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Synthesis and Characterisation of Biocompatible Polymer-Conjugated Magnetic Beads for Enhancement Stability of Urease

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Abstract We reported natural polymer-conjugated magnetic featured urease systems for removal of urea effectively. The optimum temperature (20–60 °C), optimum pH (3.0–10.0), kinetic parameters, thermal stability (4–70 °C), pH stability (4.0–9.0), operational stability (0–250 min), reusability (18 times) and storage stability (24 weeks) were studied for characterisation of the urease-encapsulated biocompatible polymer-conjugated magnetic beads. Also, the surface groups and chemical structure of the magnetic beads were determined by using attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR). The all urease-encapsulated magnetic beads protected their stability of 30–45 % relative activity at 70 °C. A significant increase was observed at their pH stability compared with the free urease for both acidic and alkaline medium. Besides this, their repeatability activity were approximately 100 % during 4th run. They showed residual activity of 50 % after 16 weeks. The importance of this work is enhancement stability of immobilised urease by biocompatible polymer-conjugated magnetic beads for the industrial application based on removal of urea.

Keywords Fe_3O_4 nanoparticles · Fe[NiFe]O_4 nanoparticles · Magnetic polymeric beads · Urease encapsulation · Urease stability

Introduction

Scientist have a growing interest for magnetic materials especially ferri- and ferromagnetic materials due to their chemical and physical properties. They have been used in the area of bioscience, medicine and industrial applications like purification, separation, immunoassay,

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drug delivery, magnetic resonance imaging, tissue engineering, diagnosis, hyperthermia, biomolecule immobilisation, biosensor and toxic elements removal from industrial waste water [1–6]. These materials offer important advantages: controllable particle size, monitoring, nontoxicity and easily separation. The surface properties of magnetic nanoparticle allow functionalizing magnetic nanoparticles by various functional groups for a range of applications.

In recent years, scientists are interested in design of new magnetic polymeric materials with different magnetic nanoparticles [7]. Magnetic polymeric materials have different sizes, different shapes and different surface area; they are porous or nonporous materials. Generally, the magnetic polymers comprise a magnetic centre and polymeric coating. The structure of the polymeric material can be varied due to the aim of the application. A variety of polymers such as pullulan, polystyrene, polyacrylamide, poly(*N*-isopropylacrylamide), poly(L-lactic-co-glycolic) acid, albumin and cellulose have been used for preparation of these magnetic polymeric materials [8–13]. Emulsion polymerisation, photochemical polymerisation, in situ polymerisation, dispersion polymerisation, precipitation polymerisation, suspension polymerisation and radical polymerisation have been investigated for preparation of magnetic polymeric materials [14–19].

Poly[β-(1 → 4)-2-amino-2-deoxy-D-glucose], chitosan, is a biocompatible polymer that is composed of amino and hydroxyl groups and is derived from deacetylation of chitin. It is also antibacterial, nontoxic and biodegradable. Chitosan shows cationic behaviour in acidic solutions due to the presence of amino groups and so it has affinity for metal ions [20, 21]. Alginate consists of mannuronic acid and guluronic acid and is one of the most successful biopolymer for encapsulation because it can easily form a soft gel. It has anionic charged due to carboxyl groups [22].

Possibilities of more efficient enzymatic catalysts have been investigated since enzyme immobilisation has been developed. The main purpose of immobilisation is to improve the stability properties of enzymes like thermal stability, pH stability, reusability and storage stability [23, 24]. Encapsulation, one of the immobilisation methods, is based on the entrapment of an enzyme with a network that allows the input of substrate and output of products. In this method, the enzyme molecules are free and is not bound to the matrix [25].

Urease (EC 3.5.1.5), the model enzyme of this study, catalyses the hydrolysis reaction of urea to carbon dioxide and ammonia. Urease is used to calculate the amount of urea in biological solutions, in order to remove urea from blood, waste water, fruit juice and foods [26].

In this study, an alternative approach involving the preparation of four different polymeric biocompatible magnetic beads and their usage for urease immobilisation was offered. The magnetic beads were chitosan-magnetite (CM), chitosan nickel ferrite (CNF), chitosan-coated alginate-magnetite (CAM) and chitosan-coated alginate-nickel ferrite (CANF) beads. During preparation, the polymeric biocompatible magnetic bead, urease enzyme, was encapsulated into the beads, and then the performance of removal of urea was investigated. Our main aim was the improvement of stability properties of urease by encapsulation in different magnetic polymeric beads. The characteristic properties of encapsulated urease systems were compared with free urease. We report an economic and easily applicable method for urease immobilisation. The results showed that stability properties of urease were improved by the magnetic polymeric beads.

Experimental

Materials

Urease (EC 3.5.1.5), urea, alginic acid sodium salt, chitosan, $FeSO_4 \cdot 7H_2O$, $Fe_2(SO_4)_3 \cdot H_2O$ and $Ni(NO_3)_2 \cdot 6H_2O$ were purchased from Sigma. All other chemicals used were of analytical grade.

Urease Activity Assay

The activities of the urease (free and immobilised) were analysed using the Berthelot method [27]; 1.94 ml phosphate buffer (50 mM, pH 7.0), 10 μl urea solution (0.1 mM) and 50 μl enzyme solution were added to the reaction tube, and the mixture was stirred for 10 min at room temperature; 500 μl phenol reagent and 500 μl hypochloride reagent were added to the reaction tube and incubated at 50 °C for 5 min. The urease activity was measured at 630 nm spectrophotometrically. One unit urease activity includes hydrolysis of 1 μmol urea/min at 25 °C, pH 7.0.

