase? In the capillary model, as the colloidal particles are transported along the capillary space by the eluent flow, their Brownian motion will also cause them to move in radial directions, and these radial excursions will in turn lead to their sampling and adopting the various fluid velocities across the capillary. But as they approach the capillary wall (the packing–eluent interface, that is), their finite size will prevent them from visiting the regions of slowest velocity, and the larger the particles, the more they are excluded (Figure 4). Here then is the source of selective distribution of particles, the second requirement for chromatography. As a result of the coupling of these two effects, larger particles will have a greater mean velocity than smaller particles and all particles will have a higher velocity than the fluid carrying them. The predictions of this simple capillary model are therefore in qualitative harmony with the two key features of HDC as usually practiced. The reader may wish to show how the model explains another important feature of HDC, namely, that the smaller the diameter of the packing particles, the greater the selectivity of the column. There is in this regard a noteworthy difference between HDC and

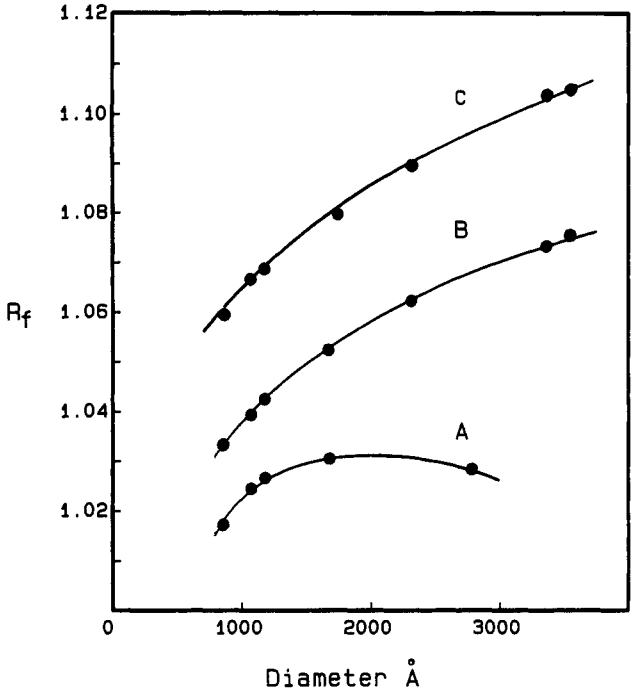

Figure 5. The rate of elution of particles in HDC is dependent not only on the size of the colloid but also on the ionic strength of the eluent. The data of curves A, B, and C were obtained in eluents containing respectively 0.176, 0.0046, and 0.000 425 M sodium chloride.

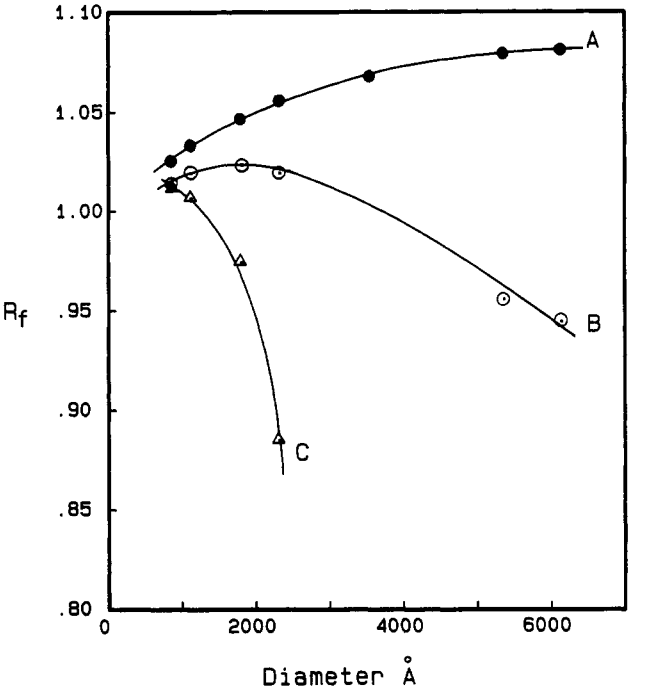

Figure 6. The elution behavior of polystyrene latex particles in high ionic strength environments (curve B in 0.4 M NaCl; curve C in 0.2 M NaCl) compared with "normal" HDC behavior (curve A).

conventional chromatography where selectivity is independent of particle size.

