

# Magnetic nanoporous microparticles for biosensors and bioreactors

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Magnetic nanoporous silica microparticles (MMPs) are increasingly popular supports for enzyme immobilisation and for use in flow-through bioassays systems and biosensor configurations. The high specific area of MMPs allows the immobilisation of large amounts of enzyme in a protective matrix. MMPs can be trapped by a magnetised physical transducer, or injected and retained in a microflow system in close proximity to a detector. High sensitivity and selectivity can be achieved in assays of analytes in complex samples. The use of MMPs has also great potential value in fields other than bioanalysis, such as bioremediation and medical applications, but these are not covered in this article.

The performance of enzyme-based biosensors and bioreactors in bioanalytical applications can be considerably enhanced through judicious exploitation of the properties of magnetic microparticles by allowing enzyme reactions to occur close to the detection system [1 and references cited therein]. Successful commercially-available instruments (e.g. from BioVeris Technology) exist that are based on such concepts. They combine microflow devices with superparamagnetic beads that are coated with a biocomponent that allows the detection by electrochemiluminescence of analytes such as antigens, drug compounds, target DNA etc. Depending on the label used (antibody, protein, oligonucleotide, etc.) the coated beads can be used in immunoassays, protein binding studies or nucleic acid-based interaction studies. Typical applications for such systems are in the fields of life sciences, clinical diagnostics, environmental testing as well as in industrial and biodefense markets, etc. One major attraction of using magnetic micro or sub-micro-sized beads is that they can be readily removed from the reaction medium by a magnet, allow-

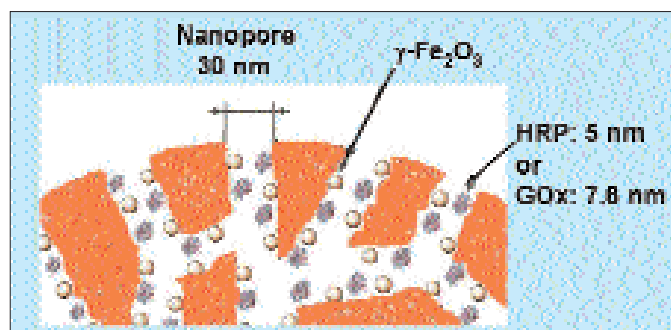


Figure 1. Schematic view of the nanoporous magnetic silica microparticles (MMPs).

ing subsequent assays to occur in a "clean" microenvironment thus improving sensitivity and selectivity. Generally, magnetic beads have an iron oxide core tightly coated with a functionalised polymer. Well defined chemistries are available for the binding to the beads of either ligands such as streptavidin or oligo cDT, or of specific recognition groups such as antibodies. The beads are generally non porous, allowing non restricted diffusional access to the immobilised label. Many fields of applications can benefit from the use of such magnetic beads either in biosensor configurations [1-3] or in micro and nanofluidics [4]. In this article we discuss the potential of porous magnetic beads as "microreservoirs" for enzyme entrapment in biosensor and bioreactor configurations.

## Nanoporous magnetic silica microparticles

Thanks to their high specific area, inherent stability, ease of functionalisation and biocompatibility, porous silica-based microparticles are well established matrices for enzyme immobilisation [5]. The combination of their porous, rigid structure and their chemistry provides a protective environment for the entrapped enzyme,

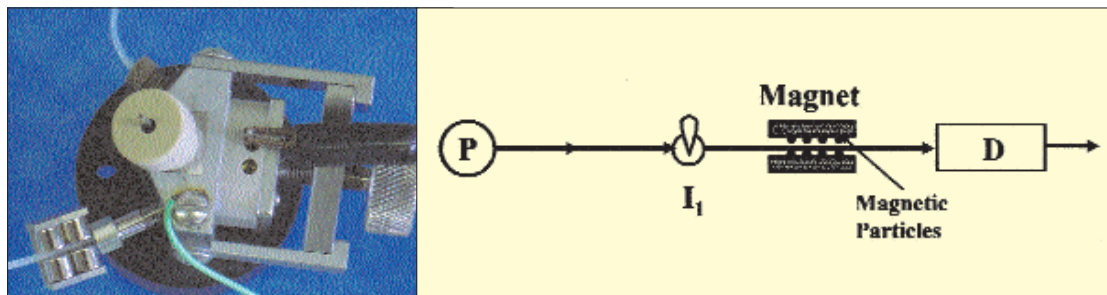


Figure 2. Aerial view of a couple of magnets surrounding a Teflon microtubing (250 µm i.d.) at the inlet of an electrochemical detector and schematic view of the corresponding flow injection set up.

thus providing increased resistance to degradation and inactivation that could otherwise be brought about by pH changes, temperature effects, proteolysis or autolysis, etc. The enzyme can be either physically or chemically immobilised onto the silica support, thus minimising subsequent leaching from the particles. In addition large quantities of enzyme (25-30 mg/g matrix) can be immobilised in a relatively small microenvironment. (It should be noted, however, that all these theoretical advantages can sometimes be partially negated in practice by factors such as restricted diffusion and/or poor access of the substrate to the active site of the enzyme). Porous silica particles can be readily magnetised by chemical deposition of maghaemite nanodots ( $\text{Fe}_3\text{O}_4$ - $\gamma\text{Fe}_2\text{O}_3$ ) inside the pores of the particles without affecting the nanopore network [Figure 1] [6]. Such particles can be prepared on a large scale and in a well characterised manner and are available commercially (Fuji Silysia Chemical Ltd., Kasugai, Japan). Different pore sizes can be obtained (from 4 to 90 nanometers diameter) with specific surface areas ranging from 40 to 700  $\text{m}^2/\text{g}$ . Since many enzymes have effective hydrodynamic diameters ranging between 2 and 10 nm they can easily be entrapped within the nanopores of the silica-based magnetic microparticles (MMPs).