Preparation of Urease-Encapsulated CM and CNF Magnetic Beads

Synthesis of Magnetite (Fe_3O_4) Nanoparticles

Co-precipitation method using ferric and ferrous salts discussed by Koneracka et al. [1] was used for preparing Fe_3O_4 magnetic particles with some modifications. Eight grammes of $Fe_2(SO_4)_3 \cdot H_2O$ and 2.8 g of $FeSO_4 \cdot 7H_2O$ were dissolved in 100 ml deionised water. The solution was mixed with 75 ml of 8 M NH_3 solution and stirred at room temperature. The mixture was incubated at 80 °C for 30 min. The magnetic particles were separated and washed several times with deionised water. Then, the particles were dried in an incubator at 70 °C.

Synthesis of Nickel Ferrite ($Fe[NiFe]O_4$) Nanoparticles

Ten grammes of $Fe_2(SO_4)_3 \cdot H_2O$ and 3.7 g of $Ni(NO_3)_2 \cdot 6H_2O$ were dissolved in 100 ml deionised water. The solution was mixed with 50 ml of 2 M NaOH solution and stirred at room temperature. The mixture was incubated at 100 °C for 3 h. The magnetic particles were separated and washed several times with deionised water. Then, the particles were dried in an incubator at 80 °C.

Urease Encapsulation and Formation of CM or CNF Magnetic Beads

Six milligrammes per millilitre of chitosan solution was prepared in acetate buffer (pH 5; 50 mM). The chitosan solution was mixed with 25 mg of Fe_3O_4 or $Fe[NiFe]O_4$ particles and sonicated for 10 min. Urease in different amounts (0.5, 1, 1.5, 2 mg) was added to the mixture. Then chitosan-magnetic particle-urease mixture was added dropwise into the 0.5-M NaOH solution at room temperature. The beads were incubated in the NaOH solution. After 1 h, the beads were separated from the solution and washed with deionised water (Fig. 1).

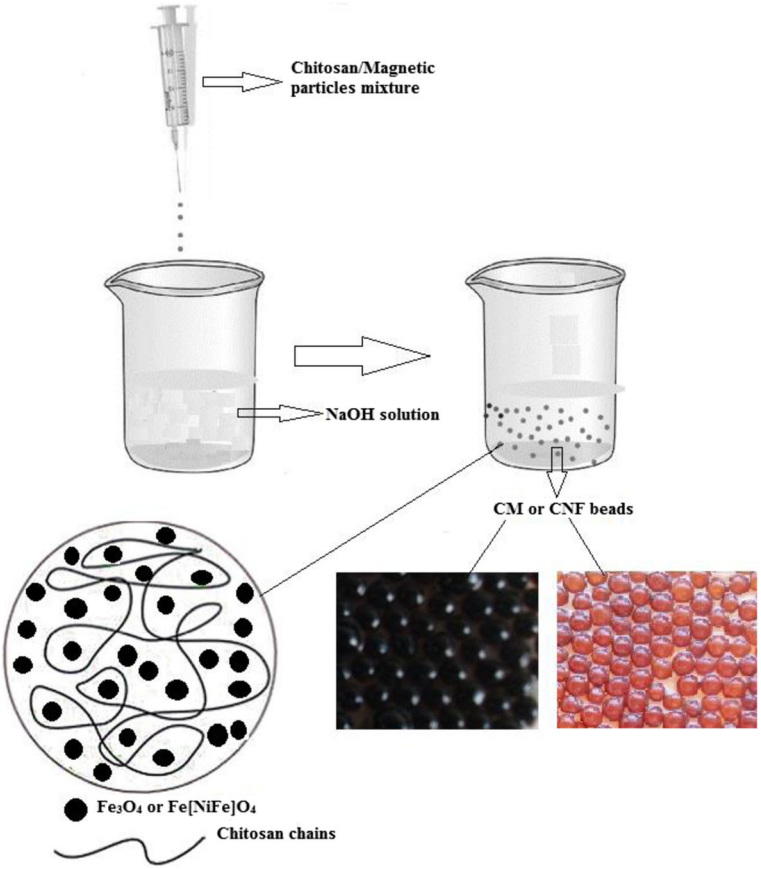


Fig. 1 The schematic illustration of preparation of CM and CNF magnetic beads

Preparation of Urease-Encapsulated CAM and CANF Magnetic Beads

Synthesis of Urease-Encapsulated Alginate Beads

Three per cent (*w*/*v*) alginate solution was prepared in deionised water. Then, urease in different amounts (0.5, 1, 1.5, 2 mg) was added to the alginate solution. The alginate-urease mixture was added dropwise into 2 % (*w*/*v*) $CaCl_2$ solution at room temperature. The beads were incubated in the $CaCl_2$ solution. After 1 h, the beads were separated from the solution and washed with deionised water (Fig. 2).

Chitosan Coating on the Urease-Encapsulated Alginate Beads

Six milligrammes per millilitre chitosan solution was prepared in acetate buffer (pH 5; 50 mM). The urease-encapsulated alginate beads were thrown into the chitosan solution and stirred for 1 h. Chitosan-coated urease-encapsulated alginate beads were separated from the solution and washed with deionised water (Fig. 2).