Although the simple "hydrodynamic" model goes a long way toward explaining much of HDC and led to its name, it does not account for all the effects we observe. For example, in aqueous eluents the R_f of a colloid depends markedly on the ionic strength of the eluent; Figure 5 shows the elution behavior of polystyrene latex particles of various sizes through columns of 20-μm cation-exchange beads using eluents of different ionic strength. So far the simple hydrodynamic model has treated the interaction of the colloidal particle and the packing–eluent interface as a purely steric one, and has ignored any electrostatic interactions that may arise due to the charged double layers at the surfaces of both the colloid and the packing. Electrostatic repulsion between the colloid and the similarly charged packing will determine how closely the former can approach the latter, and in accordance with the common understanding of double-layer interaction this distance of closest approach should increase with decreasing ionic strength. Returning to the capillary model, double-layer repulsion will tend to force particles into the faster moving core fluid with a resulting increase in R_f. This effect will be more pronounced the lower the ionic strength of the eluent, hence the behavior depicted in Figure 5.

While the data of Figure 5 may be explained in large part by a combination of hydrodynamic and electrostatic effects, at least one other observation requires some alternative explanation; under the conditions represented by Figure 5 (curve A), why do larger particles in some cases elute after smaller particles? An attractive as opposed to repulsive interaction between the polystyrene latex particles and the packing was proposed as the basis for this effect. As the ionic strength of the eluent is increased, double-layer repulsion diminishes, the colloid can approach more closely to the packing, and van der Waals attraction between the colloid and the packing will increase. If this van der Waals interaction results in a loose reversible interaction between the latex and the packing, then this should have a retarding effect on the rate of elution of the latex peak. If the attractive interaction increases with colloid particle size, then the reversal of slope evident in Figure 5A can be explained. This rationale persuaded me to explore eluents of even higher ionic strength, and the results are shown in Figures 6 and 7.

Here two features stand out at the highest ionic strength studied: large particles elute more slowly than smaller, the opposite of "normal" HDC, and for the larger particles at least, R_f is less than unity. At these high salt levels, electrostatic repulsion is probably completely subdued by the ionic environment and van der Waals interactions are so strong that they dominate over the hydrodynamic effect.

The effects are most interesting when latexes of different composition are studied under these high salt conditions (Figure 7). Under fixed and normal HDC conditions, that is, using a dilute eluent, the elution rate of colloids has been observed to be dependent only on its size and independent of such factors as the specific gravity, the surface charge density, and the chemical composition of the colloid. Now the observations of Figure 7 reveal conditions where the chemical composition of the colloid is important. It suggested in fact that latexes might be separated purely on the basis of this difference alone. Accordingly, using 0.4 M sodium chloride as the mobile phase, I eluted a mixture of polystyrene and poly(methyl methacrylate) latexes of almost identical size through a column packed with cross-linked polystyrene particles. The remarkable result is shown in Figure 8, a complete separation of the two components of the mixture into a polystyrene

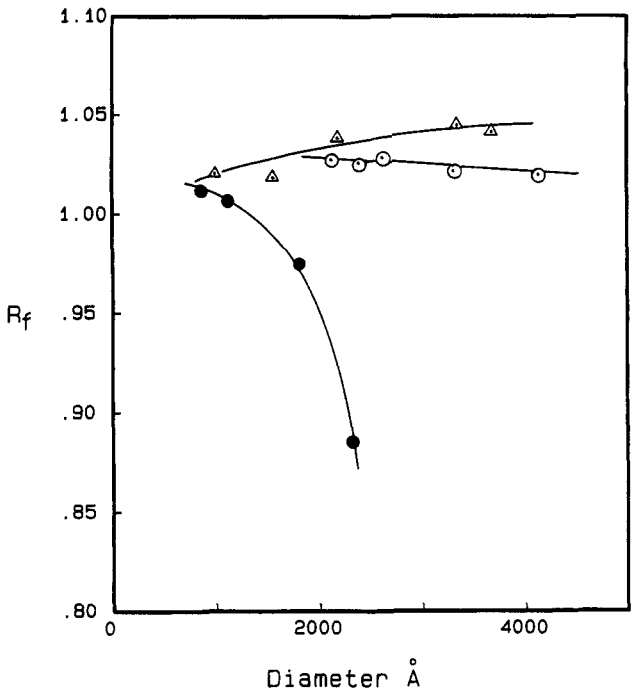

Figure 7. In high ionic strength eluent (0.4 M NaCl), latexes of different polymer composition give different elution behavior: ▲, polystyrene/butadiene; ⊙, poly(methyl methacrylate); ●, polystyrene.

fraction and a poly(methyl methacrylate) fraction.

