

A novel method for the synthesis of nano-magnetite particles

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(Received June 22, 2013, Revised March 21, 2014, Accepted March 28, 2014)

Abstract. A novel and simple method for the synthesis of nano-magnetite particles is disclosed. In the novel procedure, Fe²⁺ is the only source of metal cation. Carboxymethylcellulose (CMC) is used as the structure directing agent. The phase analysis of the nano-particles was performed using XRD and electron diffraction techniques. Size and morphology analysis was performed using light scattering and TEM techniques. The effect of NH₄OH solution (32 wt. %) at different CMC concentrations on the size distribution of the final magnetite powders is studied. An optimal base concentration exists for each CMC concentration leading to minimal agglomeration. There exists a minimum CMC concentration (0.0016 wt. %), lower than that no magnetite forms. It is shown that using the new method, it is possible to immobilize a lipase enzyme (*Candida Rugosa*) with immobilization efficiency larger than 98 % with a loading more than 3 times the reported value in the literature. The latter phenomenon is explained based on the agglomerate state of the nano-particles in the liquid phase.

Keywords: chemical synthesis; precipitation; light scattering

1. Introduction

Magnetic nano-particles are gaining ever-expanding industrial applications. Their unique magnetic properties allow them to be used in a broad range of engineering applications such as magnetic recording media, magnetic inks, seals (Tartaj *et al.* 2003) and as carriers for immobilization of enzymes (Liu *et al.* 2003, Huang *et al.* 2003, Liu *et al.* 2003). Magnetite nano-particles are considered as biocompatible super-magnetic materials and are of sound interest in biomedical applications like magnetic resonance imaging, clinical diagnosis, and magnetic drug targeting and enzyme immobilization (Cheng *et al.* 2005).

Nano-magnetite is a spinel ferrite and therefore the synthesis recipe mostly uses Fe²⁺ and Fe³⁺ cations in equimolar concentrations. There exist few synthesis procedures were only Fe²⁺ is the only source of the metal iron. Rabelo *et al.* (2001) reported the fabrication of size-controllable magnetite nano-particles by alkaline oxidation of ferrous ion adsorbed in sulphonated meso-porous styrene divinylbenzene copolymer. They observed that nano-magnetite size increased by ion-exchanging the sulphonated polymeric template with ferrous aqueous solution at increasing ion concentration. Kumar *et al.* (2001) reported the preparation of magnetite nano-rods by the

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sonication of aqueous iron (II) acetate in the presence of beta-cyclodextrin. Huang *et al.* (2003) reported an interesting method for the production of magnetite shells on polystyrene spheres as cores from the reaction of FeCl_2 with diethylene glycol in aqueous solutions.

This work introduces for the first time the production of nano-magnetite particles from the reaction of FeCl_2 (in the absence of Fe^{3+} cations) using carboxymethylcellulose (CMC) in aqueous solutions. It is shown that CMC can act as a structure directing agent (SDA) for magnetite formation. Up to now, CMC has been frequently used as a bio-compatible polymer as a dispersing agent for use in a variety of in-situ applications (Sipos *et al.* 2003). The resultant product is known as CMC-magnetite conjugate (Wibowo *et al.* 2013). However, any reported synthesis procedure for the production of CMC-magnetite conjugate uses equimolar concentrations of Fe^{2+} and Fe^{3+} cations. The present work actually produces CMC-magnetite conjugates using CMC as SDA and Fe^{2+} as the sole source of iron metal. XRD and electron diffraction techniques have been used for characterizing the produced samples. TEM analysis has been used for the determination of the size of the primary particles (nano-particles) constituting the actual tight agglomerates produced. The size of agglomerates has been determined by laser light scattering technique. Size and morphology analysis was performed using light scattering and TEM techniques. The effect of CMC concentration and amount of base (NH_4OH) addition on the phase and agglomerate size of the resultant products has been investigated. It is the opinion of the authors of this work that the “agglomerate size distribution” in the liquid phase has a determining role in the performance of nano-magnetite particles especially in processes involving adsorption and surface chemical reaction. To assess the exposed surface of the agglomerates, a specific lipase enzyme (*Candida Rugosa*) has been immobilized on the nano-magnetite particle agglomerates of minimal size obtained through the experiments via carbodiimide activation. The loading of the enzyme as a function of magnetite content of the aqueous mixture has been determined by assaying the activity of the residual enzyme in the liquid phase. 4-nitrophenyl palmitate (pNPP) has been used as substrate for determination of the activity of the enzyme. The maximum loading of enzyme is then compared with the reported value in the literature.