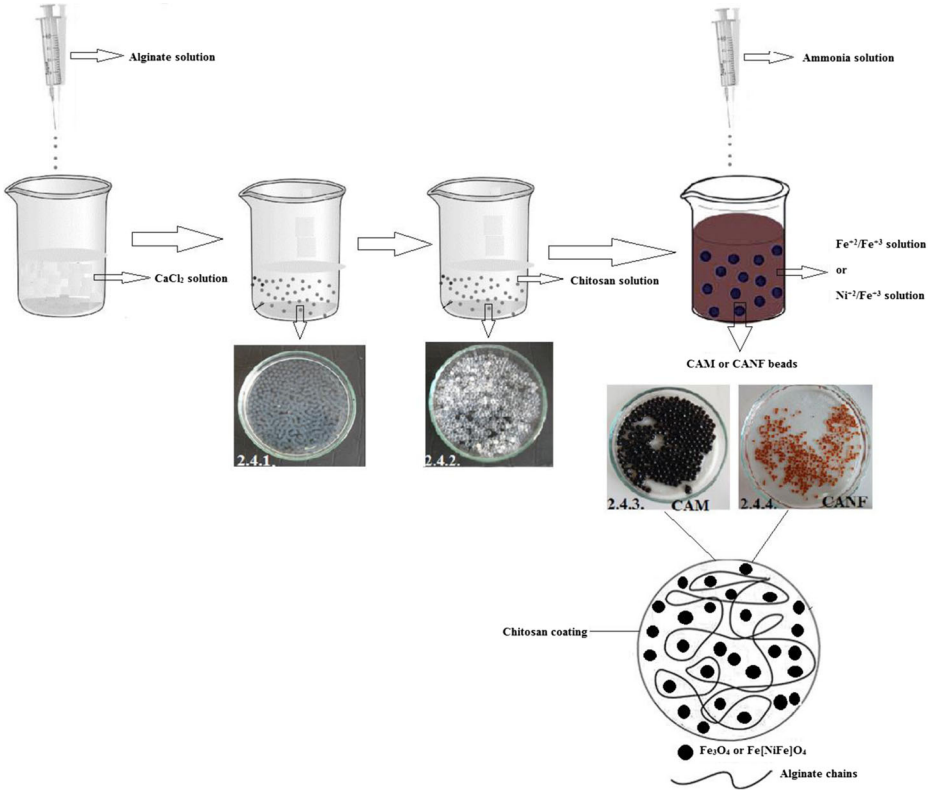


Fig. 2 The schematic illustration of preparation of CAM and CANF magnetic beads (*2.4.1.*, urease-encapsulated alginate beads; *2.4.2.*, chitosan coating on the urease-encapsulated alginate beads; *2.4.3.*, urease-encapsulated CAM magnetic beads; *2.4.4.*, urease-encapsulated CANF magnetic beads)

Preparation of Urease-Encapsulated CAM Magnetic Beads

In 100 ml deionised water, 7.99 g of $Fe_2(SO_4)_3{\cdot}H_2O$ and 2.78 g of $FeSO_4{\cdot}7H_2O$ were dissolved. Chitosan-coated urease-encapsulated alginate beads were thrown into the Fe^{+2}/Fe^{+3} solution and stirred for 20 min (mixture); 22 % ammonia solution was added dropwise into the mixture and incubated for 15 min. CAM beads were separated from the solution and washed with deionised water (Fig. 2).

Preparation of Urease-Encapsulated CANF Magnetic Beads

In 100 ml deionised water, 9.97 g of $Fe_2(SO_4)_3{\cdot}H_2O$ and 3.64 g of $Ni(NO_3)_2{\cdot}6H_2O$ were dissolved. Chitosan-coated urease-encapsulated alginate beads were thrown into the Ni^{+2}/Fe^{+3} solution and stirred for 20 min (mixture); 22 % ammonia solution was added dropwise into the mixture and incubated for 15 min. CANF beads were separated from the solution and washed with deionised water (Fig. 2).

Characterisation of Urease-Encapsulated CM, CNF, CAM and CANF Magnetic Beads

Surface groups and chemical structure of the beads were analysed using attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR; Thermo Scientific Nicolet iS-5 ATR-FTIR Spectrometer).

The morphologies of the magnetic beads were observed by scanning electron microscopy (SEM) using JEOL JSM-7600F model.

The optimum temperatures of encapsulated and free ureases were investigated from 20 to 60 °C using the standard urease activity determination procedure. The optimum pH values of encapsulated and free ureases were analysed in citrate, acetate, phosphate and Tris-HCl buffers (50 mM), ranging from pH 3.0 to 10.0 under assay condition.

To define the maximum velocity of the enzymatic reaction (V_m) and the Michaelis-Menten constant (K_m) for encapsulated urease systems and free urease, activity assay was studied for different concentrations of urea solutions under assay conditions.

Influence of temperature was determined by varying temperature at 20–70 °C. While all the other reaction conditions were constant, pH stability was studied at varying pH values in the range 4.0–9.0. Citrate, acetate, phosphate and Tris-HCl buffer solutions (50 mM) were used for adjusting reaction pH. For the both parameters, incubation time was 1 h.

The operational stabilities of the encapsulated urease systems were investigated in a continuous tank reactor system with mechanical stirring by measuring urease activities at regular time. The operational stabilities of encapsulated urease systems were monitored for 250 min.

The determination of reusabilities of encapsulated urease systems were repeated 18 times under standard assay conditions. The reaction time for each activity measurement was 10 min, and then the particles were separated and washed with pH 7.0 (50 mM) phosphate buffer. After each activity assay, the encapsulated urease systems were reintroduced into a fresh medium.

To investigate the storage stabilities of encapsulated urease systems, the beads were stored into 0.2 % sodium azide solution at 4 °C. The activity of the beads was determined at specific time intervals.

Results and Discussion

Urease-Encapsulated Biocompatible Polymer-Conjugated Magnetic Beads

Immobilised ureases have been studied with many other carriers and many methods. Chitosan is attractive for these applications because of its chemical properties. On its surface, it includes amino and hydroxyl groups, so, it is positively charged at low pH. This positive charge can increase electrostatic interactions with enzyme molecules. Also, the functional groups of amino acid residues (especially indole of tryptophan, thiol of cysteine, amino of lisine, imidazole of histidine, carboxylate of aspartate and glutamate) contribute adsorption of enzyme molecules at the surface. Because these functional groups are electron donor and they interact with transition metals by van der Waals, electrostatic, hydrophobic interactions. Therefore, in this study, chitosan and magnetic nanoparticles were used in combination. We used Fe_3O_4 and $Fe[NiFe]O_4$ magnetic nanoparticles, because of their easy separable (Fig. 3) and monitorable structures. Also, the magnetic nanoparticles increased the mechanical and chemical properties of the polymers.

