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DEMONSTRATION OF THE PRINCIPLE OF PARAMAGNETIC CHROMATOGRAPHY FOR RESOLVING MIXTURES OF PARTICLES

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SUMMARY

Particles which bind sufficient quantities of the paramagnetic cation Er^{3+} behave paramagnetically, and can be captured by a suitably-designed magnetic field. As the binding of Er^{3+} is ionic, the affinity of a given particle for Er^{3+} can be purposefully modulated by altering the ambient conditions. In the present communication, we describe one way in which these properties can be exploited to separate discrete populations of particles from heterogenous mixtures. We call this approach paramagnetic chromatography.

INTRODUCTION

We have shown that certain types of particle bind sufficient numbers of paramagnetic lanthanide ions, such as Er^{3+}, to behave paramagnetically when placed in a magnetic field[1,2]. Graham and Selvin[3] have confirmed this observation. The magnetic susceptibility of each particle is a function of the amount of Er^{3+} bound to it. As Er^{3+} binds ionically, primarily to ligands with oxygen donor atoms[4], the amount of Er^{3+} sequestered by a given particle depends upon that particle's chemistry. This conclusion presents the theoretical possibility of separating particles by rendering them selectively paramagnetic on the basis of their affinity for Er^{3+}. As this affinity is chemically determined, we have the potential to separate particles on the basis of their chemistry. This is a departure from many traditional methods of separation.

There are a number of potential approaches to achieving magnetic separations based on differential affinity for Er^{3+}. Here we demonstrate the principle behind one such approach, using commercially available particles. The three types of particle, latex, chelex and a sulphonated ion-exchange bead, differ in their affinities for Er^{3+}

under the various experimental conditions employed; we have been able to separate them accordingly.

EXPERIMENTAL

Theory

The three types of particle used in this demonstration were selected on the basis of their affinities for Er^{3+}. Latex (polystyrene) presents hydrophobic side chains which have little ability to sequester Er^{3+} when suspended in an aqueous medium. Chelex ion-exchange beads have carboxyl functional groups with a pK_a in the pH range 3–4. These beads attach to Er^{3+}, and become consequently magnetized, at pH values in excess of about 4. Conversely, they release Er^{3+}, becoming demagnetized, when the ambient pH falls below the pK_a value. The ion-exchange beads AG 50W-X8 bear exposed sulphonate groups whose pK_a value lies in the range pH 0–1.5. Er^{3+} binds to these particles under all but the most acidic of experimental conditions.

Consequently, it should prove possible to resolve magnetically a mixture of these particles in the following way: upon their suspension in a solution containing Er^{3+} at pH 6.5, the two types of cation-exchange bead, but not latex, should be magnetized. When pumped slowly between the poles of a suitably designed magnet, the magnetic field will retain the magnetized particles, while repelling the particles of latex. These are carried away in the suspending fluid, their elution being aided by the paramagnetic properties of the fluid phase.

Following elution of the latex particles, the remaining magnetized particles are washed with 10 mM hydrochloric acid. This lowers the pH to 2, thereby demagnetizing the particles of chelex, which elute from the field along with the wash. Only the sulphonated particles now remain in the magnetic field. These can be recovered following demagnetization with a high concentration of EGTA which competitively strips Er^{3+} from the beads.

Materials

From Biorad Labs. (Richmond, CA, U.S.A.) were purchased: Chelex 100, minus 400 mesh (diameter 45–75 μm) and 200–400 mesh (diameter 75–150 μm); AG 50W-X8, minus 400 mesh (diameter 38–63 μm) and 200–400 mesh (diameter 63–150 μm). Particles of latex (diameter 45 μm) and red latex (diameter 6 μm) were obtained from Polysciences (Warrington, PA, U.S.A.).

