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Large-scale separation of magnetic bioaffinity adsorbents

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Abstract

Flat magnetic separator was used to separate magnetic bioaffinity adsorbents from litre volumes of suspensions. Both magnetic cross-linked erythrocytes and magnetic chitosan were efficiently separated; at least 95% adsorbent recovery was achieved at maximum flow rate (1680 ml min^{-1}). Using this system low amounts of trypsin were concentrated from large sample volumes using magnetic erythrocytes as affinity adsorbent.

Introduction

Magnetic separation techniques have many interesting applications in biotechnology (Dunlop *et al.* 1984, Setchell 1985, Šafařík & Šafaříková 1997). They can be efficiently used to isolate rare biologically active compounds from difficult-to-handle samples. These isolation techniques are able to perform the adsorption process or affinity interaction and subsequent adsorbent separation even in the presence of particulate diamagnetic contaminants. Development of magnetic separation procedures enabling isolation of the magnetic adsorbents or carriers from large volumes of raw materials is of great importance.

Laboratory-scale magnetic separators used routinely in mining or coal industry could be used for efficient separation of magnetic bioaffinity adsorbents. Davis tube magnetic separator (a matrix-free, flow-through magnetic separator used mainly for the analysis of iron ores) enabled to separate ca. 90% of biopolymer magnetic affinity adsorbents under the optimal conditions (Šafařík *et al.* 2001). As this magnetic separator is not readily available to researchers working in biotechnology we have tried to develop another large-scale separation process based on readily available, magnetic components. A preference was given to permanent magnets, due to the safety of manipulation while working with water solutions and suspensions. Several companies produce flat magnetic separators that are usually used to capture the coarse magnetic impurities from the stream of raw materials. Such magnetic separator forms the basis of a new magnetic separation system enabling magnetic bioaffinity adsorbents to be separated from litre volumes of suspensions.

Materials and methods

Materials

Magnetic cross-linked bovine erythrocytes (average diam. 65 μm; 159 mg Fe_3O_4 ml^{-1} sedimented adsorbent) and magnetic chitosan particles (average diam. 47 μm; 25 mg Fe_3O_4 ml^{-1} sedimented adsorbent) were prepared as described previously (Šafařík & Šafaříková 2001, Šafaříková & Šafařík 2000). 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.

Magnetic separation system

The separation system employs a flat magnetic separator constructed at SVUM, Prague, Czech Republic (Šafaříková *et al.* 1996). The separator has a trough shape (magnetic plate is placed in a construction made of steel sheet) with the dimensions 320 × 205 × 70 mm. The maximum values for normal and tangential components of maximum magnetic flux density are 0.19 T and 0.205 T, respectively. The steel sheet construction enables placement of a glass plate in a predefined height above the magnet surface. A plastic bag used for the collection of urine (volume 2 l, dimensions 210 × 190 mm, having both inlet and outlet tubes on the opposite sides, produced by Porges S.A., France; the filter placed at the inlet tube inside the bag was removed) was placed on the magnet surface and connected to the peristaltic pump or reservoir on one side and to the waste flask on the opposite side. The position of the glass plate determines the thickness of the bag after filling with water and the maximum distance from the magnet surface. The magnetic separation system is shown in Figure 1.

Separation of magnetic particles

An appropriate amount of magnetic adsorbent was suspended in water; total volume ranged between 1 and 10 l. The mixed suspension was pumped to the water-filled plastic bag using a peristaltic pump or the suspension flew to the bag from the reservoir placed above the separator. Then 0.5–1 l of water were pumped in to enable the 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 bag after its removal from the magnetic separator and the volume of the recovered material was measured. The magnetic separation can be performed using various thickness of the bag (changing the position of the glass plate) and two basic positions of the bag with respect to the flat magnet (above or under the magnet).

Isolation of trypsin

Bovine trypsin (Léčiva, Czech Republic; 20 mg) was dissolved in 3 l of water. Magnetic erythrocytes (20 ml; sedimented volume) were added and the mixture was incubated under occasional stirring for 1 h.

