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Immobilization of Urease on (HEMA/IA) Hydrogel Prepared by Gamma Radiation

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In the present study, the copolymeric hydrogels based on 2-hydroxyethyl methacrylate (HEMA) and itaconic acid (IA) were synthesized by gamma radiation induced radical polymerization, in order to examine the potential use of these hydrogels in immobilization of *Citrullus vulgaris* urease. Gelation and Swelling properties of PHEMA and copolymeric P (HEMA/IA) hydrogels with different IA contents (96.5/3.5, 94.4/5.6 and 92.5/7.5 mol) were studied in a wide pH range. Initial studies of so-prepared hydrogels show interesting pH sensitivity in swelling and immobilization. *C. vulgaris* urease was immobilized on HEMA/IA (92.5/7.5) at 6 kGy with 41.3% retention of activity. The properties of free and immobilized urease were compared. Immobilized urease maintained a higher relative activity than free urease at both lower and higher pH levels, indicating that the immobilized urease was less sensitive to pH changes than the free urease. The K_m value of the immobilized urease was approximately 2 times higher than that of the free urease. Temperature stability was improved for immobilized enzyme. The free form exhibited a loss about 80% of activity upon incubation for 15 min at 80°C. The influence of various heavy metal ions at the concentration of l mM was improved after enzyme immobilization. The immobilization of *C. vulgaris* urease on HEMA/IA (92.5/7.5) at 6 kGy showed a residual activity of 47 % after 4 reuses.

Keywords: Radiation, poly(HEMA/IA), swelling, immobilization, urease

1. Introduction

Hydrogels are of interest in biomedical applications because of their tunable chemical and three-dimensional physical network structures, high water content in an aqueous medium without dissolution, good mechanical properties, and (1,2).

High-energy radiation has been successfully applied for more than a few decades for synthesis, modification and sterilization of hydrogels. Furthermore, this area is growing continuously and a large number of papers have been published in recent years. Intelligent or smart hydrogels have been developed as stimuli responsive materials, which can undergo volume changes in response to changes in temperature, pH and antigen concentration. Poly(2-hydroxyethyl methacrylate) P(HEMA), is a favorable biomaterial because of its excellent biocompatibility and physicochemical properties similar to those of living tissues (3,4). It also exhibits good chemical and hydrolytic stability and good tolerance for entrapped cells. Because of these unique characteristics, P(HEMA) is one of the most extensively studied materials in tissue engineering (3,4), and has also been widely used as the backbone for synthesizing stimuli-responsive hydrogels. The potential for the substitution of acrylic and methacrylic acid in hydrogels with itaconic acid is high. The advantage of itaconic acid is a great hydrophilicity, it has two -COOH groups with different pKa values, so that very small amounts of IA, smaller than of acrylic acid, render good pH sensitivity to hydrogels.

Immobilization of enzymes on insoluble supports has been an attractive process in enzyme technology, playing a decisive role in their application to industrial processes. Immobilization is advantageous because it extends the stability of enzyme, provides significant reduction in the operational cost and also facilitates the recovery of the enzyme (5). There are numerous applications of immobilized enzymes in analysis, industrial production, biotechnology, biomedical engineering and other applications (6–11). A number of different techniques and support materials have been used to immobilize different enzymes (12).

Urease (urea aminohydrolase, EC 3.5.1.5.), catalyze the hydrolysis of urea to form ammonia and carbon dioxide.

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Urease has been immobilized on various materials, such as cyanuric chloride DEAE-cellulose ether (13) film (14), diatomit, silica, porous glass (15). Chaudhari et al. (2005) (16) have shown that urease can be successfully immobilized on porous silicon fabricated by the usual electrochemical method (17).

It is impossible to predict the changes of physical and chemical properties of enzymes after immobilization. These properties include activity, rate of reactions, substrate specificity, influence of inhibitors and activators, Michaelis constant, activation energy, optimum pH and resistance to denaturation. Therefore, the techniques of immobilization are usually chosen experimentally (8,18).