2. Experimental

FeCl_2 (MERCK) was used as the source of iron. Carboxymethylcellulose (industrial) was used as structure directing agent. The pH of the solution was adjusted adding NH_4OH (32 wt. %, MERCK).

The synthesis procedure is as follows: FeCl_2 is dissolved in oxygen-free water. Then, CMC is added as an aqueous solution (0.0016 wt. %, oxygen-free). Finally, by adding the base (oxygen-free) to raise the pH, nano-magnetite forms in less than a minute under vigorous agitation (100 rpm). The precipitates were washed several times with distilled water and ethanol and finally dried in a freeze-dryer. Nano-magnetite particles were also produced by the **conventional** method (Kumar *et al.* 2001) using FeCl_2 and FeCl_3 (MERCK) in proper amounts. No dispersant was used.

For the enzyme immobilization section of the work, lipase (EC3.1.1.3) from *Candida Rugosa* (no.L-1754), 4-nitrophenyl palmitate (pNPP) as a substrate of lipase, carbodiimide and Gum Arabic were purchased from Sigma, Triton X-100 and Tris (hydroxymethyl aminomethane) purchased from Merck.

The enzyme immobilization procedure has been as follows: 50-70 mg of nano-magnetite was added to 2 cm³ phosphate buffer (0.003 M, pH = 6), the mixture was sonicated for 10 min after

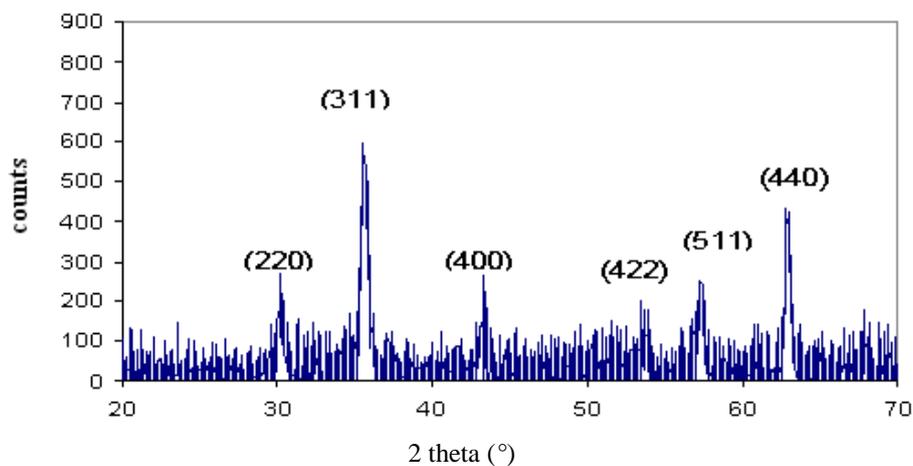


Fig. 1 The XRD pattern of a sample produced using 0.5 cm³ CMC solution and 5 cm³ base solution

adding 0.5 ml of carbodiimide solution (0.025 g cm⁻³ in phosphate buffer 0.003 M, pH = 6). After activation by carbodiimide, 2.5 cm³ of lipase (2 g cm⁻³) was added and the reaction mixture was sonicated for 30 min at 4°C. Finally, the mixture was centrifuged at 3000 rpm.

The precipitate was separated from the supernatant liquid and washed sequentially with phosphate and Tris buffer. By assaying the activity of unbound lipase in the supernatant, the amount of unbound enzymes after the immobilization process was investigated. The activity of bound lipase was determined by measuring the initial oxidation rates of pNPP at room temperature following the increase of paranitrophenol (pNP) concentration at a wavelength of 410 nm, using a Hitachi Spectrometer. A solution of pNPP was prepared by dissolving of 0.5 mg pNPP in 100 cm³ ethanol and afterwards 100 ml phosphate buffer solution was added to the mixture. The mixture was gently heated until the solution was clear. To initiate the enzymatic reaction, 100 μlit of a lipase-bound magnetite nano-particles solution (10 mg cm³) was added to 2 cm³ pNPP solution. The mixture was agitated for several minutes by vortex and finally 1 cm³ Na₂CO₃ solution (0.1 M) was added to the solution for quenching the activity of the enzyme. The amounts of Fe₃O₄ nano-particles used were 0.02, 0.20, 1.00, 2.00, 4.00, 10.00 mg cm⁻³. The precipitate was further used for activity and stability measurement. The hydrolysis reaction kinetics of free and bound lipase were investigated by assaying the hydrolysis of pNPP (0.27-1.33 mM) by lipase (1mg cm⁻³) in pH = 7.4 at 37°C.

