Preparation and Characterization of Novel Magnetite-Coated Ion-Exchange Particles

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Magnetite was incorporated into ion-exchange gel particles in two different modes. By a conventional procedure, magnetite could be dispersed throughout the particles; in addition, a novel method was discovered which led to a porous layer of magnetite coating the particles. Magnetite-coated particles have ion-exchange capacities that are virtually identical to the capacities of untreated particles, for the adsorption of a small (12 kDa) protein. Kinetics of protein binding are somewhat retarded by diffusion through the coating. These magnetic particles were designed for use in magnetically stabilized fluidized beds; induced magnetic interactions were found to be much stronger between magnetite-coated particles than between dispersed magnetite particles.

Introduction

A magnetically stabilized fluidized bed (MSFB) consists of a fluidized bed of magnetically susceptible particles over which a uniform magnetic field is applied. The usual field orientation is such that field lines run parallel to fluid flow. Magnetic dipoles are induced along the magnetic field lines, so that particles show a tendency toward vertical alignment. To obtain complete stabilization, the magnetic interactions between particles must be sufficient to counter the fluidizing forces which tend to mix particles. Since particles in a magnetically stabilized bed are free of motion, the stabilized condition can easily be determined visually.

The MSFB has recently been proposed as an adsorptive contactor in downstream bioprocessing.[1-3] A fluidized bed can handle solutions containing suspended solids, which would quickly clog a conventional packed column. The addition of magnetic stabilization renders sorptive particles in the bed stationary with respect to one another, thereby alleviating the mixing inherent in unstabilized fluidized beds. Magnetic stabilization has been found to improve adsorption efficiency in some liquid-fluidized systems.[4,5]

Magnetic stabilization behavior is used herein to indicate the strength of interparticle attractions. For a particular magnetic field strength, the transition velocity is the linear flow rate at which the bed begins to exhibit instability. As the fluid flow rate is increased, a larger magnetic field is required to stabilize the bed. Thus, the transition velocity may be increased by increasing the applied magnetic field strength or the magnetic susceptibility of particles. Figure 1 shows a typical flow vs field curve, for the particles described in this study. Each point represents the transition velocity for that particular applied field strength. Transition velocity appears to increase linearly with applied magnetic field, a finding that is consistent with theory over a short range.[6] Transition velocity curves also depend on particle physical properties such as density, size, size dispersity, magnetic composition, etc. Fluid properties such as viscosity and flow uniformity will also affect the transition behavior. In this paper, we present evidence that the location of the magnetic component in the particle can also affect interparticle attraction and hence transition velocity behavior.

Early studies of MSFBs in bioseparations have involved research in the choice of synthesis of suitable magnetic particles. Either porous or pellicular particles may be desired. Porous particles generally have higher capacities, but pellicular particles exhibit better mass-transfer kinetics. There are a number of commercially available magnetic affinity or ion-exchange particles, but these have been found to be generally too small or too light for use in a MSFB. Individual research groups have developed some suitable particles to study in MSFBs; in most cases, these have been of the pellicular type.[4,7,8] We were interested in studying the effects of magnetic stabilization in beds containing porous ion-exchange particles.

Using agarose as the particle matrix and magnetite as the magnetic component, two types of porous magnetic gel particles were studied. In the first, magnetite was incorporated as a thin permeable layer on the outside of ion-exchange particles; in the second, magnetite was dispersed into the gel prior to bead formation. When the two types of particles had similar magnetic components, it was found that magnetic stabilization was different, depending on the mode of magnetite incorporation. While particles coated with magnetite were easier to stabilize as a bed, ion-exchange kinetics were slightly retarded as a result of the coating.

Experimental Section

Materials. Ferrofluid (EMG 607) was obtained from Ferrofluidics Corp. (Nashua, NH). Magnetite powder (Fe_3O_4) was purchased from Alfa (Ward Hill, MA). Agarose powder and bovine heart cytochrome c (Type V-A) were purchased from Sigma Chemicals (St. Louis, MO). Other chemicals were reagent grade.