In this study, we performed a detailed biochemical characterization of the urease immobilization process on (HEMA/IA) (92.5/7.5) hydrogels at 6 kGy.

2. Experimental

2.1. *Materials and methods*

2.1.1. *Reagents*

2-Hydroxyethyl methacrylate (HEMA), and itaconic acid (IA) were from Aldrich (German) and were used as received. All other chemicals used in this study were of high purity and used without further purification.

2.1.2. *Plant material*

Cultivated seeds of water melon, *Citrullus vulgaris,* cv. 'Giza 1' were obtained from the Agriculture Research Center, Cairo, Egypt. Purification of *C. vulgaris* urease was carried out at the Molecular Biology Department, National Research Centre, Cairo, Egypt. The enzyme was purified 67-fold with specific activity of 149.5 unit's mg^{-1} protein.

2.1.3. *Urease assay*

Urease was measured according to Fawcett and Scott (1960) (19). All assays were carried out in duplicate and performed at 37°C for 3 min. In an alkaline medium, the enzymatically liberated NH_3 reacts with salicylate and hypochlorite to form a green colored indophenol, and the absorbance was measured at 580 nm. One unit of enzyme activity was defined as μmole urea hydrolyzed per minute at 37°C under standard assay conditions. Specific activity was expressed in units mg^{-1} protein.

2.1.4. *Protein determination*

Protein was determined by the method of Lowry et al. (1951) (20) using bovine serum albumin as the standard. The amount of immobilized enzyme protein was estimated by subtracting the amount of protein determined in supernatants after immobilization from the amount of protein used for immobilization.

2.1.5. *Buffers*

Buffers were prepared according to Gomori (1955) (21), and the final pH was checked by an EIL pH meter Type 7020.

2.1.6. *Gel determination*

A known weight of the dry copolymer was extracted in distilled water for 48 h at 100°C to determine the insoluble parts of the hydrogel. The insoluble parts, taken out and washed with hot water to remove the soluble part, were then dried and weighed. This extraction cycle was repeated until the weight became constant. The gel fraction yield in the hydrogel was determined from the following equation:

$$\text{Gel}(\%) = (w_e/w_d) \times 100$$

where w_d and w_e represent the weights of the dry hydrogel and the gelled part after extraction, respectively.

2.2. *Preparation of support material*

2.2.1. *Preparation of copolymers*

Copolymers were obtained by gamma irradiation-induced copolymerization of 20 wt% aqueous solutions of HEMA and IA mixtures with different molar ratio (96.5/3.5, 94.4/5.6 and 92.5/7.5 mol), as well as 1 g HEMA to, 40, 60, and 80 mg with taking into consideration that each composition will be studied in small glass vials and irradiated to 2, 3, 4, 5 and 6 kGy in ^{60}Co gamma rays, at a dose rate 10.28 kGy/h. After copolymerization, the vials were broken, the formed polymeric cylinder were removed and cut into discs of 2 mm thickness and 5 mm diameter. All samples were washed in excess water to remove the unreacted component and then air dried at room temperature. In order to determine final composition of gels, residual mixture after washing of gels were collected. The amount of uncrosslinked IA was determined by titration of extract against NaOH (0.05 mol/l) in the presence of phenolphthalein indicator till it reached to the end point to know the actual itaconic acid will be involved in the gel structure.

2.2.2. *Immobilization procedure*

The enzyme immobilization was carried out by gently mixing end-over-end at room temperature over night the polymer, previously washed repeatedly with distilled water, with the enzyme dissolved in 10 mM sodium phosphate buffer, pH 7.0. Aliquots of the supernatant were drawn up to verify the advancement of the immobilization.

The total uncertainly for all experiments ranged from 3–5%.

3. Results and discussions

3.1. *Preparation of hydrogels by radiation copolymerization*

The radiation technique is a sterilization technique used in many applications. During polymerization and crosslinking reactions, all monomers react together with applied γ-rays irradiation. This process is used for sterilization of hydrogel systems at the same time.

