Use of High-Gradient Magnetic Fields for the Capture of Ferritin

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High gradient magnetic field separations processes (HGMS) have been utilized for a number of applications in the separation of >1 μm ferro-and paramagnetic particles. Among the materials that have been separated using this technique are red blood cells (Frank et al., 1982), or tailings (Khalafalla, 1976), and sewage (Watson, 1973). The normal mode of operation in these processes begins by placing a ferromagnetic wire mesh into a uniform magnetic field to create large magnetic field gradients near the surface of the wires. While passing through the magnetized matrix, the desired material is separated from a bulk liquid stream via magnetic-field-induced capture onto the wire mesh. Recovery of the captured material can then be accomplished by deactivating the magnetic field. In effect, the process is a magnetic filtration of material from the liquid stream. Variations on this general theme involve using susceptibility gradients (Takayasu and Kelland, 1984) and using magnetic forces in concert with centrifugal forces for separations (Zimmels, 1986).

The imposition of a high-intensity, high-gradient magnetic field on a column packed with ferromagnetic spheres has allowed capture of the protein ferritin from an aqueous-based buffer solution. The ability of the system to capture ferritin was dependent upon the magnitude of the charge of the molecule, with significant capture occurring only at or near the isoelectric point.

Description of the Technique

In general, the ability of an HGMS system to capture particles depends upon the particle size and the magnitude of the magnetic susceptibility of both the particle and the surrounding fluid media. Solution of the equations describing the force balance between the interaction of the particle with the magnetic field gradient and induced relative motion through the viscous bulk liquid yields a set of trajectory equations for the capture of the particle on the surface of the ferromagnetic matrix (Watson, 1973; Cummings et al., 1976; Schewe et al., 1980). If one is contemplating the capture of macromolecular entities ($\leq$ 100 Å), it is evident that extremely large field gradients must be used.

Ferritin is the major iron storage protein found in mammals (Crichton, 1973). It is comprised of about 4,500 atoms of ferric iron surrounded by a proteinaceous shell. The molecular weight of ferritin is approximately 900,000 with the weight being roughly equally distributed between the iron core and the shell. The shape is essentially spherical with a core diameter of 75 Å and an outer diameter of 125 Å. Due to the iron core, ferritin displays a strong interaction with magnetic fields (superparamagnetism) which makes it a logical candidate for investigating HGMS of macromolecules. Horse spleen ferritin obtained from Sigma Chemical Company was used in this study. The protein was 20% iron by weight and had an isoelectric pH of 4.4.

The design of the column in which capture was demonstrated is shown in Figure 1. A 6 T magnetic field was imposed on a bed of ferromagnetic steel spheres packed in a 5.0-cm long by 0.46-cm ID stainless steel column. The ferromagnetic spheres (Nuclear Metals Inc.) were made of 779 steel and had an average diameter of 100 μm. The magnetic field was generated by a superconducting solenoid manufactured by Cryomagnetics Inc. During experiments, an aqueous phosphate or phthalate buffer solution was pumped through the column. To begin, the feed was changed from a pure buffer solution to one containing 2.4 gm/L ferritin in the buffer. The concentration of ferritin in the exit stream of the column was then monitored by an on-line spectrophotometer at 520 nm. The step-change in ferritin concentration in the feed caused an associated "break-through curve" at the column exit as the column was being "loaded." When the concentration of ferritin in the exit stream was no longer changing, the magnetic field was turned off. After one additional column residence time had elapsed, the feed stream was once again returned to pure buffer to wash out the column. This procedure was carried out at pH values between 2.0 and 7.0. Under each set of pH conditions, experiments were carried out both with and without activation of the magnetic field during column loading.

Results

Figure 2 is a plot of the column breakthrough curves generated at pH 7.0, both in the presence and absence of the magnetic

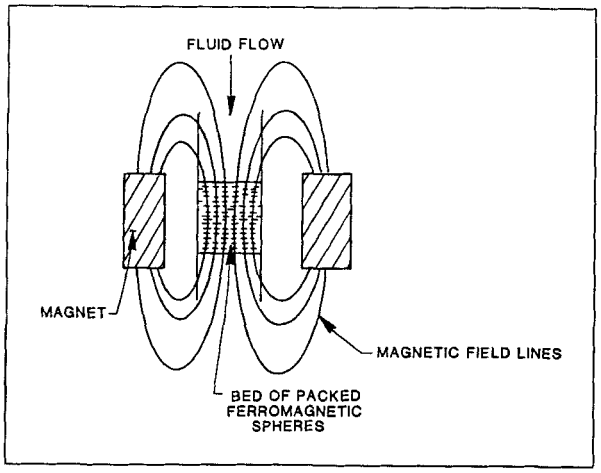

Figure 1. Packed-bed apparatus used in initial experiments.

field during column loading. These plots are in arbitrary units and have been offset for visual clarity. The curves are essentially indistinguishable. From this representation, it is easy to see that the magnetic field had no effect on the ferritin as it flowed through the column. The sigmoidal shape of the curves suggests simple dispersion through the packed column and little, if any, adsorption of ferritin on the packing in either case.

Figure 3 contains plots of some magnetic field runs at pH's of 7, 5.2, and 2.6. As the isoelectric point of ferritin is pH 4.4, these runs represent cases in which the protein is relatively highly negatively charged, slightly negatively charged and highly positively charged, respectively. Table 1 summarizes the quantitative aspects of these results. Integration of the absorbance signals during loading indicates that although ferritin input rate to the column was the same in each case, more material exited the column during the pH 7 run than in the other two instances.