Silica particles with diameters ranging from 3 to 10  $\mu\text{m}$  have been used, but the smaller particles are generally preferred since they show slower settling out of suspension and are also suitable for assays in microfluidic systems. MMPs are superparamagnetic (i.e. retain no residual magnetism after the magnetic field is removed) and are strongly attracted by a permanent magnet which can be located in a classical flow injection system (FI), in a capillary electrophoresis set up, in miniaturised lab-on-chip devices or at virtually any position in a flow line.

## Bioreactors

To evaluate their performance in FI systems, MMPs on which glucose oxidase (GOX) had been immobilised, were injected in a flow injection manifold (injection valve I1) followed by injection of the glucose sample to be analysed [6]. The MMPs were retained by the magnet(s) and the enzymatically-liberated hydrogen peroxide was detected by a platinum working electrode [Figure 2]. This configuration offers good reproducibility of the recorded signals (RSD below 1.5%) and enables quantification down to micromolar glucose concentrations. Hundreds of injections can be carried out with the same MMP reactor. The storage stability of the enzyme-immobilised MMPs depends on the particular immobilisation methods used as well as on the characteristics of the enzyme, but periods of longer than five months stability have been reported [6, 7]. If necessary the MMPs can be readily washed away by removing the magnets or by increasing the flow rate. There is minimal consumption of biocomponent and the enzyme-immobilised MMPs do not adhere to the inner wall of the microflow channel, in contrast to the situation occurring with soluble enzymes.

The kinetic and diffusion characteristics of the substrate with respect to the enzyme-immobilised MMPs mean that the flow rate has an important effect on signal intensity. In the above example optimal signal intensity was observed at flow rates below 5  $\mu\text{L}/\text{min}$ . High selectivity can be achieved in such conditions by carrying out differential measurements i.e. by recording the signal in both the presence and absence of the MMPs. This is of particular use when studying complex samples. Basically any type of detection system that can be coupled on line (e.g. electrochemical, optical, mass spectrometry) can be considered.

## Biosensors

As mentioned above, MMPs can also be attracted onto a magnetised electrode for the electrochemical monitoring of the product of an enzymatic reaction at the electrode-solution interface [Figure 3]. In contrast to other electrochemical biosensor designs, either naked or modified electrodes can be used. In addition, the enzyme-loaded MMPs are readily trapped at the electrode surface and can easily be renewed if necessary.

Interestingly, the MMPs do not form a barrier that could block the diffusion-based access of the analyte to the electrode surface. Only a 6% decrease of the voltamperometric signal was detected in the presence of the microparticles compared to the naked electrode. In a study of the biotransformation of pharmacologically interesting compounds using a biosensor based on MMPs immobilised with horseradish peroxidase (HRP), it was observed that the efficiency of peroxidation at the HRP-MMP electrode was 35% [8].

Numerous procedures are available for enzyme immobilisation on silica. In this context, it was recently observed that the deposition of gold nanorods in the MMPs, and subsequent functionalisation by aminothiols, provided useful anchoring sites for stable enzyme loading [1]. MMP-based biosensors allow both the quantitative assay of enzyme substrates and cosubstrates and also allow inhibition studies to be easily carried out. Such studies have clearly shown that the immobilised enzyme is protected from inactivation by inhibitors. Thus, in experiments involving the screening of thiol compounds known to react with a quinoneimine structure generated in a HRP-MMP based biosensor, it was found that the immobilised enzyme was less sensitive to thiol inhibitors than soluble HRP enzyme [8]. Electrodes of different sizes and configurations can be used in addition but physical transducers other than electrodes, such as vibrating quartz crystals and optical wave guides are also suitable for use in MMP trapping and biorecognition assays.

## Conclusion

Nanoporous magnetic silica microparticles allow the loading of high amounts of enzymes into a protective microenvironment, enabling a variety of bioanalytical applications to be carried out. Currently some

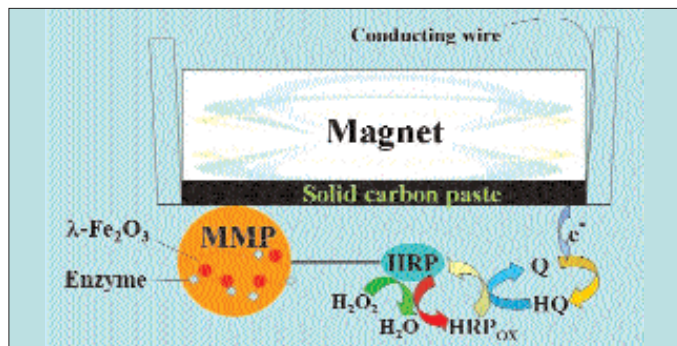


Figure 3. Diagram of a magnetised carbon paste electrode with a trapped MMP, showing oxidation of hydroquinone (HQ) to quinone (Q) by the enzyme HRP in the presence of hydrogen peroxide, and subsequent electroreduction of the quinone (Q).

efforts are being directed towards the development of biomimetic silica nanospheres for improved enzyme immobilisation [9].

In short, magnetic beads in general are attracting ever-increasing interest for use in bioanalysis applications. This is due not only to the commercial availability of well defined functionalised nano and microparticles but also because sensitive and selective high throughput assay systems can be achieved [4,10] when the microparticles are integrated into microfluidic or microchip set-ups

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