When the HEMA/IA/H_2O mixture is irradiated with gamma radiation, free radicals are generated in the aqueous solution. Random collision of the formed radicals with the monomers resulted in the formation of crosslinked copolymers of HEMA/IA. To confirm the crosslinking and incorporation of IA with HEMA monomer to form HEMA/IA copolymer; FTIR spectroscopy is shown in Figure 1.

The C–C vibrational band at 1040 cm^{-1} indicates that the free radicals generated from the vinyl C=C groups of both IA and HEMA monomer chains. The stretching vibration bands characterized for alkyl group $-CH_3$ at 3100 cm^{-1} and hydroxyl group –OH at 3600 cm^{-1} are still present in the copolymer HEMA/IA spectrum without effect by gamma radiation.

The percent gelation of the investigated hydrogels is presented in Table 1. It is observed that as the (IA) content increases, the gel fraction yield decreases. This behavior is observed for all compositions and this is due to the higher affinity of HEMA to crosslink through the formation of hydrogen bonding via –OH groups.

3.2. *pH dependence of swelling*

The equilibrium swelling (q_e) of the HEMA/IA hydrogels in the water and nutrient medium of the cells was calculated from the following relationship:

$$q_e = w_t - w_0/w_0$$

Here w_t is the weight of swollen gel at time t and w_0 is the weight of the dry gel. The state of the swelling behavior of HEMA/IA hydrogels in water depends mainly on the osmotic pressure difference between both the inside and the surrounding gel caused by the redistribution of mobile ions. Figure 2 shows the swelling of the HEMA/IA gels with different composition under different pH buffers (2.5, 3.5, 4.50, 5.5 and 7) at 37°C. Our results confirm that PHEMA is almost not pH sensitive (22). On the other hand, hydrogels with IA show very interesting pH behavior. The equilibrium degree of swelling (qe) of copolymeric hydrogels slightly increases with pH up to pH 3.5. A further change in pH leads to a significant jump in (qe) due to ionization of the itaconic acid first carboxylic group around pH 4. The first and second dissociation constants of IA are $pKa_1 = 3.85$, $pKa_2 = 5.45$, respectively (23). After pH 5.45, only a slight increase in (qe) is evident. In all compositions, the maximum extent of swelling was reached at pH 7.40, this being due to the complete dissociation of acidic groups of IA at this pH value. Such behavior of these copolymeric hydrogels with IA recommends them for application as immobilization of urease.

Table 1. Effect of acid content on the value of gel fraction yield for the (HEMA/IA) hydrogels, at doses 3 kGy

Composition of P(HEMA/IA) hydrogels	*Gel fraction yield (%)*
100/0	98
96.5/3.5	95
94.4/5.6	92
92.5/7.5	88

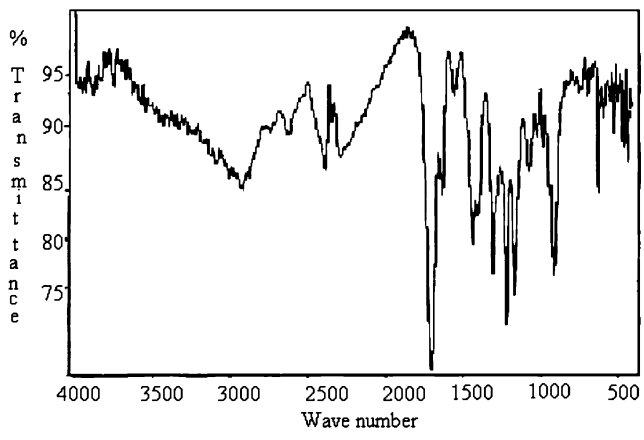

Fig. 1. FTIR spectroscopic presentation of (HEMA)/IA hydrogels.

