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Low-temperature magnetic modification of sensitive biological materials

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ABSTRACT

Nano- and microparticles consisting of magnetic iron oxides were prepared by microwave-assisted synthesis from ferrous sulfate. They were applied to the direct magnetic modification of sensitive and labile non-magnetic biomaterials (such as plant and seagrass based materials and enzymes immobilized on non-magnetic carriers) at temperatures −20 and −80 °C. These conditions warrant stable magnetic modification of non-magnetic biomaterials, e.g. cellulose, lignocellulose, or starch based ones. We have also magnetically modified enzymes immobilized on non-magnetic carriers, for example lipase immobilized on cellulose powder and glucose isomerase immobilized on solid carrier granules. It was observed that a substantial fraction of the activity is maintained by this method and that all the magnetically modified enzymes were stable during eight repeated reaction cycles with only a negligible decrease in activity over time.

1. Introduction

Magnetic materials have found enormous amount of applications in various areas of science and technology. They have been used e.g. in various areas of biology and medicine [1], molecular biology [2], analytical chemistry [3] or for waste water treatment [4]. In order to increase the number of potentially applicable magnetically responsive materials, important non-magnetic particulate materials with interesting catalytic, adsorption or other properties can be magnetically modified. Such a modification is usually based on the deposition of magnetic iron oxides nano- or microparticles on the surface or within the pores of the treated materials. Previously published methods are mainly suitable for magnetic modification of stable inorganic or organic materials. These methods are often based on the alkaline precipitation of ferrous and ferric salts in the presence of the modified material, followed by heating of the aqueous suspension to form different types of magnetic iron oxides, on the intensive heating of treated materials impregnated by iron or nickel salts, by magnetic fluid treatment etc. [5].

Despite the fact that different types of magnetic modification procedures have been successfully used for the preparation of large number of magnetically responsive materials [6], often the conditions used during these processes (e.g., high temperature, extremes of pH value, presence of organic solvents) do not allow to use such procedures for magnetization of sensitive biological structures or enzymes and other biomolecules immobilized on solid carriers. However, several gentle procedures have been already developed, e.g., for magnetic modification of prokaryotic and eukaryotic cells; different methods such as direct or polymer-mediated deposition of magnetic nanoparticles on the cell surface have been used [7,8]. Alternatively magnetic modification of mammalian cells (e.g., stem cells) has been performed using superparamagnetic iron-oxide nanoparticles which had been taken up by cells during cultivation by endocytosis [9].

In this paper we describe a simple magnetization procedure, which can overcome the steps incompatible with sensitive materials and biologically active compounds. Method presented in this article is a modification of the recently described procedure [10], based on the direct modification of the treated material by suspension of magnetic iron oxides particles synthesized by microwave assisted procedure and fixed on material at elevated temperatures [10,11]. Magnetization of sensitive materials was performed at subzero temperatures; the non-magnetic materials were modified by mixing with appropriate amount of magnetic particles suspension followed by freezing at temperatures −20 or −80 °C.

2. Experimental

Materials: *Candida rugosa* lipase (EC 3.1.1.3), immobilized *Streptomyces murinus* glucose isomerase (GI; EC 5.3.1.5) (Sweetzyme®

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IT Extra, a product of Novozymes Corp.), 4-nitrophenyl butyrate, ferrous sulfate heptahydrate, potassium hydroxide, montmorillonite K10 and sodium (meta)periodate were obtained from Sigma, USA, while cellulose powder was from Fluka, Germany. Potato starch and 4-nitrophenol were from Lach-Ner, Czech Republic. Fructose was from Roth, Germany. Spent black tea leaves, spent coffee grounds, spruce sawdust, biochar and powdered peanut husks were obtained locally, marine grass *Posidonia oceanica* (Neptune balls) was collected on beach in Sardinia, Italy and biogenic iron oxides were collected from a small water stream near Ceske Budejovice, Czech Republic. Reagent for measurement of glucose concentration was obtained from BioSystems, Spain. Different types of NdFeB permanent magnets (e.g., $D=20$ mm, height 10 mm, declared remanence 1.2 T) were used for magnetic separation.

Immobilization of enzymes: Lipase (*Candida rugosa*) was immobilized on cellulose powder using a standard procedure. 50 mg of cellulose powder was oxidized by 1.5 mL of 1% (w/v) sodium (meta)periodate in 0.1 mol L^{-1} sodium acetate buffer pH 4 and shaken for 20 h at room temperature in the dark. Activated cellulose was thoroughly washed with water and then 1.5 mL of lipase (1 mg mL^{-1}) in 50 mmol L^{-1} potassium phosphate buffer pH 7.5 was added and shaken gently for 20 h at 4 °C. Then, cellulose with immobilized lipase was washed with buffer to wash out the unbound enzyme.

Commercial immobilized glucose isomerase (*Streptomyces murinus*) was supplied in the form of granules (Sweetzyme® IT Extra, a product of Novozyme Corp., granules size 1–3 mm).