Particle Syntheses. Spherical agarose particles were formed by an emulsion procedure described by Hjerten[9] and then

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cross-linked and functionalized with carboxymethyl exchange groups.[10] Particles were then sized by sieving. A size fraction of 212–355 μm was chosen for the work herein. All particles were kept in distilled water; over long storage periods, 5% ethanol was added as antimicrobial.

In some experiments, S-Sepharose Fastflow (Pharmacia) was used instead of the particles made as above. S-Sepharose is a commercial ion-exchanger consisting of a cross-linked agarose matrix functionalized with sulfopropyl groups. The S-Sepharose media was sieved to yield a particle size range of 90–165 μm.

Some magnetic particle batches were made by a ferrofluid treatment. Both S-Sepharose particles and cation-exchangers made in-house could be treated by the following procedure: Approximately 7 mL of ferrofluid was added to 10 mL of cation-exchange media (clear-white in color) and 5 mL of water. This mixture was shaken for about 15 h. At this point, the cation-exchange particles were sieved (90-μm mesh) from the diluted ferrofluid suspension. The ferrofluid suspension was still quite dark brown, indicating that all the magnetite had not been adsorbed into particles. Particles were washed, and labeled "type 1" particles. These particles were dark brown. The "leftover" ferrofluid suspension was then added to 7 mL of *fresh* cation-exchange media, and the mixture was shaken. After only a few hours, the liquid part of the mixture appeared to be fairly clear, indicating that most of the magnetite had been removed from suspension. At this point, cation-exchange particles were removed from the liquid by sieving (90-μm mesh). These dark brown particles were labeled "type 2" particles[3].

In a few experiments, a third type of magnetic particles were employed. These particles had powdered magnetite instead of ferrofluid as the added magnetic component. Magnetite powder was simply stirred into the agarose gel (made in-house) prior to bead formation. These particles, which contained dispersed magnetite powder, were labeled "type 3" particles.

Protein Binding Studies. A measure of specific binding capacity was used as a quick and inexpensive test of some binding properties. Cytochrome c solution (20 mL, 1 mg/mL in 0.025 M pH 7 sodium phosphate buffer) was added to 1 mL of media and shaken for 5–10 min. Protein concentration in the solution was measured before and after media exposure by optical absorbance (λ = 408 nm). Percent protein bound was calculated by difference.

Protein binding kinetics and adsorption isotherms were also generated using cytochrome c. Solutions were buffered as above, but initial protein concentrations were 0.75 mg/mL. Protein concentration in the solution was monitored with a UV–vis diode-array spectrophotometer (HP Model 8452A) at 280 nm. A mixture of 1 mL of particles and 200 mL of cytochrome c solution was continuously stirred and sampled into a quartz flow-through cell by means of a peristaltic pump. Optical absorbance was measured every 10 s, with integration over 0.5 s. Particle uptake by the pump was prevented by placing a filter cloth over the uptake line. Pump uptake and solution stirring speed were adjusted so as to prevent particles from collecting on the filter cloth, thus ensuring even sampling. Sampling continued until absorbance readings remained essentially constant.

Iron Determinations. A standard analytical method was used to determine iron by the formation and subsequent detection of a colored iron (II) orthophenanthroline complex.[11] Sample preparation involved digestion of particles in concentrated HCl, followed by appropriate dilution and pH adjustment. Results could be expressed as percent Fe or percent Fe_3O_4. In some cases, it was suspected that part of the iron actually existed as Fe_2O_3 (particularly in the ferrofluid). This would have led to slight errors in calculating percent magnetite, since the iron content in the two oxides differs by a few percent.

Magnetization Measurements. A vibrating sample magnetometer was used to generate hysteresis plots of magnetization (emu/g) vs applied field (Oe) for particles containing magnetite from different sources. The two different "magnetites" (ferrofluid-derived vs powdered Fe_3O_4) exhibited different magnetization behavior. Over a limited applied field range, a constant correction factor could be approximated. Ferrofluid-derived magnetite exhibited magnetizations ≃3.2× greater than magnetite in powder form. This difference is probably due to some excess oxidation in ferrofluid-derived iron oxides.

