Short communication

One-step preparation of magnetically responsive materials from non-magnetic powders

Ivo Safarik a,⁎, Katerina Horska a, Kristyna Pospiskova c, Mirka Safarikova a

a Department of Nanobiotechnology, Institute of Nanobiology and Structural Biology of GCRC, Na Sadkach 7, 370 05 Ceske Budejovice, Czech Republic
b Regional Centre of Advanced Technologies and Materials, Palacky University, Slechtitelu 11, 783 71 Olomouc, Czech Republic
c Department of Biochemistry, Faculty of Science, Palacky University, Slechtitelu 11, 783 71 Olomouc, Czech Republic

A R T I C L E   I N F O

Article history:
Received 27 January 2012
Received in revised form 1 June 2012
Accepted 2 June 2012
Available online 9 June 2012

Keywords:
Magnetic fluid
Magnetic separation
Magnetic modification
Spent tea leaves
Montmorillonite

A B S T R A C T

A simple procedure has been developed for the conversion of non-magnetic powder materials into their magnetic derivatives, which is based on the mixing of the material to be modified with water based magnetic fluid. After drying, aggregates of maghemite were deposited on the treated material, enabling its magnetic separation. In this paper we provide two examples of low-cost non-magnetic materials suitable for this type of simple one-step magnetic modification. Magnetically modified spent tea leaves efficiently adsorbed organic dyes; high maximum adsorption capacity (up to 100 mg g⁻¹) was achieved. Magnetic montmorillonite was used as a carrier for the immobilization of lipase and β-galactosidase; immobilized enzymes showed long-term stability without leaching of enzyme from the support and enabled their repeated use without significant loss of activity.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Different types of magnetic nano- and microparticles are regularly used in enormous amounts of applications, starting from molecular biology up to wastewater treatment. They are mainly used for magnetic separation technologies, because such materials can be efficiently separated even from very complex mixtures using an appropriate magnetic separator. These materials belong to the group of smart (stimuli responsive) materials, and they are prepared in research laboratories using various approaches [1]. Several companies produce these materials commercially.

However, there is an enormous amount of various particulate diamagnetic materials available, which have been used in many technological applications. Alternatively, many particulate materials are produced as wastes. Many of them could become very efficient magnetically responsive materials provided that appropriate magnetic modification procedures are available.

There are several possibilities for magnetic modification of nonmagnetic (diamagnetic) materials. In most cases the magnetization procedure is based on the incorporation of particulate magnetic materials (usually magnetic iron oxide nano- or microparticles) into the structure or on the surface of modified materials. One of the modification procedures is based on the precipitation of magnetic iron oxides from a mixture of ferrous and ferric salts by a strong hydroxide in the presence of the modified material, as exemplified by the preparation of magnetic charcoal [2] and magnetic bentonite [3]. Recently water based magnetic fluids have been successfully used for magnetization of various biological and synthetic materials, such as sawdust [4–6], spent grains [7], peanut husks [8], biospecific affinity adsorbents [9,10], or dead and living microbial cells, e.g., Saccharomyces cerevisiae [11,12], Kluyveromyces fragilis [13] and Chlorella vulgaris [14]. In most cases, materials to be modified were suspended in methanol or acetate buffer and water based magnetic fluid stabilized with perchloric acid was added; during the incubation precipitation of magnetic nanoparticles (present in magnetic fluid) on the surface of modified materials occurred [15,16].

Despite the fact that a large number of non-magnetic inorganic, organic and biological materials has been successfully magnetically modified using the above described simple procedures, these methods were not 100% successful. Recently we have found that direct thorough mixing of an appropriate magnetic fluid with the modified material, followed by drying, leads to the formation of stable magnetized derivatives. Examples of magnetically responsive materials prepared by this extremely simple procedure and their possible applications are presented in this paper.

2. Experimental

2.1. Materials

Water-based ionic magnetic fluid stabilized with perchloric acid was prepared using a standard procedure [17]. The relative magnetic
fluid concentration (25.2 mg mL\(^{-1}\)) is given as the maghemite content determined by a colorimetric method [18]. Spent black tea leaves (English breakfast, Ahmad Tea, London) was obtained locally; it was repeatedly extracted with boiling water until the colorless extract was obtained and then dried at 50 °C; further extraction was performed with methanol. Montmorillonite K10, Candida rugosa lipase (EC 3.1.1.3; catalog no. L1754), Kluyveromyces lactis (β-galactosidase (lactase; EC 3.2.1.23; catalog no. G3665), 4-nitrophenyl butyrate, 2-nitrophenyl β-D-galactopyranoside and glutaraldehyde were obtained from Sigma. Bismarck brown Y (C.I. Basic Brown 1; C.I. 21000) and safranin O (C.I. Basic Red 2; C.I. 50240; a mixture of dimethyl safranin and trimethyl safranin) were provided by Sigma, USA. Crystal violet (C.I. Basic Violet 3; C.I. 42555) and methyl green (C.I. Basic Blue 20; C.I. 42585) were purchased from Loba Chemie, Austria. Malachite green (Basic Green 4; C.I. 42000) was obtained from Roth, Germany. Acridine orange (C.I. Basic Orange 14; C.I. 46005) was obtained from Merck, Germany while Nile blue A (C.I. 51180) was from Chemische Fabrik GmbH, Germany. Common chemicals were from Lach-Ner, Czech Republic.

