Surface functionalized particles in Magnetic Field enhanced Centrifugation

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Introduction

A potential use of synthetic particles is as carrier of surface functionalization. As magnetic forces can be controlled easily, a magnetic core allows for control of the particles, notably their separation after use. Possible applications are the use for separation or catalysis similar to the use of packed beds in adsorption chromatography or catalysts[1, 2]. A process for the purification of proteins consists of the three steps adsorption, separation and elution (see Figure 1)[3]. The main advantage compared to packed beds is the higher use of the functionalization by dispersion and higher volume flow in the process. While beds are limited in particle filling size to micron scale even in High Pressure Liquid Chromatography, magnetic separation is possible in submicron size. Consequently the amount of functionalization which can be packed and used on magnetic particles is high. Magnetic particles are separated by High Gradient Magnetic Separation (HGMS). The approach chosen here is Magnetically Enhanced Centrifugation [4]. In this procedure, a centrifuge is built within an electromagnet. Inside of the centrifuge, a magnetic filter separates particles and is continuously cleaned by centrifugation. Particles are separated by magnetic forces on a wire matrix, slip off the wires by centrifugal forces and are collected at the centrifuge wall. The steps adsorption, separation and elution are performed subsequently in a magnetically enhanced centrifuge with electromagnet.

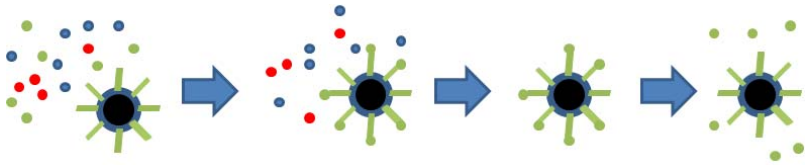

Figure 1: Different steps in separation

Particles usually consist of a ferromagnetic core (e.g. Fe_3O_4) and a Silica or polymer shell. Particle size to allow separation is usually above 200 nm and magnetization above 35 Am²/kg. Possible approachs in production are chemical germ formation, precipitation, rotating membrane pore extrusion, laser pyrolysis or a solution / spray process. On the surface ligands act as functionalization to adsorb proteins for separation. Common ligands are non-specific Silica or ion-exchange groups. For the latter the pH is set according to the isoelectric point of the target protein, which allows selective separation in feed systems with no contaminants. Selective functionalization like anti-body are possible, but expensive and currently not realistic in the scale of the machine. The particle behavior including viscosity is highly dependent on the magnetization of the particles and highly anisotropic. For particle production see different publications, e.g. [5, 6].

Objective

Objective is the separation of synthetic magnetic particles by combining centrifugation and magnetic filtration. Particles are controlled in the process by use of centrifugal forces and magnetic forces, allowing different procedures than classic HGMS devices or centrifuges do. Deposit of particles on the wire is kept low by centrifugal speed. The process is used for the separation of proteins by surface functionalized magnetic particles.

Methods

Experimental setup

All of the process steps involving particle transfer to a different liquid are done in one machine. Figure 2 shows the device (left) and the wire matrix (right) used in the machine. An electromagnet creates a magnetic field of up to 330 mT. A volume flow of up to 80 l/h during separation is applied. The centrifuge is used at 1500 rpm and an acceleration of 126 g, which is low for a centrifuge but acceptable for detaching upper particle layers from the matrix. The wire matrix serving as magnetic filter is produced by laser-cutting out of ferromagnetic stainless steel. Peeler knives are attached to the matrix for redispersion of the particles. A relative rotational velocity of 180 rpm of the matrix and the attached peeler knives is possible. The machine is sealed for sterile use. Preacceleration of the fluid is important to avoid turbulence, which impedes magnetic separation.

Separation efficiency is determined based on gravimetrical measurement. A suspension of 2 g/l demineralized water and magnetic particles is fed in the centrifuge at 1500 rpm and a magnetic field of 330 mT, except for the test done on 0 mT. After a stationary regime is reached, samples are taken.

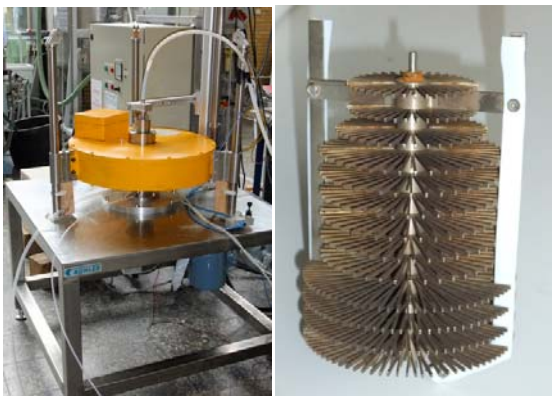

Figure 2: The magnetically enhanced centrifuge and the wire matrix including pealer knives

Separation of proteins to particles is possible as well in lab scale as in the centrifuge. In lab scale, tests were done on the separation of ovalbumin (45 kDa, pI 4.8) as model protein out of hen egg white. Hen egg white contains a high number of proteins, with ovalbumin being the one with highest proportion. In the process, the feed is filled in the empty centrifuge. For washing the centrifuge is filled at 600 rpm to cover the walls with liquid, peeler knives rotate for dispersion. Protein analysis was done as well based on SDS-Page. The particle amount is 6 g in 2 l of buffer and 25 g hen egg white. Adsorption was done outside of the centrifuge for 20 min. Then the feed was fed in the machine at 60 l/h, 1500 rpm and a magnetic field of 200 mT. In a washing step 1 l of washing water was fed in the machine.