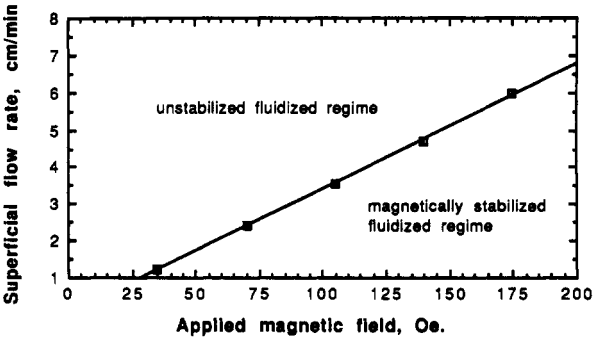

Figure 1. Typical flow rate vs applied field curve is shown. The transition velocity line was determined visually, by noting particle movements (loss of stability).

Results and Discussion

Preparation and Properties of Magnetic Particles. A special type of magnetite suspension called a ferrofluid was used in magnetizing some ion-exchange media. This ferrofluid consisted of very small, subdomain particles of magnetite (ca. 100 Å in diameter) coated with a monolayer of cationic stabilizer and suspended in aqueous solution. A ferrofluid has previously been used to postmagnetize 5′-AMP Sepharose 4B affinity media;[12] the mechanism for magnetite incorporation was not reported. By treating cation-exchange media with this ferrofluid, magnetite was expected to stick to the particles either by adsorbing onto the agarose backbone or by occupying ion-exchange sites.

When cation-exchange particles were exposed to the ferrofluid, two distinct particle types were observed, depending on the history of the ferrofluid. Different particle types were obtained, depending on whether the ferrofluid had been exposed to ion-exchange media before. Upon first exposure of a volume of ferrofluid to fresh cation-exchange media, a small quantity of the magnetite in the ferrofluid was taken up inside the particles, apparently by occupying ion-exchange sites. Resulting particles were "type 1". The remaining fluid could be decanted and reexposed to fresh media. In this second exposure, magnetite was observed to form a porous layer on the outside of particles. These particles are referred to as "type 2". The magnetite coating could be made to deposit on a number of commercially-available cation exchangers, including S-Sepharose Fastflow (Pharmacia) as well as with cation-exchange particles prepared in-house.

The two ferrofluid-derived particle types could be distinguished by appearance as well as by sorptive behavior. Figure 2 shows SEM photographs of "type 1" (dispersed) and "type 2" (coated) ferrofluid-treated S-Sepharose. Type 1 particles have a fairly smooth surface (which resembles that of untreated S-Sepharose). By contrast, type 2 particles appear very different by SEM; the surface is roughened, presumably with a coating of magnetite. Even using a light microscope, the two types can be distinguished. While both particle types are dark brown, the type 1 particles are translucent, while type 2 particles are opaque. The magnetite coating could be mechanically scraped off type 2 particles, to reveal clear gel beneath.

A quick test of binding capacity could also distinguish between the two particle types. When a solution containing 20 mg of the protein cytochrome c was exposed to

