Chapter 13
Conclusion

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The separation of proteins by High Gradient Magnetic Filtration is a promising process for the purification of media. Its success depends on four different elements: the *synthesis* of coated particles with magnetic core, a selective *functionalization* which needs to be adapted to adsorb the target matter and exclude contamination, the *separation devices* which need to fit for the process in scale, budget and separation characteristics, and the *process design*. All of these elements need to fit together and to be available at a reasonable price.

Competing technologies comprise expanded and packed bed adsorption. The core advantage of magnetic separation is the fast kinetics, similar to expanded bed adsorption, but in a continuous process combined with an easier process control and a high flow rate. The recovery rate of the adsorbent achieves, depending on the particle magnetization, is more than 99.9 %.

13.1 Particle Synthesis

For particle synthesis, different methods were presented. The core may be produced, e.g., by laser pyrolysis, which is expensive but allows an exact adjustment of properties. The particle size and the chemical content can be influenced by this method. The size variation is very narrow, allowing the production of highly defined particles.

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Another possibility is co-precipitation which showed to be versatile for the production in the range of 6–10 nm with particle clusters without any remanence. The synthesis of microgels allows as well a precise design. Microgels were obtained by achieving colloidal stability of a magnetic fluid. Dynamic membrane pore extrusion allowed the formation of vesicles; in this case, the magnetic core is encapsulated in a fatty acid for a specific coating. Spray drying results in a low-cost and an easy scalable process. It is well suited for cheap, large-scale synthesis of particles for industrial use in the 100 g scale. Mixing aqueous solutions of iron salts under alkaline conditions is possible in a continuous process and was performed in the project for the synthesis of magnetic particles in kilogram scale. The system is now on the market commercially and seems from a comparison to the other synthesis approaches to be a viable option. There are as well commercial particles available from numerous producers.

13.2 Functionalization

Synthesized particles were functionalized with an unselective silica, low selective ion exchange, and high selective antibody adsorbent. Cheap and low selective functionalization showed to work well in several, but not in all applications. Notably in the in-situ pilot-line presented, particles with a silica functionalization could be additionally shielded to prevent bacteriae from adsorbing, which resulted in an interesting process. In the food side stream, low selective functionalization did not show to be efficient though, with a nonprotein contaminant strongly reducing the activity. Antibody functionalization was developed and tested successfully. An economic analysis showed this adsorbent to be too expensive though for an implementation in the industrial pilot line.

In general, for most target systems the particle functionalization needs to be custom-made. A consequence is that in a HGMF process, the development should start with the pairing of product system and adsorbent. This is crucial and needs to be finished before the line development starts, as it directly impacts on the necessary pretreatment and further purification steps. The adsorbent hence influences the overall process design and performance.

13.3 Separation Devices

Three different separation approaches were presented, additionally a Rotor-Stator called device is on the market. They are summarized in Table 13.1. The modeling and simulation by the Discrete Element Method of particle agglomeration, deposition and detachment from magnetic wires showed the formation of needle-shaped agglomeration as expected in High Gradient Magnetic Separation. One variant is Magnetically Enhanced Centrifugation, which allows the continuous separation of particles by cleaning a magnetic wire filter by low centrifugal forces.

Table 13.1 Comparison of devices

Device	Investment cost scale (€)	Tested scale
Rotor-stator HGMS	50 000	Batch-wise 200 g particles
Halbach-based HGMS	10 000	Batch-wise 200 g particles
Continuous magnetic extraction	10 000	Continuously 10 l/h
Magnetically enhanced centrifugation	100 000	Continuously 50 l/h (to 1 m^3/h in a batch test)

It is specifically designed for large volume flows. Despite it is possible to design the device based on a permanent magnet arrangement, it is in investment more expensive than the "static" Halbach-based High Gradient Magnetic Separation and Continuous Magnetic Extraction. It allows continuous processing of high volume flows, in a significantly larger scale than the competing processes.

A Halbach-magnet-based High Gradient Magnetic Separation reactor is robust and cheap, based on permanent magnets, which allows batch-wise separation. It is easily scalable and usable in parallel. Additionally, it has a cylindrical cell including a stirrer and can therefore be used as reactor for consecutive washing and elution steps without further tank-to-tank transfer.