A Sage Instruments syringe pump Model 351 (Fisher Scientific, Pittsburgh, PA, U.S.A.) was used to pump the particles between the poles of a ferrograph magnet obtained from Telus (Peabody, MA, U.S.A.). PTFE tubing was bought from Zeus Industries (Rariton, NJ, U.S.A.).

$ErCl_3 \cdot 6 H_2O$ was purchased from Alfa Products (Danvers, MA, U.S.A.) and ethyleneglycon-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA) from Sigma (St. Louis, MO, U.S.A.).

Methods

Fig. 1 shows the configuration of the apparatus. The syringe pump was used to propel suspensions of particles, or the various washing solutions, via PTFE tubing, through a glass separating tube (102 × 3 mm I.D.). The separating tube was ar-
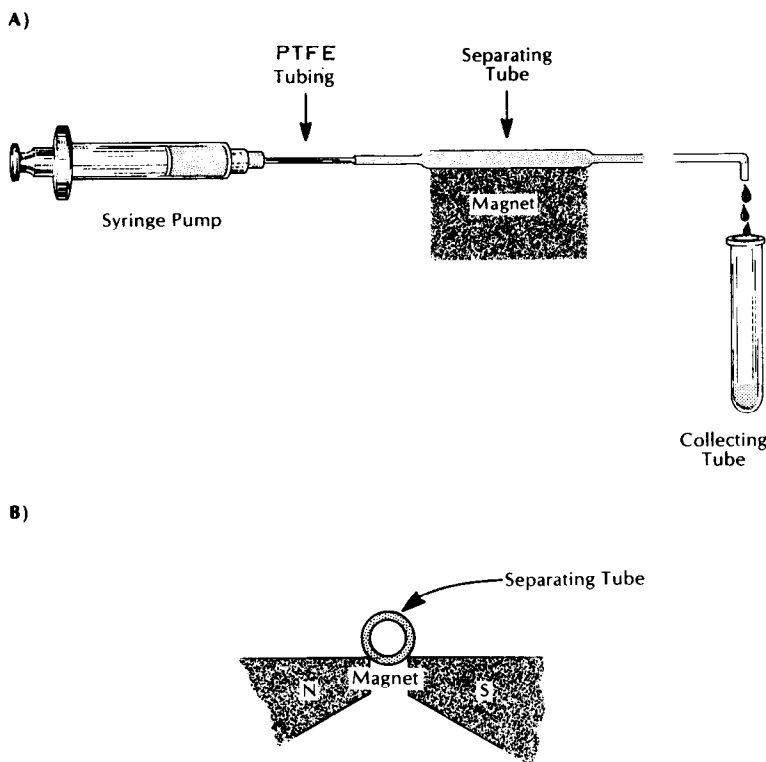
A)

B)

Fig. 1. The magnetic separation apparatus. A, Transverse section; B, longitudinal section. See text for details.

ranged along the interpolar gap of the magnet, where the field strength is strongest. The magnet employed had a maximum field intensity of approximately 18 000 G and a maximum gradient of the order of 200 000 G cm^{-1} in the vertical plane. Magnetized particles were retained in the separating tube under the influence of the field, while non-magnetized particles were eluted along with the flowing liquid. The eluate could be collected for further study. Where necessary, individual fractions were collected manually.

In a typical run, aliquots of each of the three types of beads were added to freshly prepared magnetizing solution (10 mM ErCl$_3$ in saline, pH 6.5) to give a final concentration of 100 beads/ml. A portion (1 ml) of this suspension was pumped slowly (0.6 ml/min) through the separating tube, and the eluant collected in 0.6-ml fractions. Magnetizing solution was pumped through the tube for a further 5 min, after which time it was replaced by 10 mM hydrochloric acid to lower the pH to 2. Fractions (0.6 ml) of the eluant continued to be collected as before. Following another 5 min of washing, the hydrochloric acid solution was replaced by a solution of EGTA (100 mM, pH 6.5). Fractions were collected for a further 15 min.