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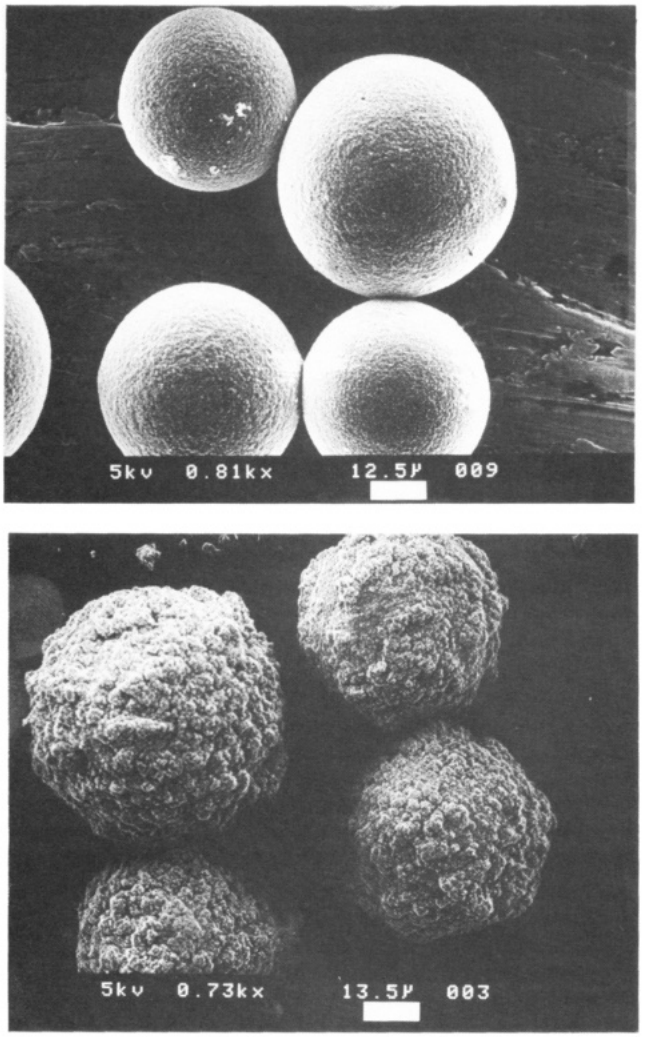

Figure 2. Top: S-Sepharose particles after "first exposure" treatment with ferrofluid (type 1 magnetic particles). Bottom: S-Sepharose particles after "second exposure" treatment with ferrofluid (type 2 magnetic particles).

1 mL of particles, different specific binding capacities were observed, depending on ferrofluid (ff) treatment:

ion exchanger	% protein bound
untreated S-Sepharose	99%
type 1 ff-treated S-Sepharose	4–30%
type 2 ff-treated S-Sepharose	97–98%

These results indicate that magnetite blocks ion-exchange sites in type 1 particles but not in type 2 particles.

Ion exchange capacity and/or kinetics might be expected to change with the addition of a surface coating on particles. Magnetite-coated S-Sepharose was compared to untreated particles, to see how protein uptake would be affected. Figure 3 illustrates that the adsorption isotherm is relatively undisturbed by the outer layer of magnetite on treated particles. Magnetite itself can act as an ion-exchanger, but its capacity is very low in comparison to that of the particles. Porous paths through the coating are apparently large enough to admit a small protein of MW ≅ 12000 Da.

The rate of uptake is slowed by the addition of a magnetite coating. In Figure 4, the ion-exchange rate for adsorption of a protein is seen to decrease after the addition of a magnetite coating. Protein uptake is 75% complete after about 100 s in untreated media; for coated media, the same degree of uptake is reached in about 200 s. The coating puts up an extra diffusional barrier that retards ion uptake by particles. If a coating thickness of 20 μm

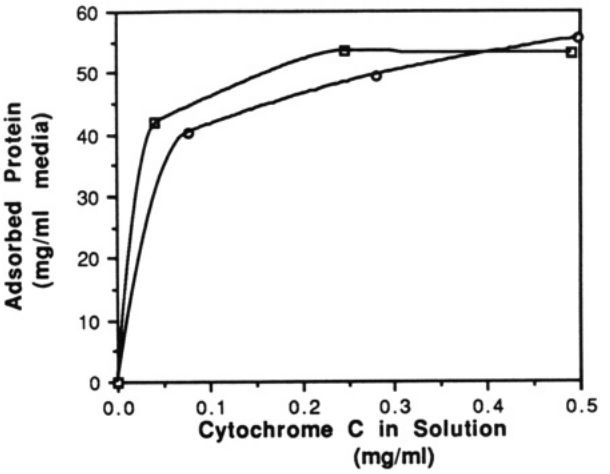

Figure 3. Adsorption isotherms for protein onto untreated S-Sepharose particles (circles) and magnetite-coated S-Sepharose (squares).

