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Separation of magnetic affinity biopolymer adsorbents in a Davis tube magnetic separator

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Abstract

A Davis tube (a matrix-free, flow-through magnetic separator used mainly in mineral processing) has been tested for separation of magnetic affinity biopolymer adsorbents from larger volumes of suspensions. Both magnetic chitosan and magnetic cross-linked erythrocytes could be efficiently separated from litre volumes of suspensions. Up to 90% adsorbent recovery was achieved under optimised separation conditions.

Introduction

Magnetic separation techniques have many interesting applications in various areas of biosciences and biotechnologies. These techniques are used especially in molecular biology for the separation of nucleic acids and oligonucleotides (Bosnes *et al*. 1997), in cell biology for the separation of target cells and cell organelles (Šafařík & Šafaříková 1999), in microbiology for the preconcentration of pathogenic microorganisms (Šafařík & Šafaříková 1999, Šafařík *et al*. 1995), in biochemistry for the isolation of various enzymes, lectins and antibodies (Šafařík & Šafaříková 2000) and in analytical chemistry for the preconcentration of the target analytes (Šafaříková & Šafařík 1999). In most cases the volumes of the treated samples are relatively low, ranging between tens of microlitres and tens of millilitres. Various commercially available magnetic separators can be used to concentrate magnetic particles from the treated samples (Šafařík & Šafaříková 1999).

Magnetic separation techniques are promising for various biotechnology applications (Dunlop *et al.* 1984, Setchell 1985). Modern biotechnology can benefit from the possible separation of the rare, biologically active compounds from difficult to handle samples (in some cases even wastes). Biomagnetic isolation techniques, due to their ability to perform adsorption process or affinity interaction with subsequent separation even in the presence of particulate contaminants, are of a special interest. Development of magnetic separation procedures enabling isolation of the magnetic adsorbents or carriers from large volumes of raw materials is of a great importance.

Large-scale magnetic separators of various types are routinely used in mining industry, raw material benefaction, ore and coal treatment and analysis etc. (Svoboda 1987). Laboratory magnetic separators employed in these industries are usually used to simulate particular separation process in a smaller scale or to analyse the magnetic separability of the tested samples. These devices could be used (either directly or only with small modifications) in biotechnology. In our experiments we tested a Davis tube magnetic separator (a matrix-free, flow-through magnetic separator originally used for the analysis of iron ores or

magnetite content in coal slurries after heavy media separation) for the separation of two types of magnetic affinity adsorbents.

Materials and methods

Materials

Magnetic chitosan particles (average diam. 47 μm; 25 mg Fe_3O_4 ml^{-1} sedimented adsorbent) and magnetic cross-linked bovine erythrocytes (average diam. 65 μm; 159 mg Fe_3O_4 ml^{-1} sedimented adsorbent) were prepared as described previously (Šafaříková & Šafařík 2000, Šafařík & Šafaříková 2001). The particles with low magnetic susceptibilities were removed by repeated static magnetic separation in flat tissue culture flasks placed in vertical position to the flat magnetic separator (Šafaříková *et al.* 1996) for 10–20 min. Strongly magnetic particles accumulated at the flask wall while weakly magnetic or non-magnetic particles accumulated at the bottom of the flasks or remained in the suspension and were removed.

Davis tube magnetic separator

The Davis tube magnetic separator consisted of an inclined glass tube (inner diam. 44 mm; outer diam. 49.5 mm; total length 780 mm; inclination 40° from vertical level) positioned between the poles of an electromagnet. The glass tube can be agitated (stroke 60 mm, rotation 68°, frequency 0.8 s^{-1}). The iron yoke (90 × 90 mm) was equipped with two pairs of coils. The diameter of iron pole pieces was 88 mm and they had a conical shape. Power supply (NB22A, produced by Elektropřístroj, Czech Republic) could be set to the voltage up to 30 V and the current up to 4 A. The suspension was fed to the upper part of the tube (filled with water) using a peristaltic pump. The bottom part of the tube was connected (via tubing) with an appropriate reservoir. A scheme of the separator is shown in Figure 1.

Separation of magnetic particles in Davis tube magnetic separator

An appropriate amount of magnetic adsorbent was suspended in water; total volume ranged between 1000 ml and 10 l. The mixed suspension was pumped to the water-filled glass tube using a peristaltic pump. Then 2 l of water were pumped in order to enable magnetic separation process to be finished and the non-magnetic particulate impurities to be washed away. The captured magnetic adsorbents were washed out from the column after switching off the magnetic field and the volume of the recovered material was measured.

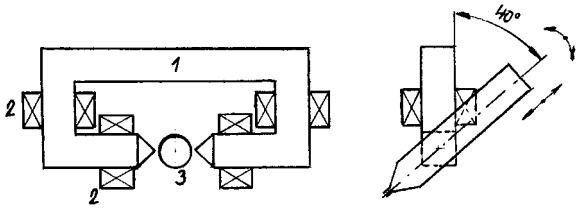

Fig. 1. Scheme of the Davis tube magnetic separator. Left, horizontal projection of the device with the removed glass tube; right, sight projection of the device with the glass tube inserted in the working position. 1 – iron yoke; 2 – two pairs of coils; 3 – glass tube holder.