Agglomerate size evaluation was performed using laser light scattering technique (Zetasizer 3000, HAS MALVERN). XRD patterns were obtained using a D-500 Siemens apparatus. HRTEM pictures and electron diffraction patterns of the nano-particles were taken using a CM 300FEG (Philips) HRTEM apparatus. Agglomerate morphology was investigated using a XL30 (Philips) scanning electron microscope.

3. Results and discussion

Fig. 1 shows the XRD pattern of a sample produced using 0.5 cm³ CMC solution and 5 cm³ base solution. The peaks correspond to magnetite and no detectable impurity phase (like other iron

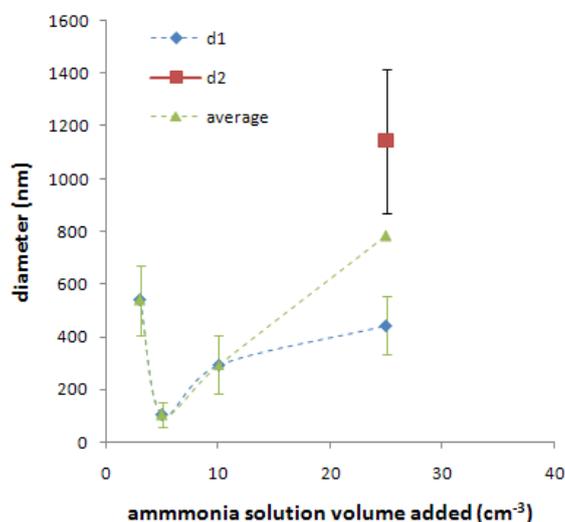


Fig. 2(a) The effect of amount base solution addition on the average diameters d_1 , d_2 and total average diameter for a constant CMC solution addition of 0.1 cm^3

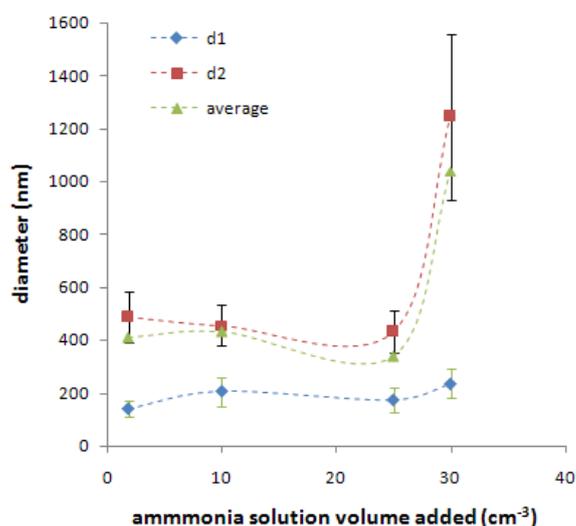


Fig. 2(b) The effect of amount base solution addition on the average diameters d_1 , d_2 and total average diameter for a constant CMC solution addition of 20.0 cm^3

oxide phases) can be observed in significant amounts. It should be emphasized that the absence of other iron oxide impurity phases will be confirmed afterwards when discussing the results of electron diffraction. XRD analysis of the products of all the other experiments that led to magnetite showed no impurity phases. Preliminary experiments showed that for a NH_4OH solution addition volume in the range of $20\text{-}30 \text{ cm}^3$, a minimum concentration of $0.0016 \text{ wt. \% CMC}$ is

needed for the formation of any magnetite. Accordingly, we used the latter concentration for the synthesis of all the other samples.

