

Magnetic composites from minerals: study of the iron phases in clay and diatomite using Mössbauer spectroscopy, magnetic measurements and XRD

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Abstract Magnetic particles as matrix for enzyme immobilization have been used and due to the enzymatic derivative can be easily removed from the reaction mixture by a magnetic field. This work presents a study about the synthesis and characterization of iron phases into magnetic montmorillonite clay (mMMT) and magnetic diatomaceous earth (mDE) by ^{57}Fe Mössbauer spectroscopy (MS), magnetic measurements and X-ray diffraction (XRD). Also these magnetic materials were assessed as matrices for the immobilization of invertase via covalent binding. Mössbauer spectra of the magnetic composites performed at 4.2 K showed a mixture

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of magnetite and maghemite about equal proportion in the mMMT, and a pure magnetite phase in the sample mDE. These results were verified using XRD. The residual specific activity of the immobilized invertase on mMMT and mDE were 83 % and 92.5 %, respectively. Thus, both magnetic composites showed to be promising matrices for covalent immobilization of invertase.

Keywords Magnetic particles · Montmorillonite · Diatomite · Immobilization · Invertase

1 Introduction

Inorganic materials have been widely used as carriers for enzyme immobilization. Their advantages are rigid structure, durability, high mechanical strength and relatively low cost [1]. Montmorillonite belongs to the smectite clays and its crystal structure consists of two tetrahedral silicate layers with an edge-shared octahedral layer of either alumina or magnesia [2]. On the other hand, diatomaceous earths or diatomite are mineral deposits of diatomaceous algae and are the major silica source on earth [3]. These minerals have many properties that make them interesting matrices for the immobilization of proteins. Some of them are chemical inertness, large surface area, high porosity and mechanical strength, besides being readily available mineral in nature. It is advantageous to use magnetic particles as matrix for enzyme immobilization because the enzymatic derivatives are insoluble in water and can be easily removed from the reaction mixture by an external magnetic field. Our group has reported several works related to biomolecules immobilized on magnetite and different magnetic composites [4–10]. The objective of the present work is to study the different iron phases in the magnetic montmorillonite clay (mMMT) and magnetic diatomaceous earth (mDE) by ^{57}Fe Mössbauer spectroscopy (MS), magnetic measurements and X-ray diffraction (XRD). It is also the objective to propose and assess the mMMT and mDE as matrices for the immobilization of invertase via covalent binding.

2 Materials and methods

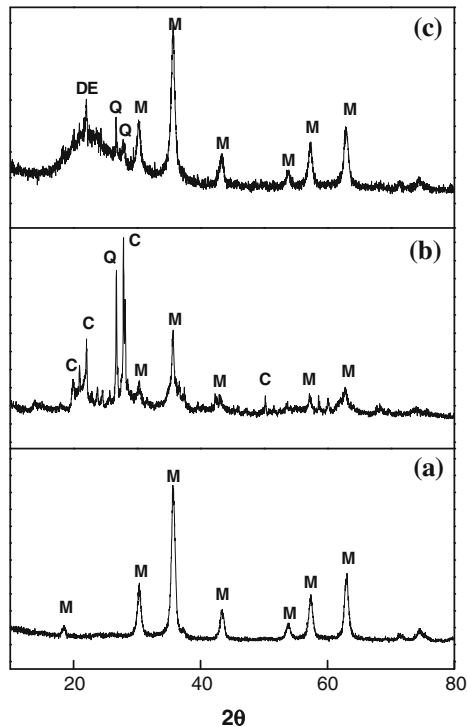
2.1 Magnetization of clays

Montmorillonite (MMT) and diatomaceous earth (DE) were kindly supplied by Minarmco S.A. (Neuquén, Argentina) and TAMER S.A. (Salta, Argentina), respectively. The synthesis of magnetic composites was performed according to Amaral et al. [11] The magnetic particles obtained were washed with distilled water and recovered by a magnetic field (Ciba Corning; 0.6 T). The mMMT and mDE were dried at 50 °C overnight.