Fig. 3 The photograph of magnetic beads attracted by magnetic bar

To determine the optimum amount of urease for the encapsulation procedure, the different urease amounts ranging from 0.5 to 2 mg were used when all of the other parameters were constant. The results are given in Table 1. We reached the optimum activity value when the amount of urease was 1.5 mg for all magnetic beads. When the urease amount was 2 mg, the activity decreased because the amount of magnetic nanoparticles, chitosan and also alginate were not enough for immobilisation. Jiang et al. [28] reported that the optimum enzyme concentration was 0.8 mg/ml for laccase-immobilised magnetic chitosan beads. In the other paper, where pullulanase immobilised magnetic kitosan beads, they reported that the optimum enzyme amount was 0.18 mg [29]. According to DeGroot and Neufeld [30], the suitable enzyme concentration was 20 mg/ml for urease-encapsulated chitosan-coated alginate beads. Urease concentration was used as 0.5 mg/ml for immobilisation on chitosan beads in the other study [31]. In our previous study, urease immobilised TiO_2/chitosan beads, and the optimum amount of enzyme was found as 1 mg/ml [32]. Wang et al. [33] reported that the amount of glucose oxidase for alginate/chitosan microcapsule was 1 mg.

FTIR and SEM Analysis

The conjugation of Fe_3O_4 or $Fe[NiFe]O_4$ magnetic nanoparticles into the beads was studied using Fourier transform infrared spectroscopy (FTIR). The FTIR spectra of the magnetite, nickel ferrite, chitosan, alginate beads, CM, CNF, CAM and CANF beads are shown in Fig. 4. All magnetic beads (CM, CNF, CAM and CANF) exhibited strong bands at 605 and

Table 1 The effect of the urease amount on the encapsulated urease activity

The amount of urease (mg)	Activity (U)[a]				Activity (U)
	CM	CNF	CAM	CANF	Free urease
0.5	0.421	0.460	0.570	0.566	0.802
1	0.636	0.736	0.655	0.680	0.809
1.5	*0.714*	*0.767*	*0.664*	*0.696*	*0.870*
2	0.486	0.520	0.615	0.624	0.866

The immobilisation conditions: 25 mg of the Fe_3O_4 or $Fe[NiFe]O_4$ particles; 6 mg/ml chitosan concentration; 3 % (*w*/*v*) alginate concentration

[a] The activities of encapsulated ureases were calculated by amounts of total beads

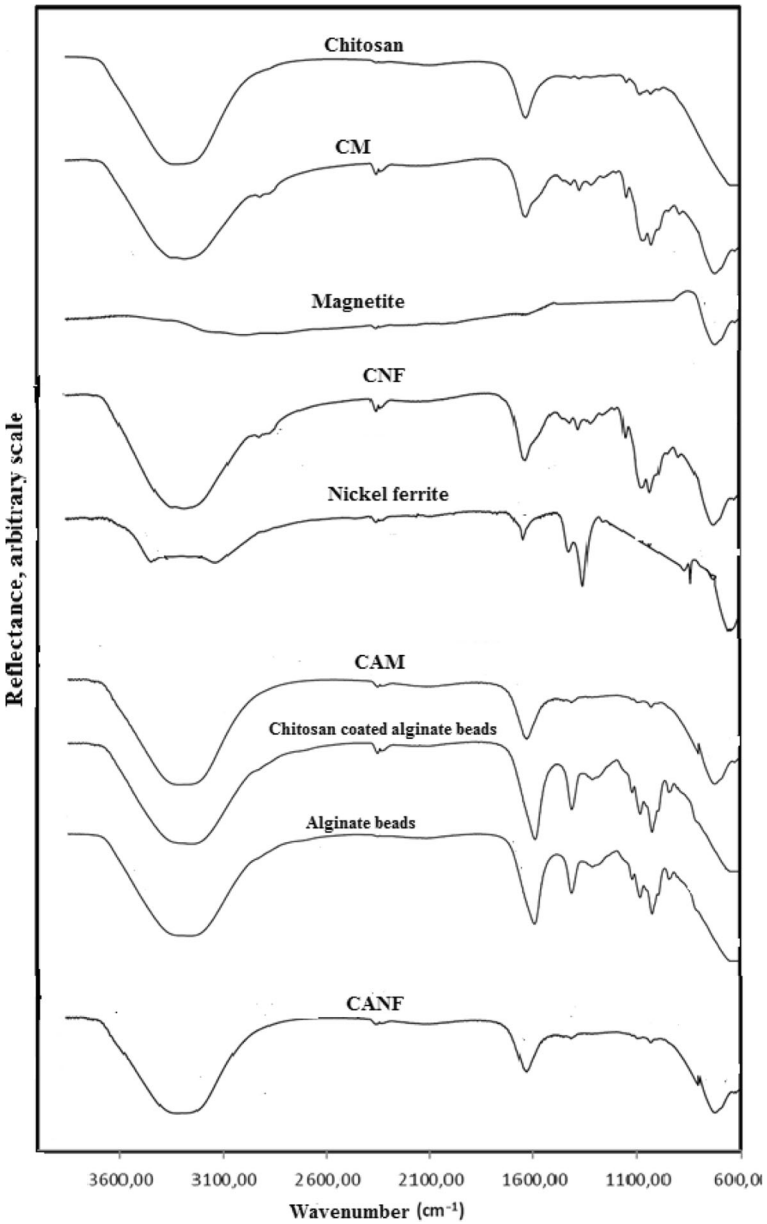


Fig. 4 FTIR spectrum of CM, CNF, CAM, CANF magnetic beads, magnetite, nickel ferrite, alginate beads and chitosan-coated alginate beads

1600 cm^{-1} due to Fe–O stretching vibrations and amino groups of chitosan, respectively. For CAM and CANF, the symmetrical and anti-symmetrical stretching of the carboxylate groups and CO band showed at 1600, 1400, 1050 cm^{-1}, respectively. As a result, the biocompatible polymer-conjugated magnetic beads were formed successfully.