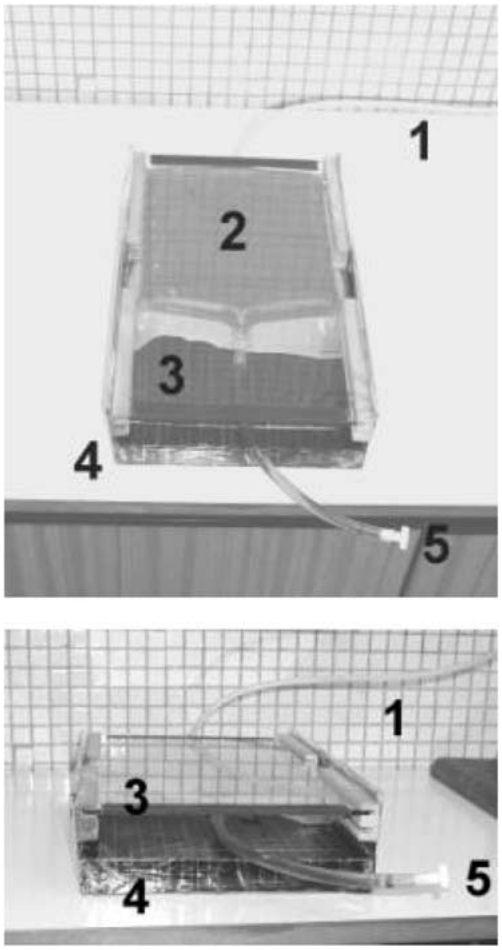

Fig. 1. Top and front views of the magnetic separation system. 1 – inlet tubing, 2 – plastic bag, 3 – glass plate, 4 – flat magnet, 5 – outlet tubing.

Then standard magnetic separation was performed, and the captured erythrocytes were washed with 1.5 l of water. Then all water was removed from the bag, and glycine-HCl buffer, pH 2.1 (40 ml) was added to elute the adsorbed trypsin. During elution step the bag was removed from the magnet. The eluted trypsin was recovered after placing the bag on the magnet and sedimentation of the adsorbent. The elution was repeated three times, the eluates we collected and trypsin activity was determined.

Other procedures

Size distribution of the magnetic particles was determined using the particle size analyzer Cilas 920 L (France). Trypsin activity was determined using BAEE (*N*-α-benzoyl-L-arginine ethyl ester; Sigma,

USA) as substrate. 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 and expressed in percents.

Results and discussion

Flat magnetic separators are commercially available from many companies. They are mainly used to capture magnetic impurities from a stream of raw materials. They are usually based on strong permanent magnets, which enable to use them without any risk during the manipulation with water-based solutions and suspensions. As such types of separators enable litre volumes of suspensions to be handled we have studied magnetic separation of two types of magnetic affinity adsorbents previously used for the isolation of biologically active compounds.

The magnetic separation unit described in this paper represents a matrix-free, flow-through system, where the separation and reaction chamber is constructed from plastic bags originally intended for the urine recovery. Such bags are inexpensive and can be changed easily. Almost all the area of the flat permanent magnet used was employed for the separation of magnetic particles. The thickness of the liquid-filled bag could be changed using the glass plate situated above the permanent magnet. This arrangement enables to change the magnetic capture characteristics of the separation system. It is also possible to change the orientation of magnetic and gravitational forces by changing the position of the bag in relation to the magnet (with the magnet below the bag, magnetic and gravitational forces act in the same direction; with the magnet above the bag, both forces are opposed). The latter arrangement is useful for the separation of magnetic adsorbents present in mixture with diamagnetic impurities.

Magnetic erythrocytes (affinity adsorbent for the isolation of proteolytic enzymes) (Šafařík & Šafaříková 2001) and magnetic chitosan (magnetic affinity adsorbent already used for the isolation of *Solanum tuberosum* lectin) (Šafaříková & Šafařík 2000) were used for magnetic separation experiments. Constant amounts of magnetic adsorbents (ranging usually between 2 and 4 ml) in 1000 ml water were delivered at the constant flow rate into the plastic bag of the magnetic separation system filled with water. Two various thickness of the bag and both magnet-bag arrangements were used. The recovery of magnetic particles was very high at all flow rates used, ranging between 95–100%. The dependence of recovery of magnetic erythrocytes on the flow rate can be seen in Figure 2.