2.2 Characterization of magnetic composites

The phases of iron present in the resulting mMMT and mDE were investigated by X-ray diffraction and Mössbauer spectroscopy. X-ray diffraction patterns were

Fig. 1 XRD patterns of (a) magnetite, (b) mMMT and (c) mDE. M = magnetite; C = montmorillonite clay; Q = quartz; DE = diatomaceous earth



measured at room temperature in a Siemens D5000 X-ray diffractometer, using $\text{CuK}\alpha$ radiation ($\lambda = 1.5406 \text{ \AA}$). Mössbauer spectra were recorded at 4.2 K in a transmission geometry using a conventional ^{57}Fe Mössbauer spectrometer employing a 50 mCi $^{57}\text{Co}/\text{Rh}$ source. The spectra were analyzed using the least squares method assuming Lorentzian line shapes and a hyperfine field distribution. The isomer shift (δ) values are relative to $\alpha\text{-Fe}$ at room temperature. Magnetization measurements were performed at 298 K in magnetic fields varying from 0 to 50 kOe (5.0 T) using a SQUID magnetometer (Quantum Design Model MPMS-5 S).

2.3 Immobilization process

The magnetic composites were silanized with aminopropyltriethoxysilane (APTES, 2.5 % v/v) stirring at 25 °C. The activation of the silanized mMMT and mDE with glutaraldehyde (10 % v/v) also was carried out stirring at 25 °C. Functionalized materials were washed several times with distilled water. The invertase from Baker's yeast (1 mL, prepared in 0.2 M sodium acetate buffer, pH 5.0) was incubated with mMMT and mDE (0.01 g) 4 °C under mild stirring. Afterwards the material was washed five times with 0.2 M sodium acetate buffer, pH 5.0. The invertase immobilized on mMMT (mMMT-invertase) and mDE (mDE-invertase) were collected by the magnetic field and the supernatants including the first two washings were used for protein determination according to Lowry et al. [12] using bovine serum albumin as the standard protein. The immobilized derivatives were stored in sodium acetate

Table 1 Mössbauer parameters

Sample	Grain size XRD (nm)	Component	δ (mm/s)	Δ (mm/s)	Γ (mm/s)	B (T)	Area %
Magnetite (RT = 300 K)	11	Sextet 1	0.31	-0.01	0.65	47.5	49.0
		Sextet 2	0.33	-0.03	1.20	43.2	51.0
mMMT (4.2 K)	25	Sextet 1	0.33	0.01	0.45	52.3	30.0
		Sextet 2	0.38	-0.12	0.60	50.0	62.5
		Doublet	0.33	0.53	0.30	-	7.5
mDE (4.2 K)	12	Sextet 1	0.33	0.01	0.47	52.3	49.5
		Sextet 2	0.28	-0.03	0.64	50.3	50.5

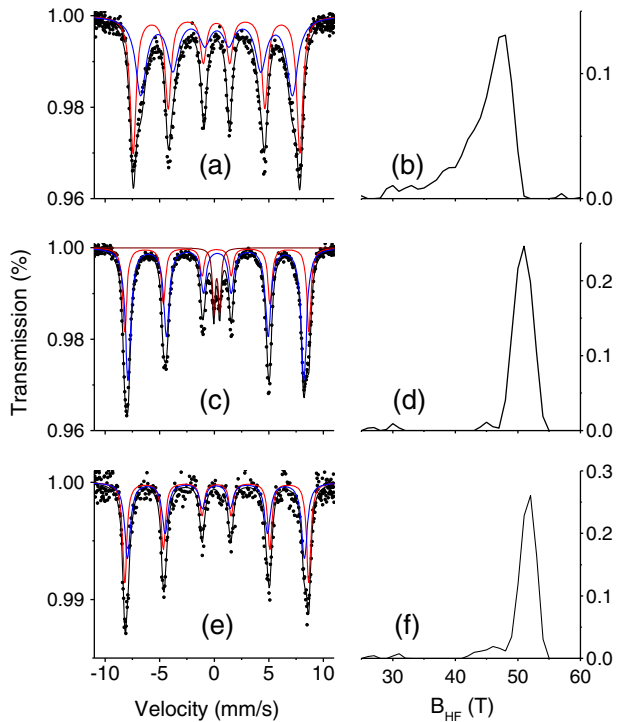
Isomer shift (δ), Quadrupole splitting (Δ), Line width (Γ), Relative area (Area) and Hyperfine field (B). Uncertainty in particle size is 0.5 nm while that the uncertainty in δ and B are 0.02 mm/s and 0.5 T, respectively. Areas are accurate within 2%

buffer at 4 °C for further use. Invertase activity was determined by using 0.15 M sucrose (10 mL) prepared in sodium acetate buffer (0.2 M, pH 5.0). After exactly 15 min of incubation at 25 °C, 20 μ L the sample was withdrawn and added to 2.0 mL of working solution in order to measure released glucose using a glucose oxidase-peroxidase (GOD/POD) enzymatic kit (Doles, Goiás, Brazil). The enzyme activity unit (U) was defined as the amount of enzyme releasing 1 μ mol of glucose per minute under the assay conditions.