SEM images of urease-immobilised CM, CNF, CAM and CANF magnetic beads are shown in Fig. 5. All the magnetic beads showed spherical morphology and had porous

surfaces. The mean diameters of the beads were in the range of 0.96 to 1.1 mm. As seen in Fig. 5e–h, chitosan completely covered the beads.

The Characterisation of Urease-Encapsulated Biocompatible Polymer-Conjugated Magnetic Beads

The enzymes are denatured and not stable at high temperature, because of their proteic structure. Thus, enzyme reaction cannot be performed practically at high temperature. The effect of temperature on the urease activity is shown in Fig. 6. The activity of free urease significantly decreased after 35 °C, when the activity of urease-encapsulated CM, CNF, CAM and CANF showed less loss after optimum temperature (40 °C for CM; 35 °C for CNF, 35–45 °C for CAM and 35–40 °C for CANF). The expanded temperature profile for encapsulated urease systems may be based on the change of the conformational integrity of the structure of enzyme via interactions with the polymers and magnetic nanoparticles. Also, immobilised ureases may acquire resistance to temperature changes by chitosan and alginate molecules. The optimum temperature values of CM, CNF, CAM and CANF were slightly different, because they had different structure and different stability.

The optimum temperature of an enzyme which is obtained from different organism may be different. Besides this, the optimum temperature can change after immobilisation. In the previous studies, the temperature effect on the enzymatic activity was studied. Kumar et al. [31] reported that the optimum temperatures of native urease and urease-immobilised chitosan beads were 65 and 75 °C, respectively, but they did not observe a large temperature profile for the immobilised one. According to Gabrovska et al. [34], the optimum temperatures of free urease and urease-immobilised poly(acrylonitrile)chitosan membrane were found to be 30 and 25 °C, respectively. In the other study, urease was encapsulated with chitosan/alginate complex beads and after immobilisation, the optimum temperature was shifted to 5 °C [35].

The effect of pH on the enzymatic activity was studied by drawing the optimum pH curves. The optimum urea-removal values were found at pH 7.5 for free urease, pH 6.5–7.5 for CM, pH 6.0–7.5 for CNF and pH 5.5–8.0 for CAM and CANF (Fig. 7). When the activity of free urease decreased rapidly at high acidic and high alkaline medium, all of the encapsulated ureases protected their activity at these pH values. The matrix material contributed to the pH stability of enzyme with additional ionic interactions. Also, the microenvironment of encapsulated urease (H^+/OH^- ion balance) may come to equilibrium by interaction of buffer solution and magnetic nanoparticles. Thus, the optimum pH profiles of all the immobilised ureases were expanded. Especially, CAM and CNF beads allow the usage in the wide pH range. After the immobilisation, the optimum pH value of enzymes often changes in the previous studies. According to Kumar et al. [31], when the optimum pH of nature urease was 7.0, it was found as 8.0 for the urease-immobilised chitosan beads. In the other study, the optimum pH values of native urease and urease-immobilised chitosan/alginate complex beads were 7.5 and 8.0, respectively [35]. Gabrovska et al. [34] reported that there is no significant difference between the optimum pH curves of the free urease and urease-immobilised poly(acrylonitrile)chitosan membrane.

After immobilisation, the three-dimensional structure of enzyme may change. Due to these changes, thermal stability of the enzymes may decrease, increase or remain unchanged. The protection of the enzymatic activity for a long time at a wide temperature profile is important for industrial processes. In order to analyse the thermal stability, free urease and encapsulated urease systems were incubated for a 1-h duration at different temperature values from 20 to 70 °C. The results are given in Fig. 8. As seen from the graph, when the free urease decreased its activity

Fig. 5 SEM micrographs of urease-immobilised CM, CNF, CAM, CANF magnetic beads; **a**, **b** urease-immobilised CM magnetic beads, **c**, **d** urease-immobilised CNF magnetic beads, **e**, **f** urease-immobilised CAM magnetic beads, **g**, **h** urease-immobilised CANF magnetic beads (the beads were dried in air)

rapidly after 60 °C (10 % relative activity at 70 °C), all the encapsulated ureases protected their activity (30–45 % relative activity at 70 °C). At 70 °C, the activity values of free urease, CM, CNF, CAM and CANF were found to be 0.080, 0.221, 0.320, 0.294 and 0.303 U, respectively. At low temperature, the encapsulated ureases had lower thermal stability compared with the free urease because the magnetic beads shrank and became a rigid structure at low temperatures. So, it is believed that the enzyme substrate interaction may be partially blocked.

At high temperatures, the decrease of activity (free urease) is a result of enzyme denaturation processes. After immobilisation, the improvement of thermal stability associated with the properties of matrix material. The matrix may absorb a significant portion of the heat in the environment, so it may protect the enzyme from denaturation. Also, the multipoint interactions with the enzyme and matrix can prevent the loss of activity. The improvement of thermal stability is explained by protecting the active centre of enzyme depending on a special configuration of hydroxyl groups of magnetic particles in the aquatic environment. In the two previous studies, it was reported that after immobilisation, the activation energy can change and can contribute to the stability properties [36, 37]. Some studies related to immobilisation of urease, chitosan-poly(glycidyl methacrylate) copolymer [38], poly(acrylonitrile)chitosan composite membranes [34] and chitosan beads [31] were used as carriers. They explained the increase of thermal stability by multiple covalent bonding between urease and the surface of carrier. In the other paper on pullunase-immobilised magnetic chitosan beads, it was stated that the thermal stability of enzyme was increased by the magnetic carrier [29].