At the end of the experiment, fractions were examined microscopically. Particles were identified by their size or colour and counted with a haemocytometer.

TABLE I

MAGNETIC BEHAVIOUR OF PARTICLES UNDER VARIOUS AMBIENT CONDITIONS

Particles were suspended in magnetizing fluid (10 mM ErCl$_3$ in saline, pH 6.5) and pumped along the magnet, as described in the experimental section. This was then followed by the passasge of more magnetizing solution, 10 mM hydrochloric acid or EGTA, and the behaviour of the particles noted by visual inspection of the separating tube and by microscopic examination of the eluates. Particles behaving paramagnetically were those trapped in the separating tube above the magnet. Diamagnetic particles eluted from the separating tube.

Type of particle	Washing fluid		
	Magnetizing solution	10 mM hydrochloric acid	100 mM EGTA (pH 6.5)
Latex			
6 μm diameter	Diamagnetic	Diamagnetic	Diamagnetic
45 μm diameter	Diamagnetic	Diamagnetic	Diamagnetic
Chelex			
45–75 μm diameter	Paramagnetic	Diamagnetic	Diamagnetic
75–150 μm diameter	Paramagnetic	Diamagnetic	Diamagnetic
AG 50W-X8			
38–63 μm diameter	Paramagnetic	Paramagnetic	Diamagnetic
63–150 μm diameter	Paramagnetic	Paramagnetic	Diamagnetic

RESULTS

Behaviour of homogenous suspensions of particles

Initially, homogenous suspensions of particles were tested to determine whether their magnetic behaviour agreed with theoretical expectations. As shown in Table I, it did. Particle size did not qualitatively influence this behaviour.

Optimizing the separating conditions

Two parameters, flow-rate and particle density, were found to influence critically the success of the magnetic separation. When the flow-rate was too low, particles settled out under gravity and did not elute cleanly. With too high a flow-rate, particles experienced flow forces in excess of the paramagnetic forces, and were thus swept indiscriminately through the magnetic field. By trial and error, an optimal flow-rate of 0.6 ml/min was determined.

Magnetized particles tended to aggregate at the place where they first encountered the magnetic field, forming a deposit with a characteristic comet-head. Under these conditions, particles at the bottom of the pile were physically trapped in the separating tube, irrespective of their magnetic properties. To minimize this complication, it was necessary to limit the number of particles per separation to under 500.

Precautions had to be taken to prevent air bubbles from entering the separating tube. These dislodged particles as the menisci of the bubble passed over them, often carrying magnetic particles from the separating tube.

Magnetic separation of latex, chelex and AG 50W-X8

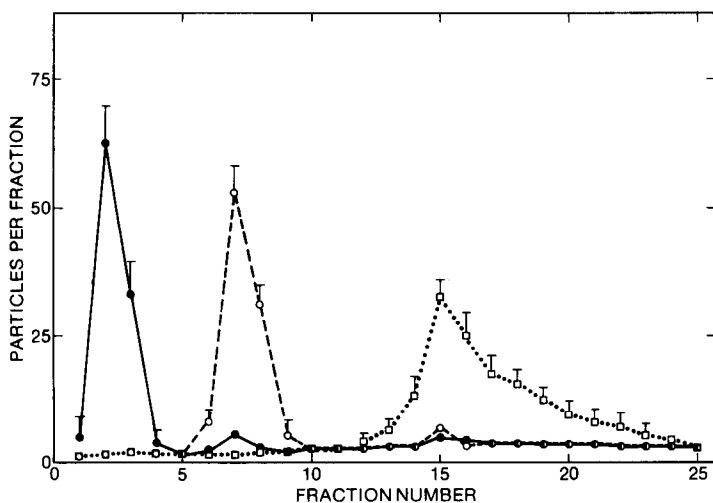A heterogenous mixture of latex, chelex and AG 50W-X8 was suspended in