Laser light scattering experiments showed that the produced powders have a bimodal size distribution generally comprehending two peaks one under 100 nm and the other between 100 and 500 nm. The general trend for size change and modality as a function of base solution added volume (constant CMC solution added volume equal to 0.1 cm^3) can be observed referring to Figs. 2(a) and (b). The bimodality (if present) is shown as two average diameters d_1 and d_2 (each corresponding to one peak in the size distribution curve) and the standard deviation as a vertical bar. The total average particle size has been calculated based on the light scattering experiments and is indicated as “average” size in these figures.

The important point behind is that considering the least amount of CMC addition (0.1 cm^3), a minimum is observed in the agglomerate average size which is slightly under 100 nm. On the other hand, if the amount of base solution addition is equal or smaller than 10 cm^3 , only a monomodal agglomerate size distribution is obtained. It is observed that if the amount of base solution addition is smaller or larger than 5 cm^3 , the agglomerate size is significantly larger than 100 nm (more than 200 nm). It seems that a rather restricted range for the base addition exists which results in low agglomeration of the particles. Fig. 2(b) shows the effect of amount base solution addition for a constant CMC solution addition of 20.0 cm^3 . Interestingly, at this higher CMC concentration, the size distribution is bimodal anywhere and there exists a significant difference between the values of the peak average diameters (at least 200 nm). On the other hand, the smaller agglomerate diameters are never smaller than 100 nm.

Fig. 3 shows an SEM micrograph of such agglomerates; although in the dry form (drying increases largely the apparent agglomerate size). It is observed that the agglomerates have a spherical shape and that are presumably consisting of smaller particles not clearly observable using the SEM technique.

Fig. 4 shows the effect of CMC solution addition for a base solution addition of 5 cm^3 (the minimum in Fig. 2(a)). The agglomerate size behavior is worth attention. At low CMC addition the distribution is monomodal (average diameter larger than 100 nm), for 5 cm^3 addition it is bimodal but with an average diameter smaller than 100 nm and for 10 cm^3 addition, it is monomodal but with a large average diameter (c.a. 300 nm).

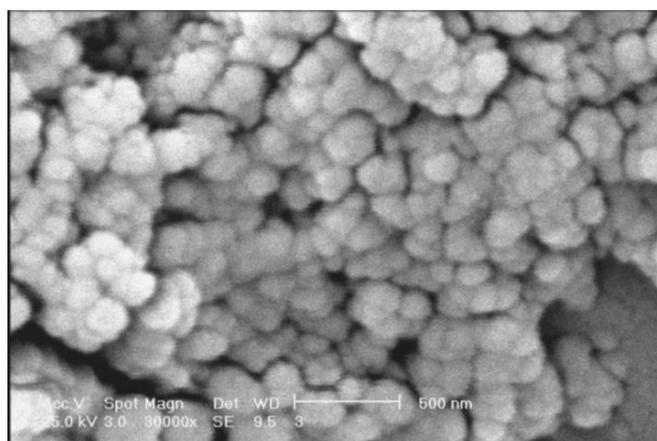


Fig. 3 SEM micrograph of a powder produced using 5 cm^3 SDA and 5 cm^3 ammonia solution

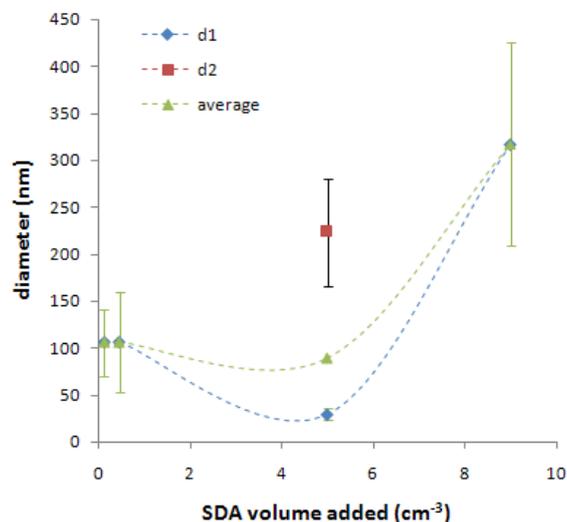


Fig. 4 The effect of CMC solution volume addition for a base solution addition volume of 5 cm³ (the minimum in Fig. 3(a))