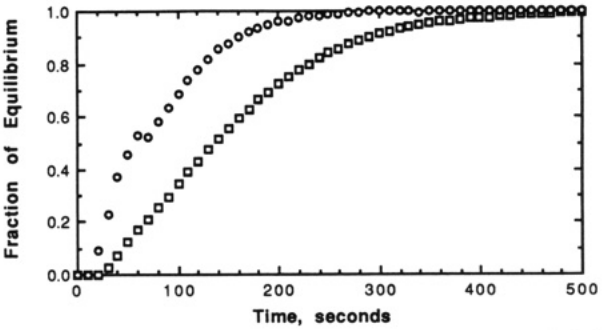

Figure 4. Kinetics of protein adsorption for untreated S-Sepharose particles (circles) and magnetite-coated S-Sepharose (squares).

is assumed, then a 100-s lag time implies that the solute diffusion coefficient throughout the magnetite coating is about 2×10^{-8} cm^2/s. This is about 2 orders of magnitude smaller than a typical diffusion coefficient for a small protein in water, and about 1 order or magnitude smaller than expected for diffusion in a gel such as S-Sepharose. Even so, ion-exchange rates are not unreasonably slow with the coated particles.

It is not known why two different particle types resulted, when ferrofluid treatment conditions were so nearly the same. The only important difference in treatments was apparently whether the ferrofluid had been previously exposed to cation-exchange particles. If so, then coated particles resulted. Simple dilution of ferrofluid was not found to cause differences in particle types; experiments involving ferrofluids diluted with water still resulted in dispersed particles upon initial contact, and coated particles upon secondary contact. pH of the ferrofluid solutions was not seen to change appreciably over the treatment time.

Magnetite particles in a ferrofluid must be in a very narrow size range around 100 Å in order to remain in suspension. A surfactant coating the surface of these tiny particles prevents agglomeration.[13] Such suspensions, or ferrofluids, can remain stable for periods of at least months. Somehow, in the second exposure of ferrofluid to ion-exchangers, magnetite particles are made to precipitate out as a rather uniform coating onto the beads. Exposing type 1 particles for longer periods of time did not result in a magnetite coating; only previously untreated

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particles could be made to form a surface layer of magnetite. This suggests that the mechanism for the formation of this coating might involve removal of the stabilizing surfactant. In the first exposure, a large amount of surfactant could be removed by ion-exchange sorption. Upon second exposure, enough additional surfactant is removed to destabilize the the ferrofluid suspension and cause magnetite deposition on particles' surfaces.

Both the type 1 and type 2 ferrofluid-treated particles remained fairly stable over time, when stored in distilled water. However, exposure to salt solutions caused the magnetite in both types to slowly desorb from the particle matrix. This suggests that the forces binding the magnetite to both particle types are primarily electrostatic. The mechanical stability of the magnetite coating in type 2 particles could be a problem; slow magnetite loss was observed under conditions of mechanical stress, such as particle mixing in a fluidized bed. Chemical or mechanical stabilization of the coating may be possible but has not yet been attempted.

Effect of Particle Structure on Interparticle Magnetic Attraction. Studies of transition velocity in MSFBs suggested that the ferrofluid-derived particles coated with magnetite (type 2) generated stronger stabilization forces than ones with magnetite dispersed within the matrix (type 1). At first, this difference was attributed mostly to the lower amount of magnetite that could be incorporated into type 1 particles. To compare the effect of magnetite incorporation mode (coated vs dispersed), the two types of particles had to be synthesized with the same bulk magnetic properties. Since ferrofluid treatment gave a low magnetite content in type 1 particles compared to type 2, dispersed-magnetite particles with a higher magnetic content were prepared by another method. Type 3 particles were prepared by mixing magnetite powder into the agarose gel prior to bead formation.