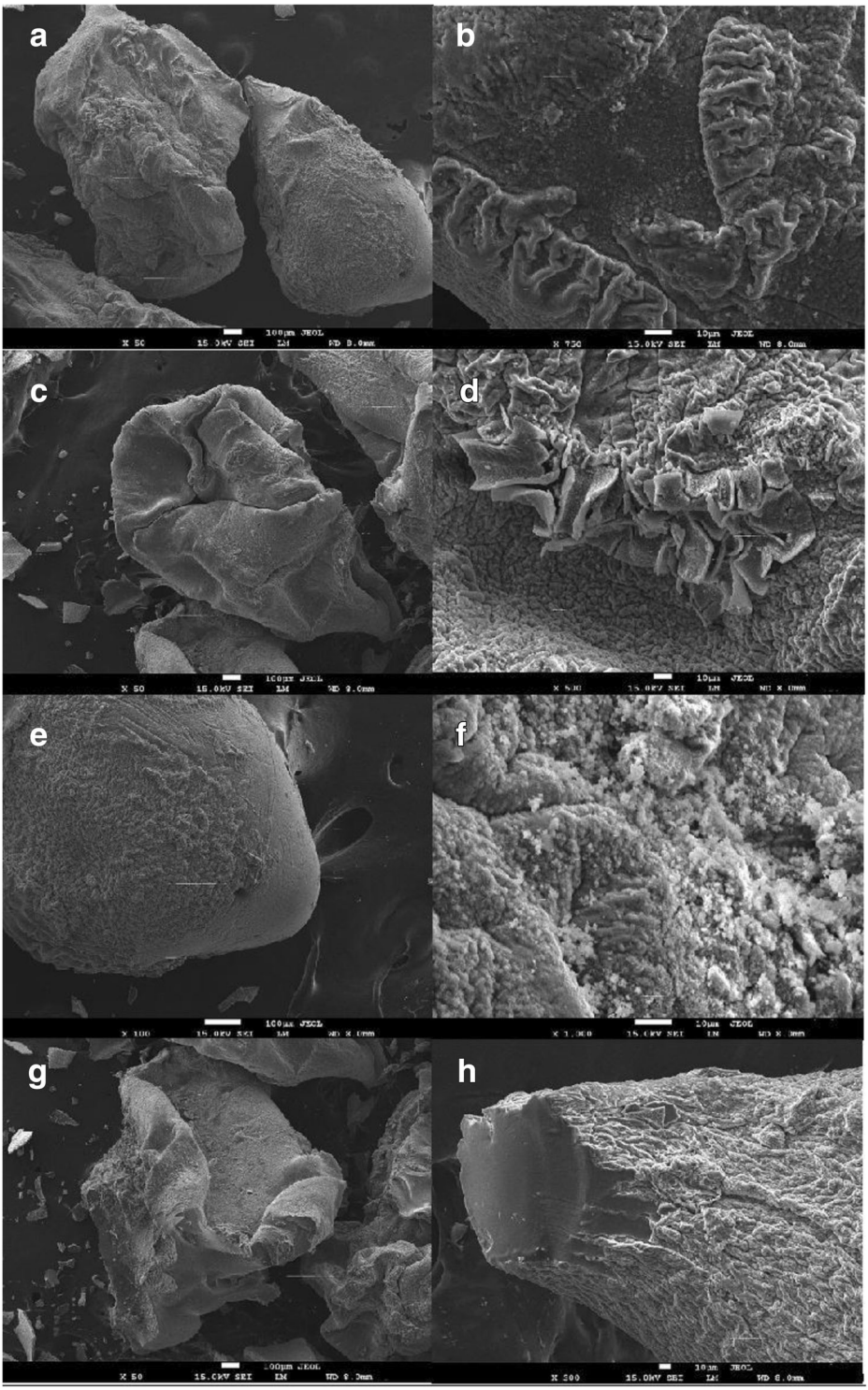
To determination the pH stabilities, free and encapsulated ureases were incubated for 1 h at pH values ranging from 4.0 to 9.0. The results are given in Fig. 9. For both acidic and alkaline media, a significant increase can be observed in all the encapsulated ureases compared with the free urease. This result shows that the conformational stability of urease increased by additional ionic interactions due to the properties of the carriers. Also, the interaction of magnetic nanoparticles and the reaction medium may provide the H+/OH− ion balance of the enzyme microenvironment. In a study about urease-immobilised chitosan-poly(glycidyl methacrylate) copolymer, they reported that the free and immobilised ureases showed similar pH stability curves [38].

One of the most important criteria for the practical application of immobilised enzymes is the performance in the continuous system. The half-life of encapsulated ureases were determined by activity assays at intervals and calculated by the equations as given below (Eqs. 1 and 2). The half-life of CM, CNF, CAM and CANF were found to be 85, 155, 43 and 49 min, respectively (Table 2).

$$\mathrm{KD} = \frac{2.303}{t} \log \frac{A0}{A} \tag{1}$$

$$\frac{t1}{2} = \frac{0.693}{\mathrm{KD}} \tag{2}$$

The kinetic parameters can alter because of the changes in enzyme (changes of three-dimensional structure, steric effects and diffusion effects) after immobilisation. The kinetic behaviours of the encapsulated ureases and free urease were investigated by Lineweaver-Burk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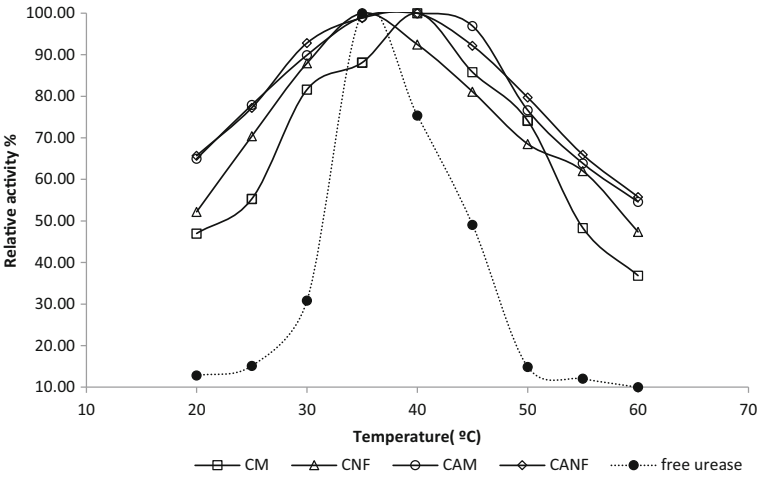

Fig. 6 Optimum temperature profiles of free and encapsulated ureases (the amount of urease, 1.5 mg/ml, at the standard activity conditions)

plots. The results are given in Table 2. Compared with the soluble urease, increase in the K_m values and decrease in V_m values of encapsulated ureases were observed. The reasons of the increase of the K_m values may be the decrease of conformational flexibility of enzyme, steric effects caused by the carrier and diffusion effects after immobilisation. This result is generally expected in the immobilised enzyme studies. According to Kara [35] and Prakash [39], after immobilisation, decreased in V_m and increased in K_m were observed for the immobilised catalase.