Magnetic modification of non-magnetic materials and immobilized enzymes: Magnetic iron oxides nano- and microparticles were prepared from ferrous sulfate heptahydrate at high pH by microwave assisted synthesis, as described previously [10,11]. 1 g of $FeSO_4 \cdot 7H_2O$ was dissolved in 100 mL of distilled water in 800 mL-glass beaker and pH was gradually increased by the addition of potassium or sodium hydroxide solution (1 mol L^{-1}) dropwise under stirring up to pH ca. 12; during this process a precipitate of iron hydroxides was formed. Then, this suspension was diluted up to 200 mL with distilled water and treated in microwave oven (700 W, 2450 MHz) at maximum power for 10 min. The formed particles of magnetic iron oxides were repeatedly washed with water. Suspension for subsequent magnetic modification procedures was prepared in a ratio of 1 part of completely sedimented particles and 4 parts of distilled water/buffer (1+4, v/v). Then, 1 g of non-magnetic material to be modified (e.g. cellulose powder, spent black tea leaves, spent coffee grounds, spruce sawdust, powdered peanut husks, marine plant *Posidonia oceanica*, biogenic iron oxides, montmorillonite K10, biochar and starch) or 1 g of immobilized enzymes (e.g., lipase immobilized on cellulose or commercial immobilized glucose isomerase (brown cylindrical granules with a particle size from 1 to 3 mm)) was thoroughly mixed with 2 mL of magnetic particles suspension to distribute the particles homogeneously on the treated material. The same mass/volume ratio was used when working with small amounts of modified materials. When needed, according to the type of treated material, excess liquid after mixing was removed with a pipette or in a desiccator under reduced pressure (when working with enzyme preparations, this procedure was performed in a cold room at 4 °C). Then, the material was put into the freezer (either −20 or −80 °C) for a few days (e.g. 7) to fix the magnetic particles on the surface or in the pores of material. After this treatment, if necessary, the remaining material moisture was removed in a desiccator under reduced pressure at 4 °C again to get the completely dry product. The magnetized materials have been usually stored in a dry state at 4 °C.

Determination of enzyme activities: Activity of immobilized lipase was determined by the hydrolysis of 4-nitrophenyl butyrate (0.5 mmol L^{-1}, dissolved in ethanol) in 50 mmol L^{-1} potassium phosphate buffer pH 7.5; the increase in concentration of yellow-coloured reaction product 4-nitrophenol was measured spectrophotometrically at 405 nm [12].

Activity of commercial immobilized glucose isomerase was determined by the isomerization of fructose to glucose; glucose was determined using BioSystems reagent; the product of coupled enzyme reactions, a pink-coloured complex, was measured spectrophotometrically at 500 nm after 20 min incubation at room temperature.

3. Results and discussion

A new procedure for the preparation of magnetic derivatives of sensitive biomaterials including immobilized enzymes has been developed, based on the recently described procedure employing microwave synthesized magnetic iron oxides nano- and microparticles (nanoparticles with diameters 20–60 nm form aggregates 0.1–20 μm in diameter; the material was characterized in detail by Zheng et al. [11]) as the magnetic label (see Fig. 1). In the original procedure the strong binding of magnetic particles to the target non-magnetic material was caused by the complete drying at elevated temperatures [10]. However, use of higher temperatures cannot be applied while working with sensitive biomaterials. That is why magnetic particles binding to non-magnetic materials at lower temperatures has been tested.

In order to develop a generally applicable magnetization procedure, useful also for magnetic modification of immobilized

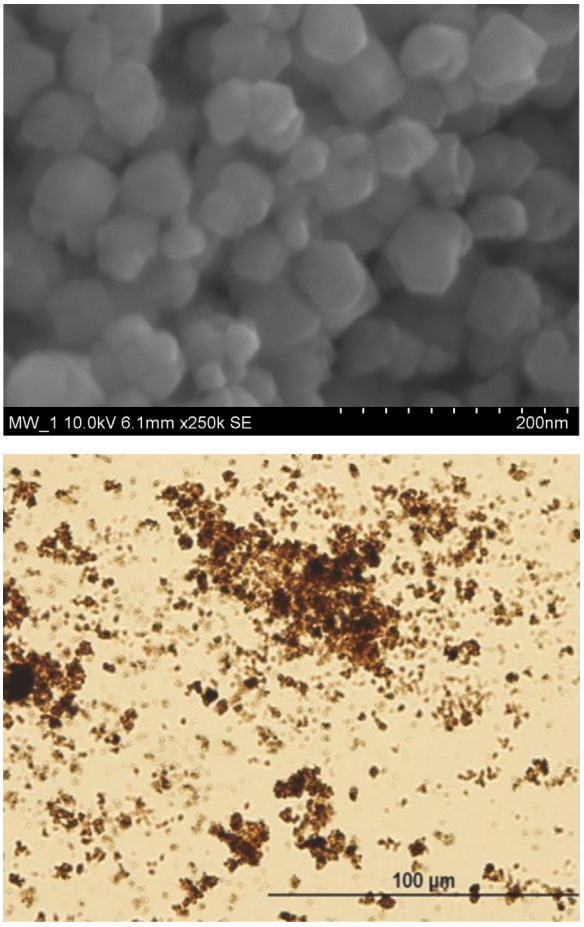

Fig. 1. Scanning electron microscopy of microwave synthesized magnetic iron oxides nanoparticles (top) and optical microscopy of the magnetic iron oxides aggregates (bottom).