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Synthesis and characterization of robust magnetic carriers for bioprocess applications

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ABSTRACT

Magnetic carriers are an effective option to withdraw selected target molecules from complex mixtures or to immobilize enzymes. This paper describes the synthesis of robust silica magnetic microparticles (SMMps), particularly designed for applications in bioprocesses. SMMps were synthesized in a micro-emulsion, using sodium silicate as the silica source and superparamagnetic iron oxide nanoparticles as the magnetic core. Thermally resistant particles, with high and accessible surface area, narrow particle size distribution, high saturation magnetization, and with superparamagnetic properties were obtained. Several reaction conditions were tested, yielding materials with saturation magnetization between 45 and 63 emu g^{-1}, particle size between 2 and 200 μm and average diameter between 11.2 and 15.9 μm, surface area between 49 and 103 m^2 g^{-1} and pore diameter between 2 and 60 nm. The performance of SMMps in a bioprocess was evaluated by the immobilization of *Pseudomonas fluorescens* lipase on to octyl modified SMMp, the biocatalyst obtained was used in the production of butyl butyrate with good results.

1. Introduction

Nanoscale materials exhibit unique properties that differ from those of their bulk counterparts. Superparamagnetic iron oxide nanoparticles (SPION) have attracted great interest because of their good biocompatibility, low toxicity, ease of synthesis, low cost of production and superparamagnetic properties [1,2]. Superparamagnetism is a particular class of magnetism present in nanoscale ferromagnetic and ferrimagnetic materials [1,2]. Superparamagnetic nanoparticles are attracted to a magnetic field but retain no residual magnetism after the removal of the field. Thus, suspended superparamagnetic nanoparticles can be separated easily from a reaction mixture, by applying an external magnetic field [3].

The magnetic removal of particles from a solution is more selective and can be quicker and more efficient than centrifugation or filtration [3,4]. In this way, magnetic separation can be used as a quick and simple method for the efficient capture of selected target species even in complex biological mixtures. Over the last years many studies concerning the applications of magnetic carriers in protein purification [5–7], enzymes immobilization [8–11] and cell separation [12,13] have been performed. However, just a few of these carriers have had real applicability in industrial scale. Interestingly, one of the main difficulties for the application of magnetic materials in industrial bioprocesses lies on the fact that these carriers were not actually designed for this purpose [4]. In fact, despite the great number of commercially magnetic carriers available for applications in biomedicine, diagnoses and lab scale biotechnology, there are no commercial carriers specially designed for industrial, large scale use [5].

Ideally, a carrier for bioprocesses industrial applications (e.g. enzymes immobilization or protein purification) should be

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thermally and chemically stable, mechanically robust and resistant to microbial attack. Besides, it should provide high surface area, with mesoporous or macroporous structure, readily activatable with different functional groups and inexpensive. In addition, an industrial magnetic carrier should present magnetic stability, high saturation magnetization and particle size large enough to enable its efficient recovery from the reaction medium [14]. It should be stressed that, since the magnetic force acting on a particle in a field gradient is proportional to the particle volume, small sizes reduce the efficiency of magnetic capture [3,4,14].

A SPION exhibits high saturation magnetization at room temperature, however, due to its high surface/volume ratio they are highly susceptible to oxidation producing non-magnetic iron oxide forms [1,15]. Thus, to ensure the SPION magnetic stability it is necessary to coat the surface of the material with a stable substance. In this context, SPION coating with silica is a good option to generate materials with high stability, nontoxic and biocompatible [2]. In addition, the silica shell can be activated with a wide range of functional groups, just using commercially available silicon alkoxides to immobilize specific ligands. The most common methodology for SPION coating with silica is the Stöber method [16], where a silica shell is formed by reactions of hydrolysis and poly-condensation of a silicon alkoxide precursor. However, silicon alkoxides are expensive, hazardous and the process for SPION coating using these reactants is time consuming [17]. The alternative use of the sodium silicate as silica source for SPION coating may be a good option, since this reactant is cheap and nontoxic. This strategy has been investigated in some studies [2,14,18]. Sodium silicate dissolution in water releases sodium ion and the reactive silicic acid ($Si(OH)_4$). However, because of the high reactivity of silicic acid in aqueous solution, the reaction control is difficult [19,20].

The use of reverse micelles may be an option to control the final particle structure. Reverse micelles are micrometer- or nanometer-sized droplets containing a polar solvent, like water, stabilized by action of a surfactant and dispersed in a non-polar solvent where reactions can be conducted. Thus, reverse micelles can work as micro- or nanoreactors. Through the use of reverse micelles it is possible to control the growth of the silica particles, its morphological structure and its pore structure [17,21–23]. This approach has been used for the synthesis of silica micro and nanoparticles [17,21] and for the synthesis of silica magnetic micro and nanoparticles [2,18,22–27], mainly using silicon alkoxides as silica source.