To study the effect of magnetite placement in particles, two batches of particles were made (212–355 μm) which had similar bulk magnetic properties. In one batch, ferrofluid treatment was used to generate type 2 magnetite-coated particles. In another batch, a known amount of magnetite powder was stirred into agarose gel before bead formation, to form type 3 particles. Iron content in both particle batches was assayed. Mass magnetization measurements were made for each type of particle; these values were converted to magnetization per mass of Fe_3O_4. Magnetite from the two sources exhibited different magnetization behaviors

	applied field, Oe			
	35	70	94.5	105
magnetite from ferrofluid	17.3	30.4	36.4	39.5
magnetite powder	5.1	8.9	11.6	13.7

Ferrofluid-derived magnetite may exhibit a higher magnetization if part of it has actually been oxidized to γ-Fe_2O_3 (maghemite), a reaction that could be facilitated by the presence of surfactant coating.[14] Maghemite has a higher magnetization than magnetite, under these field conditions.[15] Furthermore, the size of the iron oxide particles can influence their magnetization behavior.[16]

Over the applied field range in the above table (35–105 Oe), magnetizations for the two types of magnetite differed by a factor of about 3.2 (±0.3). Therefore, for dispersed-magnetite particles to have bulk magnetic properties similar to coated-magnetite particles, they would have to contain about 3.2 times as much magnetite (by wt %). Accordingly, a batch of magnetite-coated particles were made which were assayed at 5.6% magnetite, while a batch of type 3 dispersed-magnetite particles were made which contained 17.2% magnetite.

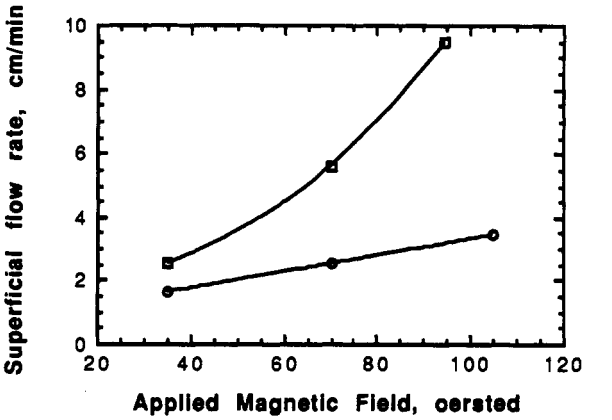

Figure 5. Transition velocity curves for type 3 (dispersed-magnetite) particles (circles) and type 2 (magnetite-coated) particles (squares).

Since the two particle batches described above had similar bulk magnetization behavior, they could be used to study the effect of magnetite placement on induced interparticle attractions. It was expected that magnetic forces induced between particles in a magnetically stabilized fluidized bed might be greater if the magnetic component was coated on the surface of particles rather than dispersed throughout the particle matrix. With surface-coated particles, the average distance between magnetic poles on adjacent particles is smaller and thus the magnetic interactive forces should be greater. The transition velocity curves in Figure 5 support this idea; a bed of magnetite-coated particles exhibit a greater degree of magnetic stabilization than a similar bed with dispersed-magnetite particles. At an applied field intensity of about 95 Oe, the magnetite-coated particles are stabilized in a fluidized bed with flow rates up to 9.5 cm/min, while stabilization in a similar bed containing dispersed-magnetite particles is possible only up to flow rates of about 3.2 cm/min. Thus, the allowable operating range of a MSFB could be greatly influenced by the design of magnetic particles comprising it.

There are a number of factors which complicate rigorous comparison of the transition velocity differences as seen in Figure 5. Physical properties such as size dispersity could not be precisely controlled. Also, a correction factor had to be devised for the different magnetite sources. This led to particle densities which were about 10% different for the two types. However, the higher density of dispersed-magnetite particles should have made them easier to stabilize, since bed expansion would not be as great.