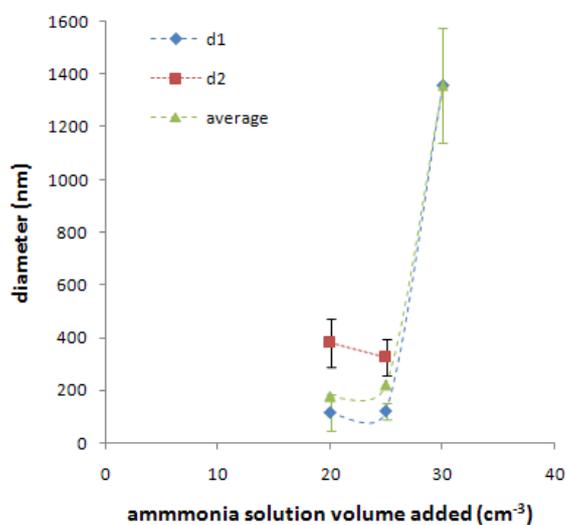


Fig. 5 The effect of NH₄OH addition volume on the size distribution of the nano-magnetite particles produced by the conventional method (no CMC, equimolar Fe²⁺ and Fe³⁺ in the precursors)

For comparative means, the effect of NH₄OH addition volume on the size distribution of the nano-magnetite particles produced by the conventional method (no CMC, equimolar Fe²⁺ and Fe³⁺ in the precursors) has been investigated and the results are shown in Fig. 5. It is observed that also in this case, a minimum exists for the average agglomerate size as a function of base solution addition. Figs. 6(a) and 6(b) show the HRTEM pictures of the crystallites obtained by the

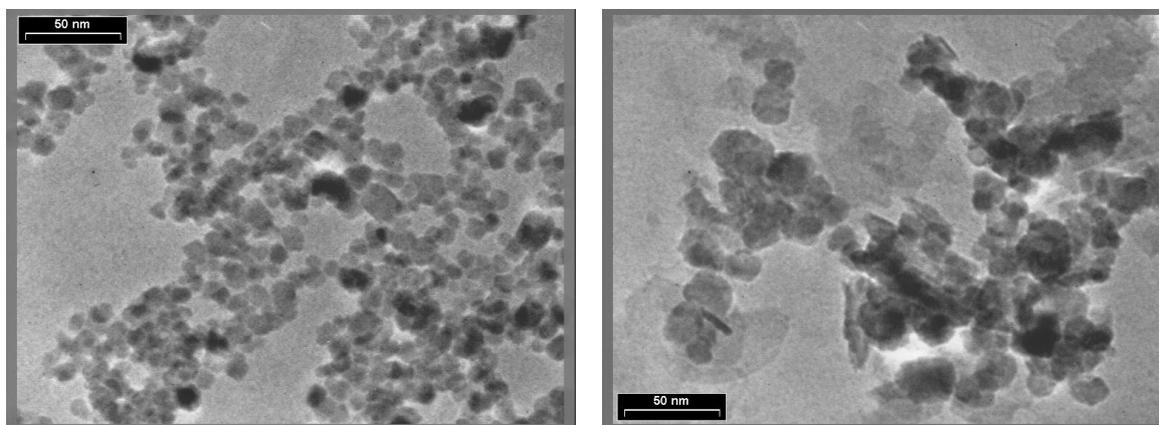


Fig. 6 HRTEM pictures of the crystallites obtained by (a) the conventional and (b) new method (0.1 cm^3 CMC solution and 5 cm^3 base solution addition)