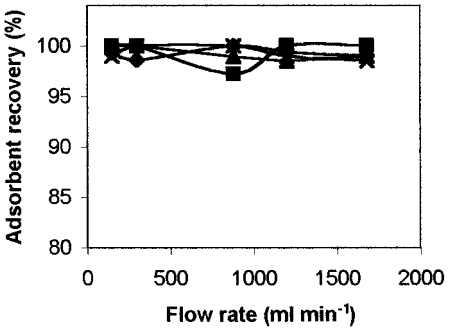

Fig. 2. Dependence of magnetic erythrocytes recovery on the flow rate during magnetic separation in the magnetic separator. Magnetic erythrocytes (3 ml) were suspended in 1000 ml of water. The mixed suspension was fed to the water-filled plastic bag. Then 0.5–1 l of water were pumped to finish the magnetic separation process and to wash the non-magnetic particulate impurities away. The magnetic separation was performed under various conditions (two values of bag thickness and two magnet-bag arrangements). The captured magnetic adsorbent was washed out from the bag after its removing from the magnet and the volume of the recovered material was measured. The adsorbent recovery is expressed in percents of the applied adsorbent volume. ♦, Bag thickness 18 mm, bag above the magnet; ■, bag thickness 30 mm, bag above the magnet; ▲, bag thickness 18 mm, magnet above the bag; ×, bag thickness 30 mm, magnet above the bag.

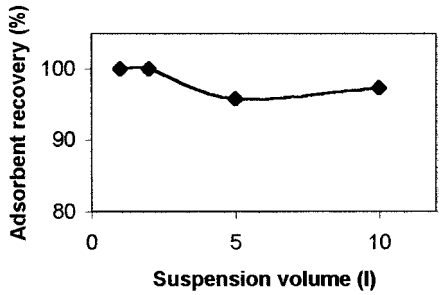

Fig. 3. Dependence of magnetic erythrocytes recovery on the volume of suspension fed to magnetic separator. Magnetic erythrocytes (3 ml, sedimented volume) were suspended in 1, 2, 5 and 10 l water. The mixed suspensions were fed to the water-filled plastic bag at a flow rate 1680 ml min^{-1}. Then 1.5 l of water were pumped to finish the magnetic separation process and to wash the non-magnetic particulate impurities away. The bag thickness was 18 mm, plastic bag was placed above the magnet. The captured magnetic adsorbent was washed out from the bag after its removing from the magnet and the volume of the recovered material was measured. The adsorbent recovery is expressed in percents of the applied adsorbent volume.

The flat magnetic separator produced sufficient pre-concentration of magnetic adsorbents also from

substantially larger volumes of suspensions. We tested the separation of constant volumes of magnetic erythrocytes (3 ml) from 1, 2, 5 and 10 l of water suspension (flow rate 1680 ml min^{-1}, bag thickness 18 mm, magnet below the bag). The suspension volume had almost no effect on the adsorbent recovery – see Figure 3. At least 95% adsorbent recovery was achieved during magnetic separation.

The plastic bag can serve not only as a separation vessel but also as a reactor. This means that, after magnetic separation, the captured magnetic adsorbents can be treated in an appropriate way (e.g., elution of adsorbed compounds) without the need to transfer them into another vessel. We demonstrate the isolation of low amount of trypsin from large volume of sample using magnetic erythrocytes as affinity adsorbent. The recovery of trypsin activity was 40%.

As can be seen from the results, flat magnetic separators could be used for large-scale separation of selected magnetic affinity adsorbents. The matrix-free magnetic separation system is suitable especially for separations of magnetic microparticles from suspension systems containing diamagnetic impurities. Magnetic separator can be used in two basic arrangements with respect to the positions of the permanent magnet and the plastic bag.

Plastic bags can be used both as separation and reaction vessels. Thus elution of the adsorbed compounds can be done very easily without the need of adsorbent manipulation. This one-bag procedure might be especially useful while working outside the laboratory. The bags can be also used for the magnetic suspension transport.

The described easy-to-use, portable and inexpensive magnetic separation system can find many interesting applications in biotechnology research or further development of magnetic solid-phase extraction (Šafaříková and Šafařík 1999).

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