3 Results and discussion

The XRD patterns of the magnetic particles are presented in Fig. 1. The 2θ peaks at 18.44°, 30.32°, 35.75°, 43.32°, 53.89°, 57.34° and 62.96° are attributed to the crystal planes of magnetite at (111), (220), (311), (400), (422), (511) and (440) respectively [13]. The characteristic peaks of magnetite and quartz were observed in all magnetic composites. By analyzing the XRD patterns it is observed that the magnetite is the most predominant crystalline phase in mDE, while that in mMMT the aluminosilicates are the predominant crystalline phase. The mMMT and mDE exhibited broad and low intensity peaks in the base line (Fig. 1b and c). This broad X-ray structure suggests an amorphous component in the prepared composite [14]. The grain size estimated from the main reflections of each diffractogram, by using the Scherrer formula, is shown in Table 1. In this calculus we are not considering possible contributions of crystal stress. The XRD patterns of magnetite and maghemite are very similar. The main difference consists of a few low-intensity diffractions (<5 %) which are only present for the maghemite structure [15]. These diffraction lines are present in the patterns presented in mMMT and mDE (Fig. 1b and c), but this does not confirm or exclude the presence of maghemite in the composites produced. Furthermore, the fact that the low intensity peaks become visible in the XDR pattern does not prove that the transition from magnetite to maghemite took place; it could simply be due to an increase of the particle size [15]. However, in a more recent report [16], the differentiation between magnetite and maghemite was made on the basis of high angle peaks corresponding to plane (511) and (440) peak-heights and its resolution through the deconvolution.

Fig. 2 Mössbauer spectra and their corresponding p-B distribution (a) and (b) magnetite at room temperature (c) and (d) mMMT at 4.2 K and (e) and (f) mDE at 4.2 K. Scattered points are data point and the fitted spectrum is shown in black line. The subspectra shown in red and blue lines are the component subspectra corresponding to A-site and B-site iron respectively, whereas in (c) the subspectrum shown in dark line is showing doublet



The hyperfine parameters obtained by Mössbauer Spectroscopy (MS) at an appropriate temperature can be used to identify the magnetic signal of the iron oxide and to obtain information about the Fe^{3+} linking the components of the material [17]. The measurements were carried out at 4.2 K to check any superparamagnetic state present in the samples. Only sample of pure magnetite may be analyzed at room temperature (300 K). ^{57}Fe Mössbauer spectrum of pure magnetite shows two sextets (Fig. 2a). The first one (A sites) has a hyperfine magnetic field, $B = 47.5$ T, and an isomer shift, $\delta = 0.31$ mm/s; assigned to Fe^{3+} ions; the second sextet (B sites) has $B = 43.2$ T and $\delta = 0.33$ mm/s; this sextet corresponds to mixed Fe^{2+} - Fe^{3+} ions [18]. The line width (Γ) of the second sextet corresponding to the B-site is quite high. This could be because of defects in the sample, particle size distribution and presence of different iron environments which leads to intermediate iron oxidation states. Due to the high linewidth the two components in the spectrum are not fully resolved. As it is also clear from the broad hyperfine field distribution (Fig. 2b) that there may be more than two sextets present in the sample corresponding to different iron oxidation states or different iron minerals. These values are similar to the bulk material (sextet 1: $B = 49.0$ T and $\delta = 0.26$ mm/s and sextet 2: $B = 46.0$ T and $\delta = 0.67$ mm/s) [19], but the δ for second component is significantly lower, this might indicate the presence of some other iron mineral such as maghemite. The deviation in the ideal area ratio (1:2) of the iron in tetrahedral and octahedral position obtained from the subspectra area is due to the smaller particle size compared to their bulk counterpart [20].