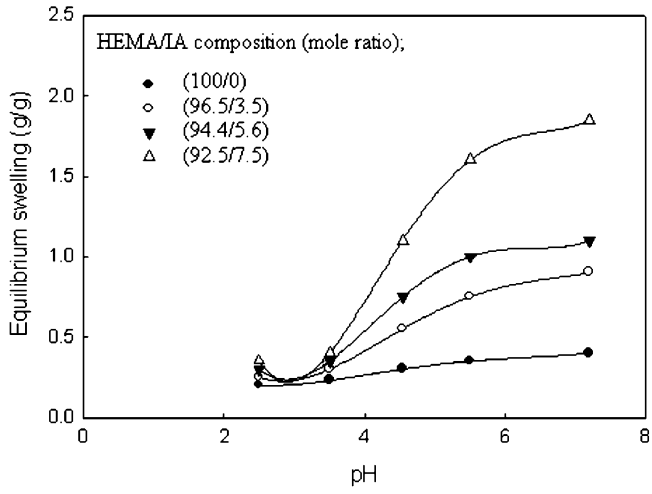

Fig. 2. Effect of pH on the equilibrium degree of swelling for different (HEMA/IA) composition, at dose 3 kGy.

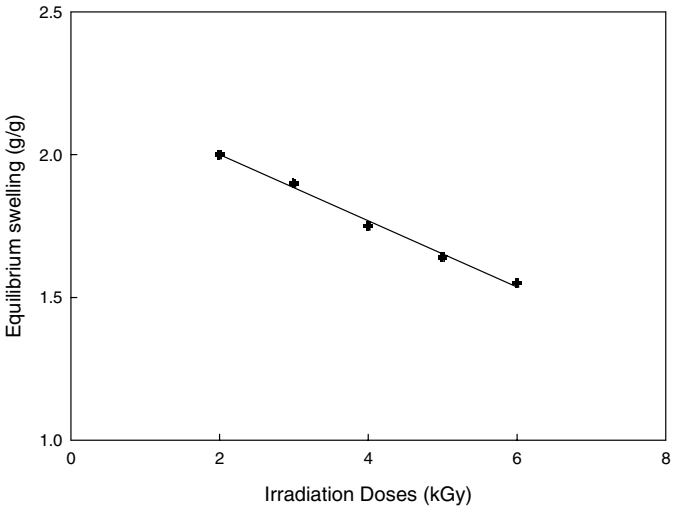

Fig. 3. Effect of irradiation dose (kGy) on the equilibrium swelling for (HEMA/IA) (92.5/7.5 mole ratio) hydrogel at pH 7.

Also, we can seen that the equilibrium swelling increases as the content of acid increase, in copolymer these results can be attributed to the two carboxylic groups per chain length of the IA copolymerized with HEMA, which enhance repulsive forces of −COOH groups with the neighboring chains. This electrostatic repulsion was responsible for the network swelling with the expected reduction of the hydrostatic pressure inside of the network. Additionally, as acid increases in the system, osmotic pressure inside the network was higher resulting in swelling of the hydrogels,

The influence of irradiation dose on the equilibrium swelling for the hydrogels prepared at HEMA/IA (92.5/7.5 mole ratio) hydrogels at pH 7 is shown in Figure 3. The equilibrium swelling decreases with an increase of irradiation doses. This is due to the enhancement of the crosslinking process at higher doses, and as a consequence, the diffusion and swelling properties are hindered by network structure formation.

3.3. *Establishment of support materials of C. vulgaris urease immobilization*

3.3.1. *Effect of monomer*

The enzyme activity of immobilized urease is expressed as enzyme units/g of polymer support. Because HEMA and IA have highly reactive carboxylic groups toward amino groups of the protein, enzyme immobilization to the hydrogels by stable covalent binding is possible. The effect of the molar ratio of HEMA to IA on immobilization efficiency was studied. 100 units of urease were attached to one gram of various support materials. The specific activities of the immobilized enzymes were 14.1, 38.3, and 105.7 U/mg enzymes for HEMA to IA molar ratios of 27.6, 16.9 and 12.3, respectively which prepared at 3 kGy. The minimum binding of urease was recorded on (HEMA/IA) (96.5/3.5) at 3 kGy with retention activity 1.2 % and specific activity 14.1 units mg^{-1} protein. HEMA/IA (92.5/7.5) support at 6 kGy wasa most efficient carrier where it has a maximum binding of urease activity with retention activity of 41.2% and specific activity 108.4 units mg^{-1} protein (Table 2). (HEMA/IA) (92.5/7.5) hydrogel which was prepared at 6 kGy was thus chosen for its highest total enzyme activity.