2.2. Preparation of magnetically responsive materials

In a typical procedure, 1 g of powder to be modified was thoroughly mixed in a short test-tube or a small beaker with 1 mL of water based ferrofluid stabilized with perchloric acid. The mixing with a spatula or laboratory spoon enabled homogeneous distribution of magnetic fluid within the treated material. This mixture was allowed to dry completely at temperatures not exceeding 50 °C and then it was washed with water and/or methanol. The magnetically responsive material was used in the form of water suspension, or the material was dried.

2.3. Adsorption of organic dyes on magnetically responsive spent tea leaves

50 mg of magnetically-modified spent tea leaves was mixed with 6.0 mL of water in a test tube. Then 0.1–4 mL portion of stock water solution of a tested dye (1 mg mL\(^{-1}\)) was added and the total volume of the solution was made up to 10.0 mL with water. The suspension was mixed on a rotary mixer (Dynal, Norway) for 2 h at room temperature. Then the magnetic adsorbent was separated from the suspension using a magnetic separator (MPC-1 or MPC-6, Dynal, Norway) and the clear supernatant was used for the spectrophotometric measurement. The concentration of free (unbound) dye in the supernatant \((C_{eq})\) was determined from the calibration curve. The amount of dye bound to the unit mass of the adsorbent \((q_{eq})\) was calculated using the following formula:

\[
q_{eq} = \frac{(C_{tot} - C_{eq})}{5} \quad \text{(mg g}^{-1}\text{)}
\]

where \(C_{tot}\) is the total (initial) concentration of dye (μg mL\(^{-1}\)) used in the experiment. The value \(q_{eq}\) was expressed in mg of adsorbed dye per 1 g of adsorbent. Equilibrium adsorption data were fitted to Langmuir adsorption isotherms using SigmaPlot software.

2.4. Immobilization of lipase and β-galactosidase on magnetically responsive montmorillonite K10

Lipase and β-galactosidase were immobilized on magnetically modified montmorillonite (mag-MMT) by simple adsorption or by adsorption followed by glutaraldehyde cross-linking. For both methods of immobilization 100 mg of mag-MMT was mixed with 1.5 ml of enzyme solution (lipase: 1 mg mL\(^{-1}\), in 50 mM phosphate buffer pH 7.5; β-galactosidase: 10 μL in 1.49 mL of 100 mM phosphate buffer pH 7.0 containing 5 mM MgCl\(_2\)) and shaken for 20 h at 4 °C using an automatic rotator (20 rpm). Then, in the case of simple adsorption, the unbound enzyme was removed and magnetic material was thoroughly washed with buffer until no enzyme activity was detected. In the case of the second method of immobilization (adsorption followed by cross-linking), the unbound enzyme after adsorption was removed and 1.5 ml of 3% glutaraldehyde (GA) solution in buffer was added. This mixture was shaken for 3 h at 4 °C, then supernatant was removed and magnetic material was thoroughly washed with buffer until no enzyme activity was detected.

2.5. Lipase and β-galactosidase assays

Activities of hydrolyses immobilized on mag-MMT were determined spectrophotometrically using artificial substrates. Lipase hydrolyzed 0.5 mM 4-nitrophenyl butyrate (dissolved in ethanol) in 50 mM potassium phosphate buffer, pH 7.5 (at 405 nm) and β-galactosidase cleaved 1.5 mM 2-nitrophenyl β-D-galactopyranoside in 100 mM phosphate buffer pH 7.0 with 5 mM MgCl\(_2\) (at 410 nm). Particles of mag-MMT with attached enzyme were stirred during the reaction in buffer containing substrate, then magnetically separated to the bottom of the cuvette to stop the reaction and increasing amount of yellow-colored 4-nitrophenol or 2-nitrophenol was measured spectrophotometrically.