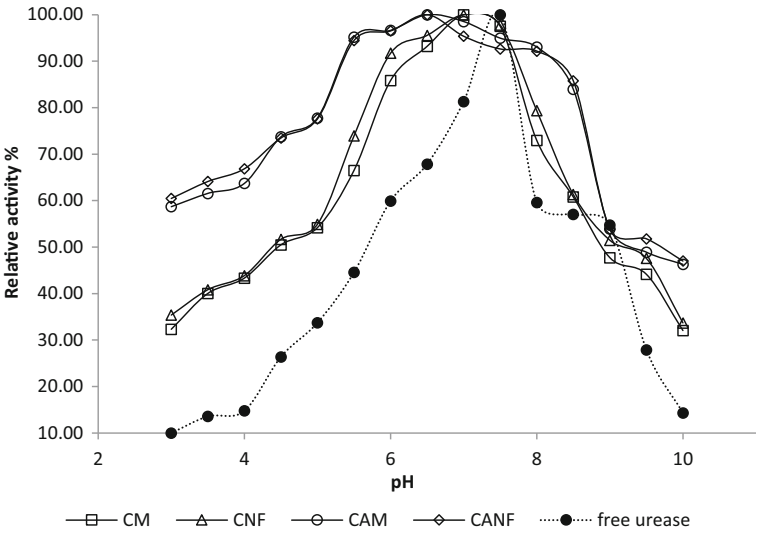

Fig. 7 Optimum pH profiles of free and encapsulated ureases (the amount of urease, 1.5 mg/ml, at the standard activity conditions)

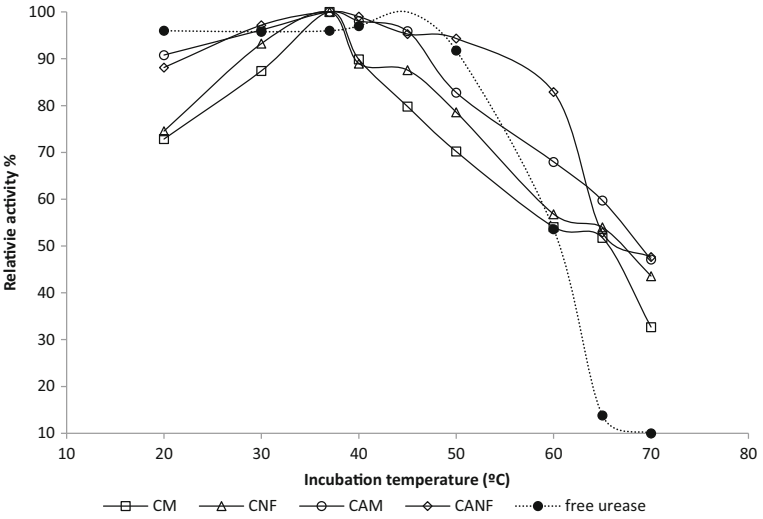

Fig. 8 The thermal stability of free and encapsulated ureases

In order to determine the reusability of urease-encapsulated magnetic beads, the beads were used 18 times for the catalytic reaction (Fig. 10). At the end of the run, the magnetic beads were separated and washed with deionised water. Then, the reaction medium was altered to fresh medium. The relative activities of CM and CNF beads were approximately 100 % during the 4th run. The beads protected their 50 % activity during the 18th run. CAM and CANF beads showed residual activity of 100 % after 5 reuses and 50 % after 14 reuses. One of the most important benefits offered by the immobilised enzymes is reusability. Therefore, many previous studies

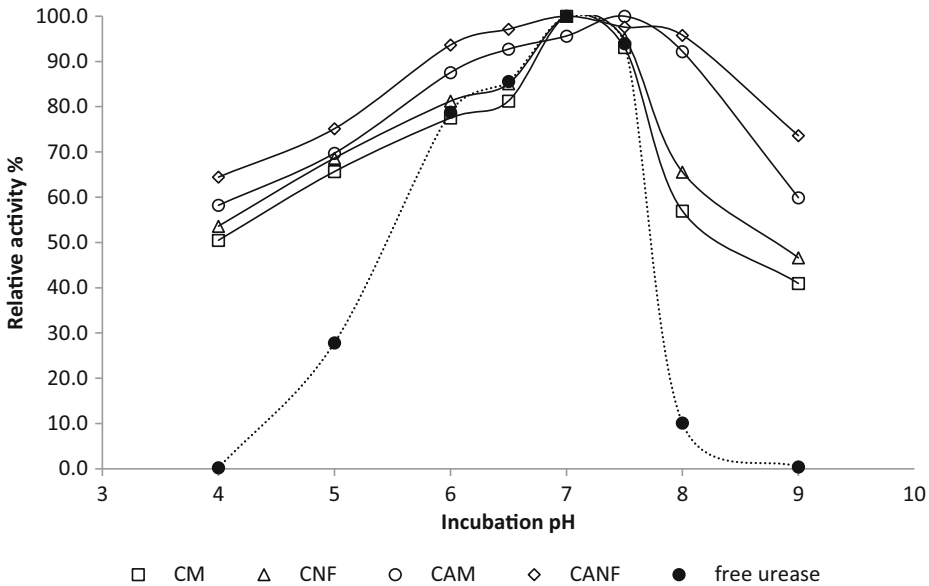

Fig. 9 The pH stability of free and encapsulated ureases

Table 2 The half-time ($t_{1/2}$) and kinetic parameters of encapsulated ureases

	$t_{1/2}$ (min)	K_m (mM)	V_m (U/mg protein)
CM	85	0.168	0.823
CNF	155	0.165	0.827
CAM	43	0.172	0.529
CANF	49	0.181	0.532
Free urease	–	0.071	1.22