3.3.2. *Effect of irradiation doses on poly(HEMA/IA) immobilization*

The effect of irradiation doses (kGy) on immobilization of urease on (HEMA/IA) (92.5/7.5) hydrogel is given in Table 2. The increase in irradiation doses from 2 to 6 kGy resulted in a corresponding increase in specific activities of the immobilized urease from 93.8 U/mg reached to 108.4 U/mg. This is due to the enhancement of the crosslinking process at higher doses.

3.4. *Immobilization of C. vulgaris urease on Cu^{2+} chelated ppoly(HEMA/IA) matrix*

Because of the easily polarized nature of their d-electron shells due to orbital valances, 1st row transition metal ions such as Cu^{2+}, Zn^{2+} and Ni^{2+} function as soft or borderline

Table 2. Immobilization of *C. vulgaris* urease on different matrix. Each value represents the average of two experiments

Support materials (HEMA/IA) Molar ratio	*Protein immobilized (mg g^{-1} support)*	*Activity immobilized (Units g^{-1} support)*	*Specific activity (Units mg^{-1} protein)*	*Retention activity %*
(96.5/3.5) at 3 kGy	0.085	1.2	14.1	1.2
(94.4/5.6) at 3 kGy	0.27	10.35	38.3	10.35
(92.5/7.5) at 3 kGy	0.35	37	105.7	37
(92.5/7.5) at 2 kGy	0.32	30	93.8	30
(92.5/7.5) at 6 kGy	0.38	41.2	108.4	41.2
	molar ratio of (HEMA/IA)-Cu at 3 kGy			
(96.5/3.5)	0.091	1.6	17.58	1.6
(94.4/5.6)	0.11	3	25.8	3
(92.5/7.5)	0.12	3.1	27.2	3.1

*100 units (0.67 mg protein) of urease enzyme were applied on 1 g of each support material.

Lewis acids according to the Lewis acid–Lewis base concepts of Pearson (24). They thus exhibit preference for nonbonding lone pair electrons from oxygen atoms in aromatic and aliphatic carboxylic–containing ligands. In the case of amino acid residues with in a polypeptide or protein, histidine, tryptophan and the α-amino group at the N-terminus are particularly favored by these borderline metal ions (25). Taking advantage of thess properties, Cu^{2+} ions was coordinated to the itaconic acid ligand and the enzyme was bound to the polymer via Cu^{2+} ions. Table 2 shows that the specific activities of the immobilized enzymes were 17.58, 25.8, and 27.2 U/mg enzymes for (HEMA/IA)-Cu molar ratios of 27.6, 16.9 and 12.3, respectively which were prepared at 3 kGy. The specific activities of the immobilized urease on (HEMA/IA) were approximately 2 times higher than that of the (HEMA/IA)-Cu^{2+}. This means that the activity of the immobilization decreases by Cu^{2+} chelated poly(HEMA/IA) matrix.

3.5. *Physico-chemical properties of free and immobilized urease on HEMA/IA (92.5/7.5) at 6 kGy*

Comparative studies of the free and immobilized *C. vulgaris* urease on HEMA/IA (92.5/7.5) at 6 kGy were carried out with respect to pH optima, Michaelis constant (K_m), effect of temperature on enzyme stability and inactivation by heavy metal ions.