Other procedures

Size distribution of the magnetic particles was determined using the particle size analyzer Cilas 920 L (France). Magnetic susceptibility was measured using the magnetic sensor MS2B (Bartington Instruments, UK). The volume of magnetic particles before and after magnetic separation was measured after 24 h of sedimentation at 1 g. The recoveries of magnetic particles after separations were calculated.

Results and discussion

The Davis tube magnetic separator is a laboratory machine originally designed to separate small samples of strongly magnetic ores into magnetic and non-magnetic fractions. It was developed in 1921 and no significant changes have been made in its design since that time. Although it is neither well-designed as a separator nor as an analytical instrument, it is widely used for estimating the amenability of iron ores to magnetic separation or for the analysis of magnetite content in coal slurries after heavy media separation (Svoboda 1987). Because this separator enables to handle litre volumes of suspensions, we studied magnetic separation of two types of magnetic affinity adsorbents previously used for the isolation of biologically active compounds.

Magnetic chitosan (magnetic affinity adsorbent already used for the isolation of *Solanum tuberosum* lectin (Šafaříková & Šafařík 2000)) and magnetic erythrocytes (affinity adsorbent for the isolation of proteolytic enzymes (Šafařík & Šafaříková 2001)) were

used for magnetic separation experiments. The size distribution of both magnetic affinity adsorbents was characterized using the particle size analyser. The data are given in Table 1.

In the separation experiments constant amounts of magnetic adsorbents (either 4.4 ml magnetic chitosan or 5.3 ml magnetic erythrocytes) in 1000 ml water were pumped at the constant flow rate into the glass tube of the magnetic separation system filled with water. In all experiments the electromagnet was run at 4 A and 30 V, giving the magnetic induction 144 mT in the centre of the gap. At lower flow rates (especially below 240 ml min^{-1}) part of the magnetic particles remained in pump tubings and in the upper part of the tube thus causing non-reproducible results. Reproducible capture of magnetic particles could be obtained at the flow rates equal or higher than 390 ml min^{-1}. The tube agitation, as well as the high flow rate, caused a higher leakage of magnetic particles and thus lower adsorbent recovery. The optimum flow rate for magnetic erythrocytes was about 390 ml min^{-1}, and for magnetic chitosan it ranged between 390–680 ml min^{-1}, without tube agitation. Under these conditions approximately 80% adsorbent recovery was reached. The dependence of recovery of magnetic adsorbents on the flow rate can be seen in Figures 2 and 3.

Non-magnetic and weekly-magnetic particles were removed from magnetic adsorbents used in the experiments using static magnetic treatment. Continuous separation in the Davis tube caused further removal of weekly magnetic particles thus lowering the overall recovery of the adsorbents. To increase their recovery we collected fractions of magnetic adsorbents captured within the separator tube under influence of magnetic field and performed the analogous experiments. As can be seen from Figures 2 and 3 the recovery of magnetic particles increased up to 85–90% under the optimal flow rate. The size distribution analysis showed that after magnetic pre-treatment in the Davis tube the average particle size increased (see Table 1). Determination of specific magnetic susceptibility has clearly shown higher values for the captured magnetic adsorbents (158×10^{-7} m^3 kg^{-1} for magnetic chitosan and 398×10^{-7} m^3 kg^{-1} for magnetic erythrocytes) than for the non-captured fraction (65×10^{-7} m^3 kg^{-1} for magnetic chitosan and 335×10^{-7} m^3 kg^{-1} for magnetic erythrocytes). The differences are caused by the non-homogeneous distribution of magnetite in the adsorbent particles and by the change of the particles diameter.

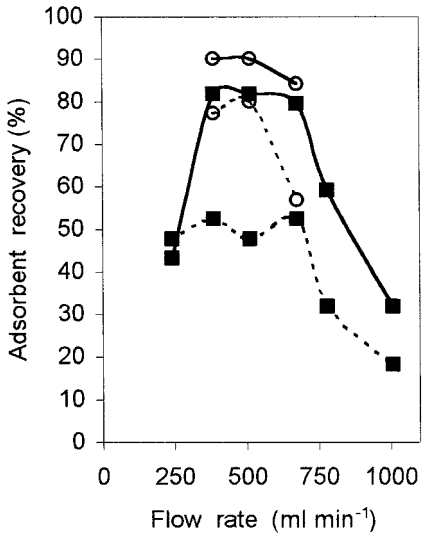

Fig. 2. Dependence of magnetic chitosan recovery on the flow rate during magnetic separation in the Davis tube separator. Magnetic chitosan (4.4 ml, sedimented volume; both original magnetic adsorbent and that one already captured during preliminary Davis tube treatment) was suspended in 1000 ml of water. The mixed suspension was pumped to the water-filled glass tube using a peristaltic pump. Then 2 l of water were pumped to finish the magnetic separation process and to wash the non-magnetic particulate impurities away. The magnetic separation was performed both with and without tube agitation. The captured magnetic adsorbent was washed out from the column after switching off the magnetic field and the volume of the recovered material was measured. The adsorbent recovery is expressed in percents of the applied adsorbent volume. Solid line – magnetic separation without tube agitation; dashed line – magnetic separation with tube agitation; filled symbols – original magnetic adsorbent; empty symbols – magnetic adsorbents after Davis tube treatment.