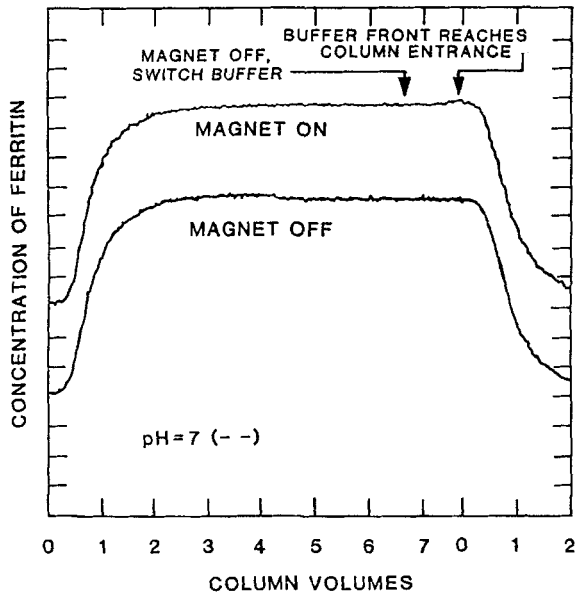

Figure 2. Comparison of Ferritin breakthrough curves and elution curves for magnetic and nonmagnetic case (pH = 7).

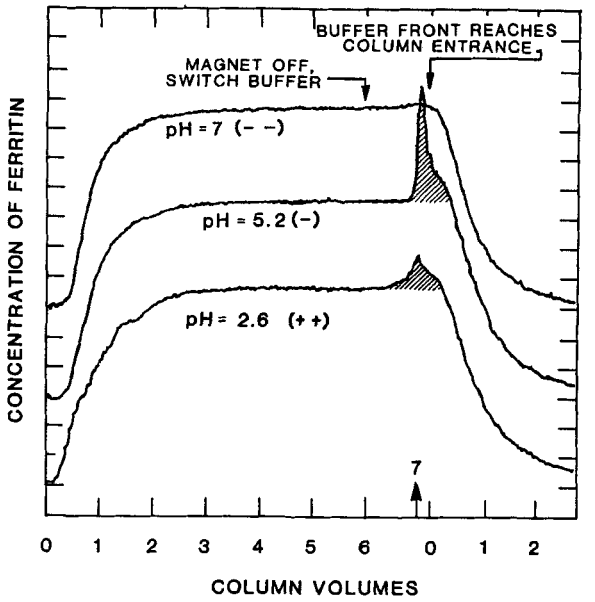

Figure 3. Ferritin breakthrough curves for magnetic cases (pH = 7, 5.2, 2.6).
Cross-hatched areas denote captured ferritin.

This signifies that there was material captured during the runs at lower pH's.

Upon deactivation of the magnet after breakthrough, an additional peak is observed in the cases where the pH is 5.2 and 2.6. This indicates that the ferritin that had been retained by the column was released when the magnet was turned off. At pH's of 5.2 and 2.6, the column retained 8 and 6%, respecitvely, of the ferritin input during loading. From these results, it is evident that as the magnitude of the charge decreases, additional ferritin is captured on the packing by the magnetic field and that it is immediately removed from the column upon deactivation of the magnetic field. The associated nonfield cases for pH's 5.2 and 2.6 were the same as that for pH 7 (see Figure 2).

When the system was run at the isoelectric point, the magnetic field would induce essentially total capture and the column would begin to plug. When the system was run at a pH of 2 (highly positively charged) the results were as in the pH 7 case: no evidence of magnetic-field induced capture was noted. Hence, the total capacity for ferritin capture in the column was relatively small away from the isoelectric point.

Remarks

If one carries out the traditional HGMS trajectory analysis on ferritin for these experimental conditions, it predicts that ferritin should be captured. However, this was not the case when the protein was run under conditions where the magnitude of its charge was beyond a critical value. This points to a weakness in the standard trajectory analysis; namely, that interparticle (molecular) interactions in solution are neglected. As one is attempting to effect the capture of small (macromolecular) entities, it becomes clear that intermolecular forces can dominate behavior of the species in a magnetic field gradient. Perhaps the capture may be more accurately described as a magnetically induced destabilization of the molecules in solution. If this is the case, one could envision a type of "isoelectric focusing" technique for the separation of charged molecules. Manipulation of

Table 1. Quantitative Ferritin Capture Data

pH	Ferritin Input During Loading mg	Ferritin Recovered During Loading mg	Ferritin Recovered After Magnet Off mg	Percent Captured %
7.0	8.4	8.4	0.0	0
5.2	8.5	7.8	0.7	8
2.6	8.5	8.0	0.5	6

the microenviornment of the liquid to cause one of a mixture of such species to become uncharged would allow that particular type of molecule to be subject to magnetic field forces and subsequent capture from solution. Furthermore, if one could design a system in which the bulk liquid had a large magnetic susceptibility (e.g., a ferrofluid), then the magnetic forces may be appreciable enough to affect even very small molecules in solution. In any case, to more fully understand these phenomena one will have to undertake a more fundamentally based approach to the study of forces involved in the interactions of charged macromolecules in viscous media under the influence of magnetic field gradients.

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