So although our initial experiments used conditions that favored separation based on steric effects, further experimentation showed where flocculation–deflocculation interactions might indeed be exploited to give particle separations that were based not only on size but in certain cases on particle composition.

It is apparent that the transport of colloidal particles through packed beds involves complex and intriguing phenomena so it is not surprising that it has engaged the attention of some colloid theoreticians. They in turn have developed quantitative models[4-6] that are in remarkable agreement with our experimental observations. So successful do these theoretical efforts appear to be that it strongly suggests that much more may be learned from a combination of theoretical effort with the type of chromatographic experimental techniques described here. It is to be hoped that some will continue the exploration of this fascinating area.

The Scope of HDC and How It Compares with Other Methods of Particle Separation

While HDC has been applied to a wide variety of colloidal particles,[7] it is not restricted to particulate material nor is it restricted to chromatography in packed beds. Thus, Prud'homme and co-workers have applied HDC to molecular weight studies of very high molecular weight water soluble polymers;[8,9] in a particularly interesting advance, Tijssen and others explored the hydrodynamic chromatography of macromolecules in microcapillaries;[10] Silebi and co-workers have published extensively on capillary HDC applied to particles.[11-14]

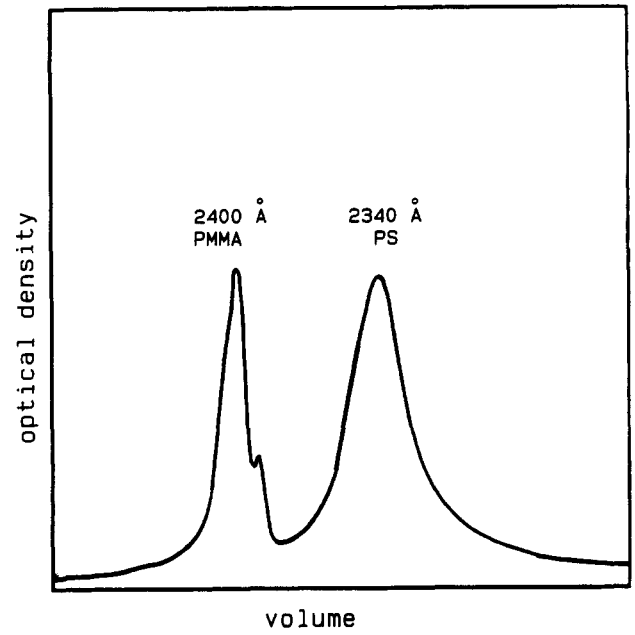

Figure 8. This separation of polystyrene (PS) particles from poly(methyl methacrylate) (PMMA) particles of almost identical size was accomplished on a 1-m column filled with 20-μm polystyrene spheres using 0.4 M sodium chloride in the eluent. (The side peak on the main PMMA peak may be due to larger particles of PMMA in the sample or of aggregates of the primary particles.)

And how does HDC stack up with several other modern techniques of particle separation[15] such as field flow fractionation, electrophoresis, and various sedimentation techniques including sedimentation FFF? While it may have less resolving power than some of these other methods, HDC appears to be quite unique in that its measurements depend only on particle size and not on such other factors as particle density or surface charge. This is not the case for these other methods.

Conclusion

This exploration of the transport of colloids through packed beds has been rewarding in a number of ways, not the least of which has been the discovery of a fast and precise means of particle size analysis. But I believe that there is much still to be learned and that continued research in this interesting milieu of hydrodynamics and surface chemistry cannot fail to add to our understanding of the fascinating state of matter that we call colloids.

It is my pleasure to acknowledge the contributions of my many colleagues and associates over the years, particularly Roland Hamburg, Marty Langhorst, Ron Pelletier, Frank Saunders, Dewey Scheddel, and Jitka Solc.

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