The hyperfine magnetic fields for mMMT (sextet 1 equal to 52.3 T and sextet 2 equal to 50.0 T) and mDE (sextet 1 equal to 52.3 T and sextet 2 equal to 50.3 T)

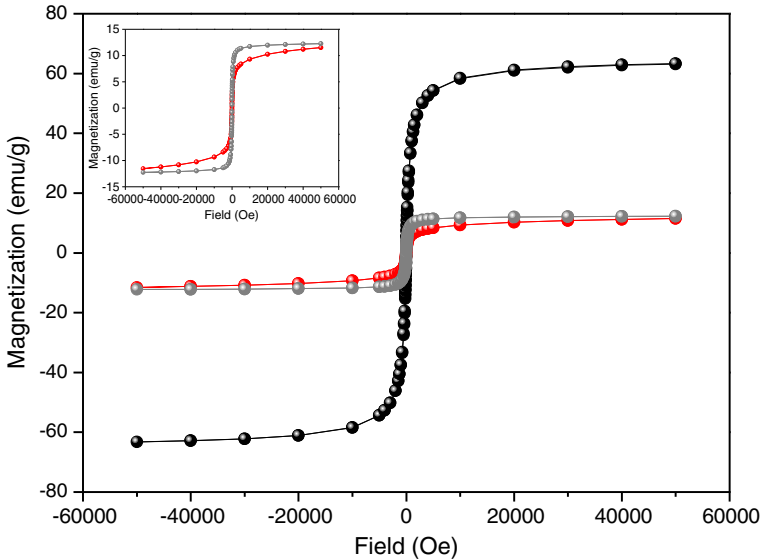


Fig. 3 Magnetization measurements for the magnetite (*black*), mMMT (*red*) and mDE (*gray*). The inset shows a magnified view of the magnetization curves of the mMMT and mDE

showed slightly higher values than pure magnetite (Fig. 2c, e and Table 1). In addition to the signals relating to magnetite, Fig. 2c (mMMT) also shows a doublet having an isomer shift equal to 0.33 mm/s and an area equal to 7.5 %. This doublet emanates from ferric iron in a non-spherical local surrounding, maybe coming from the rim of the iron oxide core, i.e., the magnetic relaxation effect which is attributed to the presence of superparamagnetism as well as the ferromagnetic nanoparticles [20]. The absence of a doublet in the Mössbauer spectrum of mDE suggests that there is no non-magnetic and non-spherical iron surrounding present in the mDE sample. The mMMT and mDE spectra (Fig. 2c and e) show an increase in the B sites compared to the spectrum for pure magnetite, whereas the intensity of the A sites (Fe^{3+}) decreases. Table 1 shows the hyperfine parameters obtained from fitting the Mössbauer spectra. The percentage area reported under the curve of the lines of best fit is related to the composition of the obtained materials. According to these spectra and the hyperfine parameters, it is evident that the mMMT showed a higher increase in the B sites than mDE as compared to the pure magnetite, i.e., the presence of the clay caused more modifications in the magnetite compared to diatomaceous earth.

The magnetic properties of magnetite, mMMT and mDE particles were measured by applying an external magnetic field at 298 K. The saturation magnetization for magnetite, mMMT and mDE was determined by the magnetization curve at maximum magnetic field. As shown in Fig. 3 the saturation magnetization of mMMT and mDE was around 10 emu g^{-1} lower than the value of 60 emu g^{-1} found for the magnetite particles at 298 K. The decreased saturation magnetization can be attributed to surface effects, such as a magnetically inactive layer produced by the disordered surface [21]. In addition, the magnetite, mMMT and mDE particles exhibited superparamagnetic behavior.

Important parameters in the immobilization process such as reaction conditions of enzyme, solid support and linker determine the biochemical, mechanical and kinetic properties of the immobilized enzyme. The magnetic composites proposed as matrices for the immobilization of invertase via covalent binding showed excellent results. Thus, the residual specific activity of mMMT-invertase and mDE-invertase was 83 % and 92.5 %, respectively. No decrease in specific activity was observed, suggesting the potential application of these magnetic composites from minerals of low cost as matrices for the immobilization of invertase or other enzymes.

4 Conclusion

The synthesis of magnetic composites from two minerals of low cost as well as the study of the iron phases in mMMT and mDE was successfully performed. X-ray diffraction measurements of mMMT and mDE exhibited similar peak compared to those of magnetite and showed that the montmorillonite clay and diatomaceous earth minerals does not significantly interfere with the structure of synthesized magnetite. Through Mössbauer spectra we observed that the nanoparticles of mMMT are composed by a mixture of magnetite and maghemite whereas the mDE showed a pure magnetite phase. All magnetic particles displayed superparamagnetic behavior in agreement with particles size distribution at the nano scale. The residual specific activity of the mMMT-invertase and mDE-invertase was 83 % and 92.5 % respectively. Finally, these results suggest that mMMT and mDE are promising matrices for covalent immobilization of invertase and could be used for the immobilization or purification of other enzymes of industrial interest for biotechnological applications.

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