Physical properties of the particles affect their behavior as a fluidized bed. Bed expansion behavior in unstabilized fluidized beds is given by the equation

$$\frac{\epsilon^3}{1-\epsilon} = \frac{150\mu U}{g(\rho_p - \rho_l)D_p^2} \quad (1)$$

where U = linear superficial flow rate (cm/s), ρ_p = particle density (g/cm^3), ρ_l = fluid density (g/cm^3), D_p = particle diameter (cm), ϵ = bed void fraction (liquid fraction of total bed volume), and μ = fluid viscosity (g/cm s). The expression $\epsilon^3/(1-\epsilon)$ describes the degree of bed expansion, increasing as the fluidized bed expands. For solids fluid-

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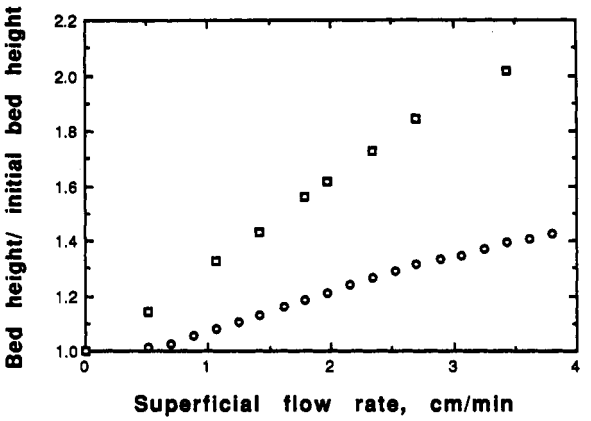

Figure 6. Bed expansion curves for the type 3 (dispersed-magnetite) particles (circles) and type 2 (magnetite-coated) particles (squares). These are the same particle batches as were used in the transition velocity comparison given in Figure 5.

ized by liquids, this equation is generally applicable for $\epsilon < 0.8$.[17]

From eq 1, it can be seen that smaller, lighter particles will undergo a greater amount of bed expansion at any given flow rate. More highly expanded beds should be more difficult to magnetically stabilize. Figure 6 shows fluidized bed expansion curves for the type 2 and type 3 particles used in transition velocity comparisons. These curves were generated with no applied magnetic field, and thus reflect particle physical properties such as density and size. The type 3 (dispersed-magnetite) particles undergo a much lesser amount of bed expansion than do type 2 (magnetite coated) particles, due mostly to their greater density. If similar magnetic interactions were generated in these beds, one would expect the type 3 particles to have higher transition velocities. The fact that the opposite behavior is seen further supports the idea that coated magnetite particles generate stronger magnetic stabilization forces.

It is interesting to note that the coated-magnetite particles could be magnetically stabilized even under conditions of considerable bed expansion. For 95-Oe applied field, a bed of magnetite-coated particles could be stabilized even after the bed had expanded 2.2× (relative to a settled bed). For a bed of dispersed-magnetite particles under similar conditions, magnetic stabilization could not be attained above a bed expansion of 1.3×.

In future work, a quantitative evaluation of the magnetic stabilizing forces in the above systems will be attempted. A better understanding of the assembly mechanism for the magnetic coatings is also desired. Mechanical and chemical instability hinder the use of the ferrofluid-derived magnetic particles in adsorptive processes. If applications warrant, attempts may be made to stabilize these particles.

Conclusions

Magnetite can be made to deposit as a thin permeable layer on the surface of ion-exchange beads. The coated type of magnetic particle will be advantageous if one wishes to maximize induced magnetic attractions between particles, as in a magnetically stabilized fluidized bed. This iron oxide coating allows the passage of at least small proteins (MW ≅ 12 000 Da), but somewhat retards their uptake. An improvement in the mechanical stability of the magnetite coating will be required for many applications.

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Registry No. Fe_3O_4, 1309-38-2; agarose, 9012-36-6; S-Sepharose Fastflow, 115251-16-6.

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