This paper describes the synthesis of robust silica magnetic microparticles (SMMPs) specially designed for applications in bioprocesses. SMMPs were obtained in reverse micelles nanoreactors using inexpensive sodium silicate as silica source. The SMMPs prepared following this methodology have high SPION concentration, mesoporous structure, high and accessible surface area, narrow particle size distribution and very high saturation magnetization.

2. Materials and methods

2.1. Materials

For SPION preparation were used $FeCl_3{\cdot}6H_2O$ 99% (Sigma–Aldrich, USA), $FeCl_2{\cdot}4H_2O$ 99% (Sigma–Aldrich) and NH_3(aq) 28% (Vetec, Brasil). For SMMps synthesis, sodium silicate (Na_2SiO_3), Triton-X 100 (laboratory grade), cyclohexane 99%, all from Vetec, and ammonium sulfate 99% (NH_4SO_4) from JT Baker (USA). For the SMMp surface modification triethoxy(octyl)silane 97% (OCTEO) and toluene 99% from Sigma–Aldrich were used. For the lipase immobilization experiments were used commercial lipase from *Pseudomonas fluorescens* (PFL), bovine serum albumin and tributyrin all from Sigma–Aldrich. For the synthesis of butyl butyrate were used butyric acid (99%), 1-butanol (99%) and heptane (99%) from Sigma–Aldrich and commercial immobilized lipase IMMAPF-T2-150 (*P. fluorescens*) from ChiralVision (The Netherlands). Other reagents of analytical grade were also used.

2.2. Methods

2.2.1. SPION synthesis

Solutions of $FeCl_3{\cdot}6H_2O$ 350 mM (1000 mL) and $FeCl_2{\cdot}4H_2O$ 200 mM (1000 mL) were prepared using distilled water previously boiled by 20 min for the removal of the excess of oxygen. The solutions freshly prepared were mixed in a jacketed reactor under mechanical stirring, then 250 mL of NH_3(aq) 28% (m/m) aqueous solution was added using a peristaltic pump. The resultant solution was stirred at 60 °C and 1000 RMP by 1.0 h. The SPIONs formed were magnetically recovered, washed using distilled water and stored until their use.

2.2.2. SMMP synthesis

For the SMMPs synthesis two solutions were prepared, solutions A (*SA*) and B (*SB*). *SA* was prepared by dissolving sodium silicate in 100 mL of distilled water to the final concentration of 0.5, 1.0 or 1.75 M; next, the sodium silicate solution was dispersed in 350 mL of cyclohexane, containing 25, 50 or 100 mL of Triton-X 100, under vigorous stirring to form a stable emulsion. In a similar way, *SB* was prepared by the addition of 100 mL of a ammonium sulfate, 0.5, 1.0 or 1.75 M, to 350 mL of cyclohexane containing Triton-X 100 (25, 50 or 100 mL). A mass of SPION was added to *SA* and the mixture was vigorous stirred by 3 min, then *SB* was transferred to the reactor using a peristaltic pump (flow rate – 100 mL min^{-1}). After the total addition of *SB* to the reactor, the resulting mixture was kept under stirring (1000 RPM) at 25 °C by 1 h. The formed SMMPs were magnetically recovered and washed thrice with an acetone/ethanol solution (1:1 v/v) and twice with distilled water. After the washing process, the SMMPs were incubated in HCl solution (0.5 M) at 25 °C by 16 h, next the particles were washed twice with distilled water and stored until their use.

2.2.3. Instrumental analysis

X-ray diffraction (XRD) patterns were obtained using a Shimadzu LabX XRD-600 apparatus with a Ni filtered CuKα1 (λ = 1.5406 Å) radiation source, operating at 30 kV and 30 mA. The samples were scanned with the 2θ angle ranging from 20° to 80°, with a step size of 0.02°.

The Fourier-transform infrared spectroscopy (FTIR) analysis was performed in an FTIR-8400S apparatus (Shimadzu). The samples were diluted in KBr and pressed to form pellets. Spectrograms were recorded in the 4000–400 cm^{-1} range, with a resolution of 2 cm^{-1}.

The magnetic properties of SPION and SMMPs were measured using a superconducting quantum interference device (SQUID) magnetometer (Quantum Design). The hysteresis loops were measured in magnetic field (H) between ±70 kOe, at 300 K.

The SMMPs morphology was investigated using a Philips XL30 FEG microscopy operating at 25 kV. Aqueous suspensions containing SMMPs were deposited onto an aluminum sample holder, dried at ambient temperature and gold-coated. The scanning electron microscopy (SEM) images were recorded at distinct magnifications. The particle diameter distribution (Feret's diameter) was estimated by image analysis using the ImageJ software, appropriate SEM images were used and at least 300 particles were counted by image [48]. The energy dispersive X-ray spectroscopy (EDS) was performed in the same equipment using an Oxford Tetra Link detector.

Thermogravimetric measurements were done using a TA SDT 2960 equipment from TA Instruments. Samples were heated in open α-alumina pans from ambient temperature to 600 °C under