The Davis tube separator enabled sufficient preconcentration of magnetic adsorbents also from substantially larger volumes of suspensions. We tested separation of constant volumes of magnetic chitosan (7 ml) from 1, 2, 5 and 10 l of water suspension (flow rate 515 ml min^{-1}, without tube agitation). It was observed that with increasing the suspension volume the adsorbent recovery decreased – see Figure 4. Approximately 65% adsorbent recovery was achieved during magnetic separation from 10 l of suspension.

To determine the theoretical maximum capacity of the magnetic separator excess of both magnetic chitosan particles and magnetic erythrocytes was pumped through the system at the flow rate 600 ml min^{-1} without the tube agitation. The maximum capacity for magnetic chitosan was ca. 75 ml of the sedimented material, while in the case of magnetic erythrocytes this value reached ca. 30 ml.

Table 1. Size distribution of magnetic affinity adsorbents before and after the Davis tube treatment. D_{av} – average diameter of the particles; D_{10}, D_{50} and D_{90} – parameters indicating that 10, 50 and 90% of particles have a smaller diameter than the value given in the table.

Magnetic adsorbent	D_{av} (μm)	D_{10} (μm)	D_{50} (μm)	D_{90} (μm)
Magnetic chitosan (original suspension)	47	13	41	91
Magnetic chitosan (after Davis tube treatment)	64	21	57	116
Magnetic erythrocytes (original suspension)	65	10	52	139
Magnetic erythrocytes (after Davis tube treatment)	77	16	63	160

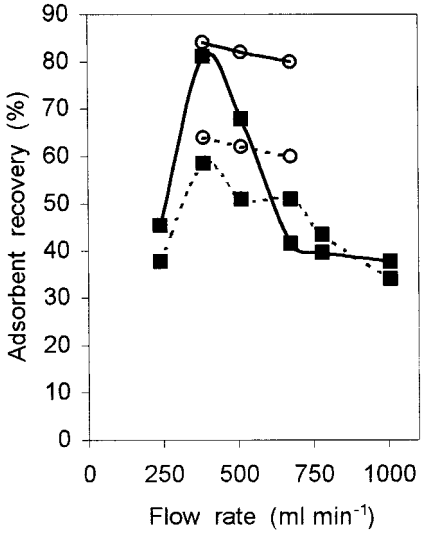

Fig. 3. Dependence of magnetic cross-linked erythrocytes recovery on the flow rate during magnetic separation in the Davis tube separator. Magnetic erythrocytes (5.3 ml, sedimented volume; both original magnetic adsorbent and that one already captured during preliminary Davis tube treatment) were suspended in 1000 ml of water. The magnetic separation conditions were the same as in Figure 2. Solid line – magnetic separation without tube agitation; dashed line – magnetic separation with tube agitation; filled symbols – original magnetic adsorbent; empty symbols – magnetic adsorbents after Davis tube treatment.

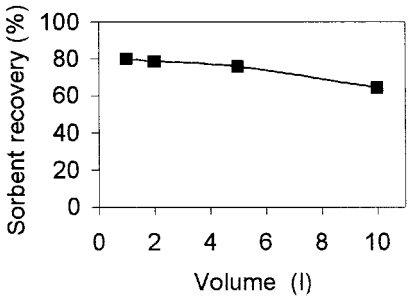

Fig. 4. Dependence of magnetic chitosan recovery on the volume of suspension fed to Davis tube separator. Magnetic chitosan (7 ml, sedimented volume) was suspended in 1, 2, 5 and 10 l water. The mixed suspensions were pumped to the water-filled glass tube at a flow rate 515 ml min^{-1} using a peristaltic pump. Then 2 l of water were pumped to finish the magnetic separation process and to wash the non-magnetic particulate impurities away. The magnetic separation was performed without tube agitation. The captured magnetic adsorbent was washed out from the column after switching off the magnetic field and the volume of the recovered material was measured. The adsorbent recovery is expressed in percents of the applied adsorbent volume.

As can be seen from the results, the Davis tube and probably other magnetic separators of the similar construction could be used for large-scale separation of selected magnetic affinity adsorbents. There is a clear advantage in using matrix-free magnetic separation systems for separations from suspension systems because the presence of a ferromagnetic matrix brings about the problems associated with keeping the matrix clean and passable long enough in real biotechnology applications. However, to use the tested matrix free magnetic separation system efficiently for real larger-scale magnetic separations it would be necessary to improve this system, especially to increase the magnetic induction within the system to enable efficient magnetic separation of magnetic adsorbents with lower magnetic susceptibilities. These improvements would enable more efficient separation of fine magnetic adsorbents, even using the agitation of the tube for efficient removal of diamagnetic particulate contaminants present in treated samples.

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