3.6. *pH optima*

The pH profiles of free and immobilized *C. vulgaris* urease preparation for HEMA/IA hydrogels are shown in Figure 4. The reaction mixture contained in 1.0 ml: 10 mM urea, 0. 33 unit of enzyme and 50 mM sodium acetate buffer (pH 5.0–5.5), sodium phosphate buffer (pH 6.0–8.0) and Carbonate bicarbonate buffer (pH 9–10). In general, immobilization would result in the shift of optimal pH of the enzyme (26,27). The change in optimum pH depends on the charge of the enzyme and/or of the polymer matrix. This change is useful in understanding the structure–function relationship of the enzyme and to compare the activity of free and immobilized enzyme as a function of pH. However, this phenomenon was not observed in the present case (28,29). The pH profile for free and immobilized enzyme was almost the same. The optimal pH value was observed around pH 7.5 for both free and immobilized urease. This suggested that the optimal pH value for enzyme activity had no marked changes after being bound to the support. The immobilized urease expressed a relatively high activity of 62.8% at pH 9, while the free counterpart just retained an activity of 38%. The immobilized enzyme had a relative activity of 63.4% at pH 5.8. This was higher than that of the free counterpart (22.9%). Such a phenomenon was probably arose from the multi-point stabilization of enzyme on the surface of the hydrogel. This would limit the transition change of enzyme conformation against pH inactivation (30). This effect was in agreement with general observations of immobilized urease on different supports (31, 13, 17,32).

3.7. K_m

The Michaelis–Menten constant (K_m) referred to the affinity of enzyme towards its substrate. The lower the K_m value means higher its affinity against its substrate. The calculated K_m values of free and immobilized urease were 3.3 and 6.25 mM, respectively (Fig. 5). The K_m value of the immobilized urease was approximately 2 time higher than

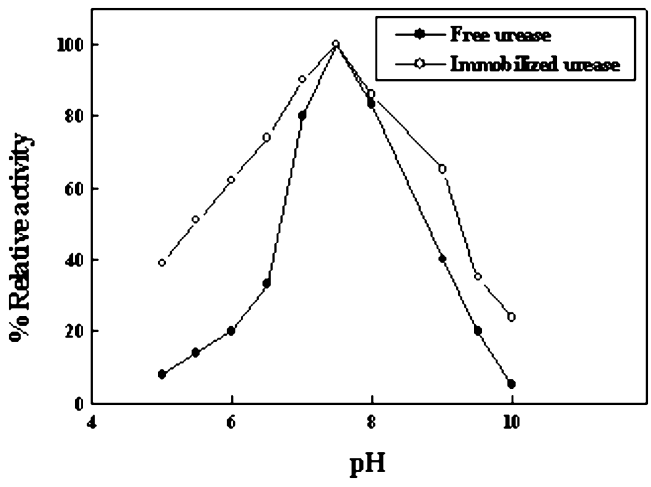

Fig. 4. pH optimum of free and immobilized urease. The reaction mixture contained in 1.0 ml: 10 mM urea, 0. 33 unit of enzyme and 50 mM sodium acetate buffer (pH 5.0–5.5), sodium phosphate buffer (pH 6.0–8.0) and carbonate bicarbonate buffer (pH 9–10). Each point represents the average of values from two experiments.

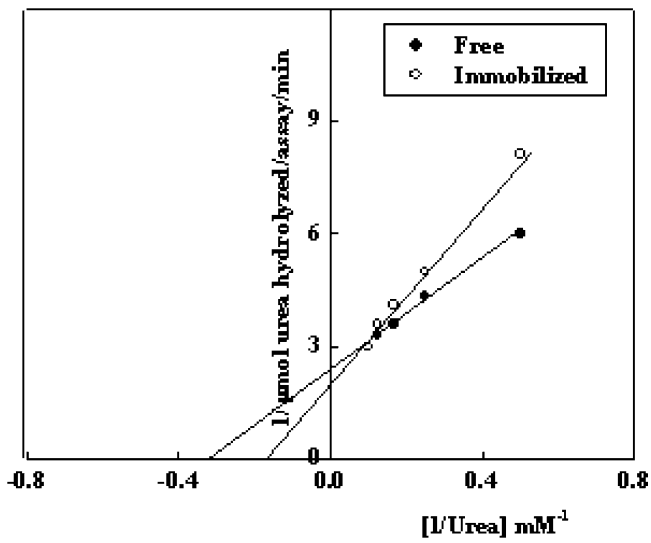

Fig. 5. Lineweaver-Burk plots ralating free and immobilized *C. vulgaris* urease activity to urea concentration. The reaction mixture contained in 1.0 ml: 120 mM sodium.