Continuous Magnetic Extraction allows the chemical separation of particles and adsorbed media. It is cheap investment-wise, based on a permanent magnet. It is slower compared to High Gradient Magnetic Separation variants such as Halbach-based High Gradient Magnetic Separation and Magnetically Enhanced Centrifugation in terms of the necessary residence time but allows the separation of small, colloidal particles. It needs the addition of tensides, but performs the extraction step not just as a magnetic separation but simultaneously a chemical separation step.

Two continuous approaches were developed within the project. With some optimization and refinement for industrial use, Magnetically Enhanced Centrifugation and Continuous Magnetic Extraction present an interesting option for the processing of large volumes.

13.4 The Processes

The particles synthesized as well as the devices set up were tested with industrial suspensions. Two different processes were presented, one being the separation of Phytase out of fermentation broth by In Situ-Separation to enhance the fermentation. The particles used were coated with nonselective Silica, combined with a coating excluding bacteriae. It showed to be possible to separate proteins in situ. The process was continued after separation, resulting in an increase of the process yield.

The separation of a protein as a pharmaceutical and food additive from a waste stream of the soy industry was an attempt to move to a large scale and set up an industrial process. Anion exchanger functionalized particles were used in combination with a pretreatment eliminating contaminating proteins. The process design and separation was possible, and several grams of protein could be separated by processing a significant amount of magnetic particles per batch. The economic perspective was reduced through the low selectivity of the particles and the activity reduction of the particles by nonprotein contaminants. A more sophisticated and more selective ligand was not available at process scale and would strongly increase the process cost. A ligand fitting to the system at a reasonable price would be the key to an economically viable process.

Recycling of particles showed to be effective in small-scale tests, and particles did not show to change behavior in repeated large-scale environments. Loss of particles in dead zones and walls of tubes, valves, and devices showed to be significant. This depends as well on the functionalization, which made specific particles adhere to walls. Elution is critical, as incomplete elution reduces the overall processes efficiency. Especially on selective particles, elution is nontrivial. The separation of the target protein from the elution medium is another challenge. Excessive washing showed in the current process to reduce the amount of the salt used for the elution, which resulted in a much higher concentrations compared to the target protein. The process advantages are the possibility for continuous use and the low number of process steps.

13.5 Outlook

The technology is in an evolved state with several different approaches available for the synthesis of particles. While some of the approaches allow an exact structuring of the particle sizes such as laser pyrolysis, some approaches can be scaled economically to a large scale, specifically taking the economic perspective into account. Particle prices for diagnostics do not apply, as the large scale results in a significant reduction of costs. Nonspecific functionalization is available at an acceptable price. Silica-coated and ion-exchange particles were produced and used effectively. Silica-coated particles delivered good results in the in situ phytase separation, after modifying the particles to exclude the adsorption of bacteriae. Cation-exchange SO_3 particles showed to be efficient as well. Anion exchange TMAP-functionalized particles were used in the industrial line for separation.

Specific functionalization showed to be nonrealistic for the current production lines. While antigen functionalization works, it was not available at process scale and exceeded the financial scope of the project. A consequence is that the choice of the process depends on the availability of cheap functionalization. A theoretic comparison showed that magnetic fishing in the current process, while not economic, is competitive with different processes like packed bed adsorption. For an economic perspective, the market price of the product and the costs of the functionalization decide on the potential of the process. In different processes it might make sense to take profit of the fast and continuous magnetic separation instead of conventional packed bed adsorption. In theory this allows much lower cycle times, an easy exchange and storing of functionalization.

Several magnetic separation devices are available at different process scales. The choice depends amongst other things on the scale and the regime of the process—batch-wise or continuous. A continuous large-scale process is possible based on Magnetic Enhanced Centrifugation. In the pharmaceutical industry, batch processes are more common, which is possible by a Halbach-based High Gradient Magnetic Separation reactor with the advantage of higher volume flow and easy exchange of functionalization compared to a packed bed. Magnetic extraction allows simultaneous magnetic and chemical separation.

The integration of these technologies in pilot lines was successful. While not every element worked out perfectly, there is no major obstacle left for a complete process integrating the developed separation devices and core particles.

Overall it could be shown that a continuous magnetic separation process can be realized in a production scale. There are still some limitations, which have to be explored for an efficient and economic use. It is now the challenge of interested companies to implement this technology into their manufacturing sites for the production of innovative, new products.