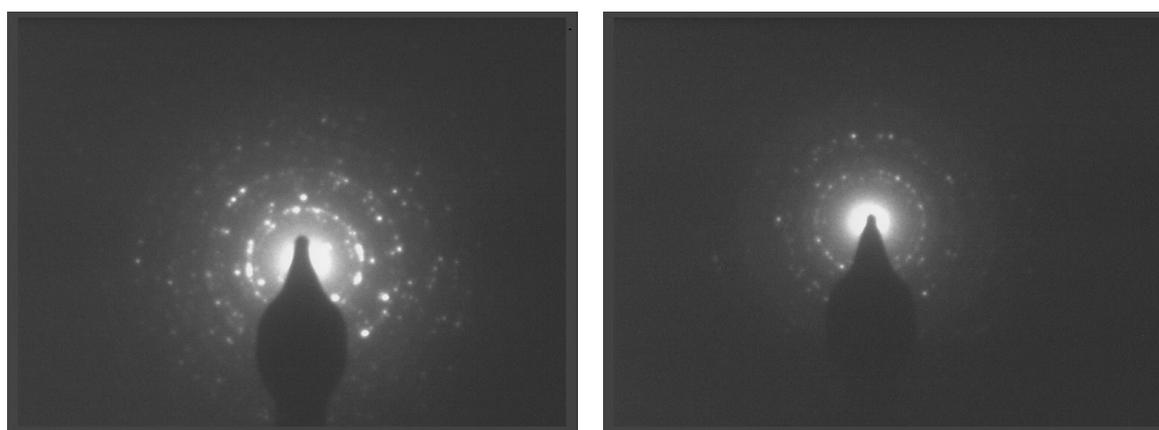


Fig. 7 The electron diffraction patterns of the crystallites obtained by (a) the conventional and (b) new methods

conventional and new method (0.1 cm^3 CMC solution and 5 cm^3 base solution addition), respectively. It is observed that the conventional method results in the production of smaller crystallite sizes (ca. 10 nm). The new method results in an average crystallite size of 25 nm. The tendency of the crystallites to agglomerate is more pronounced for the new method. Figs. 7-a and 7-b show the electron diffraction patterns of the crystallites obtained by the conventional and new methods, respectively. The distinct rings demonstrate the polycrystalline nature of both powders and correspond to a magnetite phase.

At this stage, we attempt to present a mechanism for production of nano-magnetite using the new method: After addition of the CMC solution, as the pH of the solution is raised by the addition of the base solution, Fe^{2+} cations are adsorbed onto the CMC molecules, presumably as hydrated species like $\text{Fe}(\text{H}_2\text{O})_6^{2+}$. Addition of the base solution triggers the production of iron (II) hydroxide in the form of emerald-green particles (this could have been observed visually during the experiment). At the same time, CMC acts as a mild oxidizing agent and provides the nucleation sites for Fe_3O_4 (magnetite). Afterwards, the growth process begins and eventually the

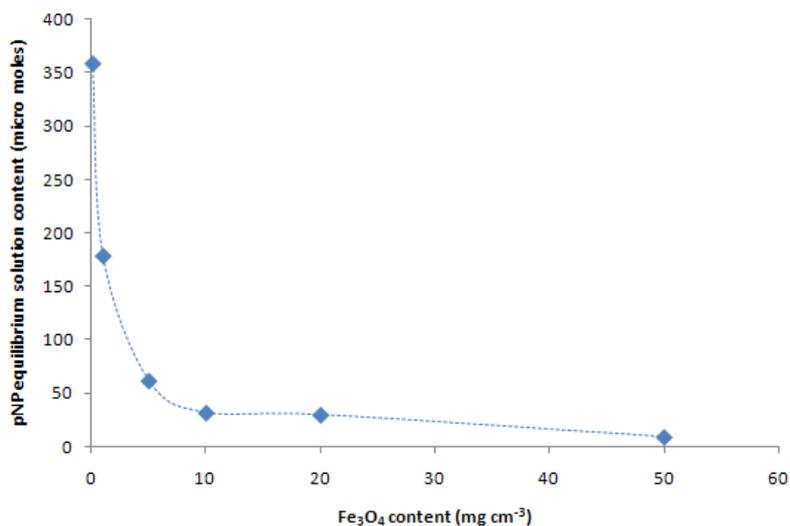


Fig. 8 Effect the amount of Fe₃O₄ nano-particles in the liquid phase on the content of pNP after a reaction time of 1 min