about urease immobilisation investigated this parameter. A comparison between similar studies on the immobilisation of enzyme and our systems is given in Table 3. When Tables 2 and 3 are analysed together, the use of CAM and CANF beads in the batch systems is considered to be more advantageous than using the continuous systems.

The storage stabilities of the encapsulated urease systems were investigated by measurement of the urease activities at regular time during 24 weeks. CM and CNF beads showed a residual activity of 50 % after 19 weeks. The relative activities of CAM and CANF beads were approximately 50 % during 16 weeks. A comparison between similar studies on the immobilisation of enzyme and our systems is given in Table 4. According to the table, our encapsulated urease systems offered great advantages in terms of storage performance.

Conclusions

In this study, we suggested urease immobilisation applications of biocompatible polymer-conjugated magnetic beads. The preparation of magnetic beads and immobilisation method were simple, safe and low cost. This encapsulation method offers an effective process for removal of urea. Also, the magnetic properties of the beads facilitate their separation from the reaction medium by a magnetic field. The characteristic properties (optimum temperature, optimum pH, kinetic parameters, thermal stability, pH stability, operational stability and

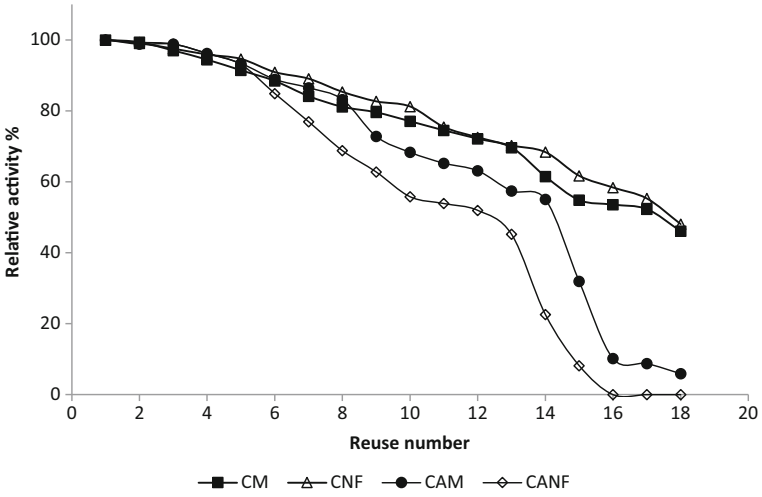

Fig. 10 The reusability of encapsulated ureases (the amount of urease, 1.5 mg/ml; 25 °C; pH 7.0)

Table 3 Reusabilities comparison between similar studies on the immobilisation of enzyme and our systems

Carrier	Enzyme	Immobilisation method	Reuse number and % residual activity	References
Chitosan/alginate beads	Urease	Encapsulation	50 % after 20 reuse	[35]
Chitosan coated Carboxymetyl celulose-alginate beads	Urease	Encapsulation	50 % after 20 reuse	[40]
Alginate beads	Urease	Cross-linking	54 % after 14 reuse	[31]
Chitosan beads	Urease	Cross-linking	80 % after 14 reuse	[31]
Magnetic chitosan beads	Laccase	Cross-linking	20 % after 10 reuse	[28]
Magnetic chitosan beads	Pullunase	Cross-linking	64 % after 10 reuse	[29]
Magnetic chitosan beads	α-amilase and glucoamilase	Co-immobilisation	50 % after 5 reuse	[41]
CM beads	Urease	Encapsulation	50 % after 18 reuse	Our study
CNF beads	Urease	Encapsulation	50 % after 18 reuse	Our study
CAM	Urease	Encapsulation	50 % after 14 reuse	Our study
CANF	Urease	Encapsulation	50 % after 14 reuse	Our study

reusability) of encapsulated ureases (CM, CNF, CAM and CANF) were compared with soluble urease, and these stability properties were improved after encapsulation of urease. Encapsulation of the urease with magnetic polymeric material may permit the dispersal of the urease molecules, whereby the active centre is not limited, thus making the activity of urease highly protected. The envisaged urea-removal systems protected their activity at 30–45 % relative activity at 70 °C. One of the most important benefits offered by the immobilised enzymes is reusability. Our encapsulated ureases protected their activity (100 %) after the 4th–5th runs. These beads showed at least 50 % activity at all the pH values (4.0–9.0). All the magnetic beads showed residual activity of 50 % after 16 weeks. The results show clearly that the biocompatible polymer-conjugated magnetic beads are effective and easily applicable for encapsulation of urease. The importance of this work is improving the stability properties of urease for industrial applications.

Table 4 Storage stabilities comparison between similar studies on the immobilisation of enzyme and our systems

Carrier	Immobilisation methods	Storage time	References
Chitosan/alginate beads	Encapsulation	10 weeks	[35]
κ-carrageenan capsules	Encapsulation	4 days	[42]
Polysiloxane polymer	Entrapment	18 weeks	[43]
polyacylamide gel	Encapsulation	45 days	[44]
Agar tablets	Entrapment	53 days	[45]
CM beads	Encapsulation	19 weeks	Our study
CNF beads	Encapsulation	19 weeks	Our study
CAM beads	Encapsulation	16 weeks	Our study
CANF beads	Encapsulation	16 weeks	Our study

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