Molar absorption (extinction) coefficients (ε) of the reaction products were determined spectrophotometrically for special media conditions used in enzyme assays. Coefficient for 4-nitrophenol in 50 mM potassium phosphate buffer, pH 7.5 at 405 nm was 13815 L mol\(^{-1}\) cm\(^{-1}\) while coefficient for 2-nitrophenol in 100 mM phosphate buffer pH 7.0 with 5 mM MgCl\(_2\) at 410 nm was 1907 L mol\(^{-1}\) cm\(^{-1}\).

2.6. Operational stability of immobilized lipase and β-galactosidase

Reusability of lipase and β-galactosidase immobilized on mag-MMT was tested as their operational stability; they were repeatedly used for 8 cycles. Particles with attached enzyme were washed with buffer between each cycle. Activities of hydrolyses were measured spectrophotometrically as described previously. Residual activities of enzymes after each cycle were determined and compared taking the initial activity in the first cycle as 100%.

2.7. Time stability of immobilized lipase and β-galactosidase

Particles of mag-MMT with immobilized lipase and β-galactosidase were stored in buffer at 4 °C for 45 days and percentage of residual enzyme activity on the carrier was determined. Possible presence of enzyme released from the support was tested during this time period by measuring the activity of free enzyme in the supernatant.

3. Results and discussion

An extremely simple procedure for the magnetization of originally non-magnetic powdered materials has been developed; during the experiments, many different inorganic and organic materials and biomaterials have been modified, such as montmorillonite, halloysite, sawdust, spent tea leaves, spent coffee grounds, spent barley grains, cellulose, starch and selected materials for liquid chromatography. Water based magnetic fluid stabilized with perchloric acid was used as a magnetic modifier. Magnetic fluid was composed of maghemite nanoparticles with diameters ranging between 10 and 20 nm (electron microscopy measurements), with a mean particle diameter ca 12.5–14 nm [15,16]. Thorough mixing of ferrofluid with the target non-magnetic materials, followed by complete drying under slightly elevated temperature, led to the deposition of maghemite nanoparticles (originally present in ferrofluid) on the surface and within the pores of the treated materials. The maghemite nanoparticle deposition is usually very stable, and during the subsequent washing steps only a very low amount of nanoparticles was released. The surface of the modified materials is covered by maghemite nanoparticle aggregates, as can be
seen on typical examples of magnetically modified materials (magnetic spent tea leaves and montmorillonite — see scanning electron microscopy pictures in Fig. 1). It can be expected that the strong binding of magnetic iron oxide nanoparticles to the surface of non-magnetic materials has been achieved by a subtle balance of van der Waals, electrostatic and hydrophobic interactions both between the magnetic nanoparticles and the treated material surface and between the adsorbed magnetic nanoparticles [19]. The magnetically modified materials could be easily separated by rare earth permanent magnets or commercially available magnetic separators (see Fig. 2).

Two different magnetically modified materials have been selected to show their application potential. Magnetic spent tea leaves were used as a biosorbent for efficient removal of organic xenobiotics, while magnetic montmorillonite was used as a carrier for enzyme immobilization.

The adsorption properties of the magnetically modified spent tea leaves were tested with seven water soluble organic dyes, belonging to different dye classes, namely acridine orange, Bismarck brown Y, crystal violet, malachite green, methyl green, Nile blue A and safranin O. The adsorption of the tested dyes reached equilibrium in approximately 60–90 min. Incubation time of 2 h was used for adsorption experiments. The equilibrium adsorption isotherms for the tested dyes are shown in Fig. 3. The experimental data follow the Langmuir isotherm equation which was used for experimental data analysis. The Langmuir model is valid for monolayer adsorption onto a surface.
The results are presented in Table 1.

As can be seen, the adsorption of the tested dyes can be successfully described by the Langmuir isotherm. Such a description allows a simple calculation of the maximum adsorption capacity, which is a very important parameter describing the adsorption process.

In the case of seven tested dyes, the highest $Q_{\text{max}}$ was found for crystal violet (100.1 mg g$^{-1}$), while the lowest $Q_{\text{max}}$ value was obtained for methyl green (30.8 mg g$^{-1}$).

Magnetically modified montmorillonite was used as a carrier for immobilization of two important enzymes, namely lipase and lactase.

The studied enzymes were immobilized both by adsorption, and by adsorption followed by cross-linking with glutaraldehyde. However, in the case of lactase, glutaraldehyde treatment caused total inactivation of the adsorbed enzyme. Table 2 shows activity of immobilized enzymes on 1 mg of mag-MMT.

During the study of operational stability, lipase adsorbed on mag-MMT and cross-linked with glutaraldehyde retained 79% of initial activity after 8 cycles. There was no decrease in activity of lipase adsorbed on mag-MMT (without cross-linking) after 8 cycles. $\beta$-Galactosidase adsorbed on mag-MMT (without cross-linking) retained 78% of initial activity after 8 cycles (Fig. 4). All immobilized enzymes were stable during the study of operational stability.