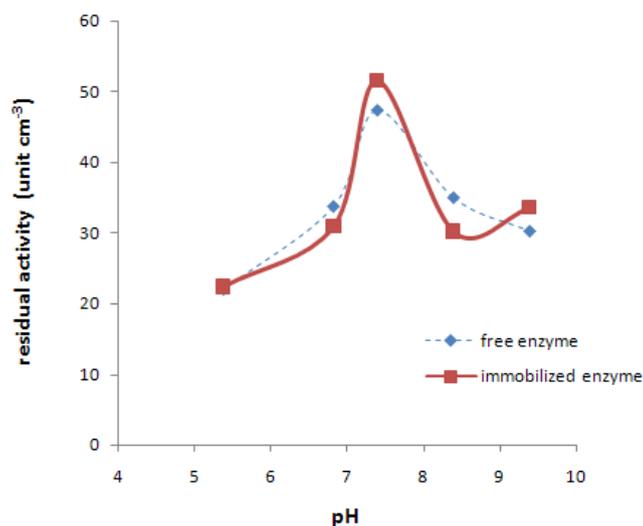


Fig. 9 Effect of pH on the activity of free and bound lipase in the range of 5.4-9.4

nano-sized magnetite crystallites are obtained.

Fig. 8 shows the concentration of pNP after contacting the pNPP solution with the nano-magnetite/immobilized lipase for a pre-determined reaction time of 1 min. It is observed that after a magnetite content of 10 mg cm⁻³, the pNP solution concentration decreases to less than 2 % of its initial value. In a similar series of experiments performed by Huang *et al.* (2003) using nano-magnetite produced by the conventional procedure, they obtained a minimum of 30 mg cm⁻³ nano-

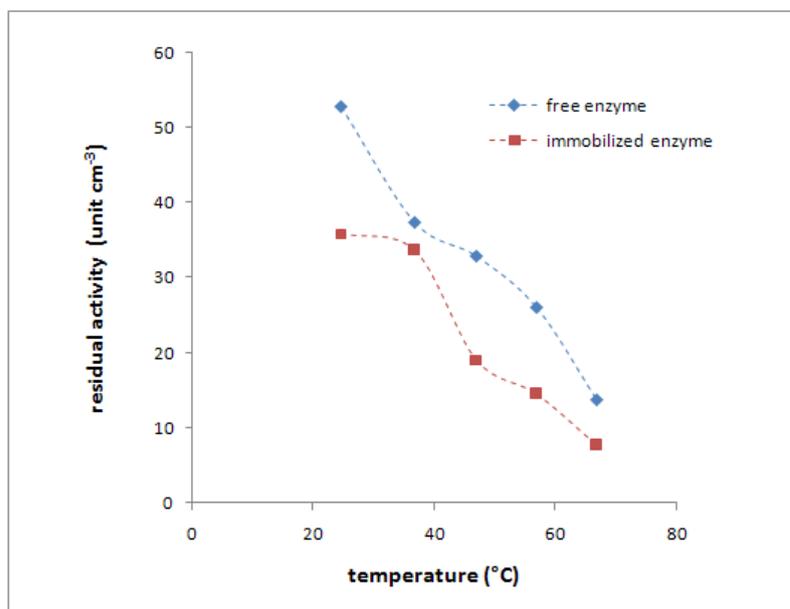


Fig. 10 Thermal stabilities of free and bound lipase in the temperature range of 25-67 °C

magnetite for the same degree of pNPP conversion. Accordingly, our results show that the nano-magnetite produced by our novel method results in a more effective enzyme immobilized composite. We attribute this characteristic to a more appropriate state of agglomeration of the initial nano-magnetite particles in contact with the liquid phase. In other words, our optimum sample has a favorable high exposed surface area.

The immobilized lipase shows a slightly better tolerance to variations in pH compared to the free enzyme (Fig. 9). The thermal stability of the immobilized enzyme on the nano-magnetite powder synthesized by the novel method is significantly better than the free enzyme (Fig. 9). Plotting of the Lineweaver-Burk diagrams also revealed the increased affinity of the enzyme to the substrate after immobilization (not shown).

4. Conclusions

A novel and simple method for the synthesis of nano-magnetite particles is disclosed. In the novel procedure, Fe^{2+} is the only source of metal cation. Carboxymethylcellulose (CMC) is used as the structure directing agent for the first time. An optimum base concentration exists for each CMC concentration leading to minimal agglomeration. There exists a minimum CMC concentration (0.0016 wt. %), lower than that no magnetite forms. It is shown that using the new method, it is possible to immobilize a lipase enzyme (*Candida Rugosa*) with immobilization efficiency larger than 98 % with a loading more than 3 times the reported value in the literature. The latter phenomenon is explained based on a more favorable state of agglomeration which provides a higher exposed surface of the nano-magnetite particles to the liquid phase.

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