Fluid was kept in the machine during washing by a rotational speed of 600 rpm for 3 min. Stirring was done at a differential speed of 112 rpm. The elution was done in 1 l 1 M NaCl solution for 10 min in the same way washing was done. Then the particles were flushed out by flushing the machine in a circle with 2 l of demineralized water at 240 l/h for 5 minutes 3 times during rotation of peeler knives and of the centrifuge at 600 rpm.

Results

Particle processing results

The separation of magnetic particles available as well on the market is possible at a separation efficiency of more than 99% up to 80 l/h at a magnetic field of 200 mT (e.g. Merck MagPrep TMA). Figure 3 gives an overview of separation efficiency for a different research grade particle kind at different volume flows. Those particles were chosen because of the fact separation is low enough to measure reliably separation efficiency at different parameters. Separation of particles degrades at high volume flows. Without magnetic field, separation is reduced to an amount of particles separated by centrifugal forces of 38% vs. 82% with magnetic field. In case of a magnetic centrifugation without matrix, separation efficiency is reduced to about 74%. This indicates that the agglomeration and separation of magnetic particles in itself is efficient. An industrial feed stream we tested containing a protein interesting for separation seems to be more efficient in separation. A possible reason for this is the fact particles in demineralized water agglomerate less due to higher repulsion of the steric layer, while the feed stream at a conductivity of about 10 µS/cm allows easy agglomeration.

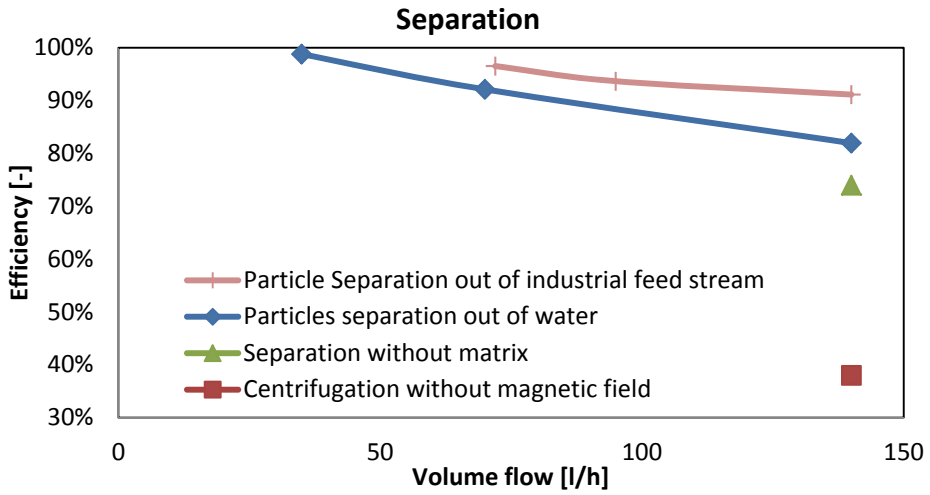

Figure 3: Separation efficiency of the centrifuge out of water / industrial stream in food industrie; 1500 rpm, 70 l/h, 2 g/l particles; magnetic field of 0 / 330 mT

Particle adsorption

The separation was first optimized in lab scale on purity and separated target protein proportion for different pH values, particle concentrations, binding time and elution conditions. Tests showed highest efficiency for a particle amount of 3 g/l (see Figure 4), with a minimum of target protein lost and negligible separation of different proteins. While at lower concentration a part of the target protein is lost, at higher concentration different proteins adsorb. A pH of 6.0 showed to be most

efficient with few other proteins binding, which was expected for an isoelectric point of 4.8 of ovalbumin.

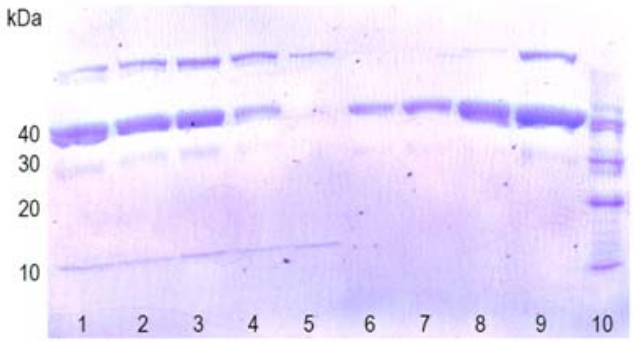

Figure 4: Adsorption at different concentrations in lab scale; 1: hen egg white diluded; 2: adsorption 0.5 g/l; 3: adsorption 1 g/l; 4:adsorption 3 g/l; 5:adsorption 10 g/l; 6: elution 0.5 g/l; 7: elution 1 g/l; 8: elution 3 g/l; 9: elution 10 g/l; 10: Marker

Separation of lab scale could be reproduced in the centrifuge. Lost particle amount was below detection limit (<99%). However a significant amount of target protein was flushed out in the washing step, while no contamination was detected, so the washing step may be omitted. In this case only 65% of particles corresponding 3.9 g were discharged. While this is a low percentage, in different experiments of particle amounts of 20 g and above discharge of 92% and more was possible.

Conclusion

Separation of magnetic particles, dispersing and their discharge in a Magnetically Enhanced Centrifuge was shown. The process can be applied for the automatic separation of protein.

Acknowledgment

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