Table 2

<table>
<thead>
<tr>
<th>Immobilization method</th>
<th>Activity of bound enzyme (nkat/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mag-MMT-lip-GA</td>
<td>3.82</td>
</tr>
<tr>
<td>Mag-MMT-lip</td>
<td>4.04</td>
</tr>
<tr>
<td>Mag-MMT-lac</td>
<td>1.20</td>
</tr>
</tbody>
</table>

The studied enzymes were immobilized both by adsorption, and by adsorption followed by cross-linking with glutaraldehyde. However, in the case of lactase, glutaraldehyde treatment caused total inactivation of the adsorbed enzyme. Table 2 shows activity of immobilized enzymes on 1 mg of mag-MMT.

During the study of operational stability, lipase adsorbed on mag-MMT and cross-linked with glutaraldehyde retained 79% of initial activity after 8 cycles. There was no decrease in activity of lipase adsorbed on mag-MMT (without cross-linking) after 8 cycles. $\beta$-Galactosidase adsorbed on mag-MMT (without cross-linking) retained 78% of initial activity after 8 cycles (Fig. 4). All immobilized enzymes were stable during the study of operational stability.

Table 2

<table>
<thead>
<tr>
<th>Dyes</th>
<th>Langmuir isotherm $q_{\text{eq}}$ (mg g$^{-1}$)</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acridine orange</td>
<td>$q_{\text{max}} = 98.8$</td>
<td>0.990</td>
</tr>
<tr>
<td>Bismarck brown Y</td>
<td>$q_{\text{max}} = 71.7$</td>
<td>0.996</td>
</tr>
<tr>
<td>Crystal violet</td>
<td>$q_{\text{max}} = 100.1$</td>
<td>0.987</td>
</tr>
<tr>
<td>Malachite green</td>
<td>$q_{\text{max}} = 66.2$</td>
<td>0.929</td>
</tr>
<tr>
<td>Methyl green</td>
<td>$q_{\text{max}} = 30.8$</td>
<td>0.990</td>
</tr>
<tr>
<td>Nile blue A</td>
<td>$q_{\text{max}} = 87.1$</td>
<td>0.958</td>
</tr>
<tr>
<td>Safranin O</td>
<td>$q_{\text{max}} = 84.7$</td>
<td>0.958</td>
</tr>
</tbody>
</table>

The studied enzymes were immobilized both by adsorption, and by adsorption followed by cross-linking with glutaraldehyde. However, in the case of lactase, glutaraldehyde treatment caused total inactivation of the adsorbed enzyme. Table 2 shows activity of immobilized enzymes on 1 mg of mag-MMT.

During the study of operational stability, lipase adsorbed on mag-MMT and cross-linked with glutaraldehyde retained 79% of initial activity after 8 cycles. There was no decrease in activity of lipase adsorbed on mag-MMT (without cross-linking) after 8 cycles. $\beta$-Galactosidase adsorbed on mag-MMT (without cross-linking) retained 78% of initial activity after 8 cycles (Fig. 4). All immobilized enzymes were stable during the study of operational stability.

Table 2

<table>
<thead>
<tr>
<th>Immobilization method</th>
<th>Activity of bound enzyme (nkat/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mag-MMT-lip-GA</td>
<td>3.82</td>
</tr>
<tr>
<td>Mag-MMT-lip</td>
<td>4.04</td>
</tr>
<tr>
<td>Mag-MMT-lac</td>
<td>1.20</td>
</tr>
</tbody>
</table>
for 45 days without significant loss of their activity (<5%) and no significant leaching of enzyme from the support was detected (Fig. 5).

4. Conclusions

As can be seen from the results, a very simple procedure for magnetic modification of non-magnetic powdered materials has been developed. The magnetic derivatives can be efficiently used e.g. as magnetic adsorbents for the removal of selected xenobiotics, or enzyme carriers. Magnetically modified spent tea leaves were used as an adsorbent for organic dyes; the maximum adsorption capacities of this material reached values up to 100 mg of adsorbed dyes per 1 g of magnetic biosorbent which is fully comparable with other described non-magnetic biosorbents [20]. Analogous materials such as magnetic montmorillonite can serve as a carrier for enzyme immobilization. Hydrolases (lipase and β-galactosidase) immobilized on mag-MMT showed long-term stability without leaching of enzyme from the support. Enzymes attached to mag-MMT were repeatedly used without significant loss of their activity.

Acknowledgments

This research was supported by the Grant Agency of the Czech Republic (project no. P503/11/2263) and by the research project LH12190 (Ministry of Education of the Czech Republic). The authors thank Jan Proska and Filip Novotny (Czech Technical University, Prague) for making SEM images.

References