Fig. 2. Separation of three types of particle by paramagnetic chromatographs. See text for details (n = 5). Key to symbols: ●, latex; ○, chelex; □, AG 50W-X8.

the magnetizing solution. As the particles would need to be identified at the end of the experiment, each class of particles was of a different size or colour. Thus in one group of experiments, red latex (diameter 6 μm) was mixed with 200–400 mesh chelex (diameter 75–150 μm) and minus 400 mesh AG 50W-X8 (diameter 38–63 μm). Although the results shown in Table I had demonstrated that size did not affect the magnetic behaviour of the particles, various other combinations of different sized particles were also used to confirm this.

The suspension of particles was pumped through the separating tube above the magnet and washed for 5 min with further magnetizing solution, before switching to 10 mM hydrochloric acid. After 5 min, the hydrochloric acid wash was replaced by a solution of 100 mM EGTA, pH 6.5, for a final 15 min exposure. Fractions were collected at intervals of 1 min, their contents identified by their size or colour, and counted with a haemocytometer.

Fig. 2 summarizes the results of the separation. During the first 5 min of the experiment, latex particles were virtually the only type of particle to elute. Only one or two particles per fraction of chelex or AG 50W-8X were seen. By 5 min, no further particles were eluting. Upon changing to a washing solution of pH 2, there was an abrupt release of chelex particles from the separating tube. A small number (1–3 per fraction) of latex particles also appeared at this time. These had presumably been formerly trapped by surrounding chelex. Elution of chelex was complete after 5 min washing with 10 mM hydrochloric acid. The remaining particles were eluted with EGTA. Unlike the elution of chelex with hydrochloric acid, particles of AG 50W-X8 were only liberated by EGTA following a lag of several minutes. Furthermore, the elution exhibited pronounced tailing. As before, small numbers of residual latex and chelex particles appeared in the AG 50W-X8 fraction.

Recovery efficiencies were over 90% and, as the standard deviation bars show, the separation was consistent from run to run.

DISCUSSION

Magnetic forces have found previous use in the isolation and separation of specific types of particles. Deoxygenated erythrocytes are weakly magnetic, and have been successfully isolated with high-gradient magnetic field[5,6]. Phagocytic cells have been removed from heterogenous cellular suspensions, following their engulfment of magnetic particles[7], while magnetic antibodies[8] or other magnetic affinity probes[9] have been employed to isolate cells with specific cell-surfasce antigens. Other designs have been applied to the capture of particles of $MnCO_3$ (ref. 10) ferritin-covered latex[11] and oxides of Mn, Cr and Al[12].

Our approach here is conceptually different from these examples. In each of the above examples, the particles behaved in an all-or-none magnetic fashion. In the present work, the magnetic susceptibilities of the particles have been experimentally manipulated for the purposes of separation. Designed only to demonstrate the principle of the method using standard particles, the present work shows how such manipulations can be achieved and exploited for paramagnetic chromatography. Changes in pH and the addition of soluble, competing chelators are just two ways of modulating the binding of Er^{3+} and thus magnetization. Although short term exposure to Ln^{3+} solutions is not toxic[13], the elution conditions for separations of biological materials will need to be milder than those employed in the present example. Such conditions include the addition of specific amounts of competing, nonmagnetic lanthanides or Ca^{2+}, and manipulations of the magnetic properties or ionic strength of the washing solutions. We have also found that elution is successful with lower (0.5–1 mM) concentrations of EGTA in saline, although the elution time is prolonged.

In addition to the method described here, which is applicable to the separation of discrete populations of particles, there exists a potential analytical function. In this case, particles of different magnetic susceptibility follow different trajectories on passing through the magnetic field, coming to rest at characteristic locations within the field. The ferrograph analyzer achieves this with the wear particles of engine oil[14] and, with less precision, with the wear particles of human joints after treatment with Er^{3+} (ref. 15).

ACKNOWLEDGEMENTS

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