that of the free urease. This increase may have resulted from conformational and steric modifications introduced by the covalent attachment of the enzyme to the support and to mass transfer resistances inherent in the morphology of the support used, thus reducing the affinity of the substrate for the active site of the enzyme. When the K_m values of HEMA/IA/urease was in agreement with that of previous immobilized urease on chitosan memberane (33); alkylamine derivative of titanium(IV)-porous silica (8) and cellulose support (34).

3.8. *Effect of temperature*

Stability of free and immobilized polymer particles (HEMA/IA) (092.5/7.5) on *C. vulgaris* urease was determined by measuring relative enzyme activity as a function of temperature in the range of 20–80°C. The activity profiles of free and immobilized enzyme at different temperature are represented in Figure 6. At 60°C, the free enzyme retained only 73.6% relative activity, while immobilized *C. vulgaris* urease retained 85.76% of its initial activity. Similarly, at 80°C, the free enzyme retained only 20% relative activity while immobilized *C. vulgaris* urease was found to retain 60% of its initial activity upon incubation for 15 min. In the case of immobilized enzyme, a significant improvement was observed against free enzyme resulting from the covalent conjugation of the enzyme molecule on the carboxyl-functionalized (HEMA/IA) hydrogel, as well as diffusional limitations of the immobilized molecules (35). Thus, the described immobilization method was observed to confer excellent thermal stability on the product. Similar results have been reported for immobilized urease on different matrices, on aminoalkylated glutaraldehyde-treated chitin (36), on glutaraldehyde-treated molecular sieve 4, (37), on alkylamine derivative of titanium (IV)-porous silica (33), on glutaraldehyde-pretreated chitosan (8) and on DEAE-cellulose activated with 2-amino-4,6-dichloro-s-triazine (34). Reddy *et al*., 2006 (32) showed that immobilized urease, upon coupling to alkylamineand arylamine glass, retained 50% of its activity when incubated at this temperature for 90 min. This improved thermo stability might be useful in the application of this system at high temperatures, avoiding the microbial contamination, as well as the solubility of substrate and products is higher; which would have good scope in industrial use.

3.9. *Inactivation by heavy metal ions*

The influence of various heavy metal ions at the concentration of 1×10^{-3} M on free urease was arranged in order of decreasing toxicity: $Hg^{2+} > Cu^{2+} > Zn^{2+} > Ni^{2+} > Cd^{2+} > Co^{2+} > Mn^{2+}$with 100, 93, 86.7, 85, 82, 80, and 98% inhibition, respectively. For immobilized enzyme, the inhibitory effect of these metal ions at the same concentration was in the order $Mn^{2+} > Hg^{2+} > Zn^{2+} > Cu^{2+} > Co^{2+} > Ni^{2+} > Cd^{2+}$ with 86, 75, 64, 61, 60, 59, and 42% inhibition, respectively (Table 3). Fahmy et al., 1998 found that, the inhibitory effect of heavy metal ions at the concentration of 10^{-3}M on free urease was arranged in order of decreasing toxicity: $Hg^{2+} > Cu^{2+} > Zn^{2+} > Ni^{2+} > Cd^{2+} > Co^{2+} > Mn^{2+}$. For immobilized enzyme, the inhibitory effect of these metal ions at the same concentration was in the order $Hg^{2+} > Cd^{2+} > Co^{2+} > Ni^{2+} > Cu^{2+} > Zn^{2+} > Mn^{2+}$. This protection may result from the structural changes introduced in urease by the applied immobilization procedure and consequently, lower accessibility of the inhibiting ion to the essential –SH groups of the enzyme active site. Krajewska (1991)(38) inactivated the immobilized jack bean urease on glutaraldehyde-pretreated chitosan membrane with heavy metal ions and compared with free urease; the relative toxicity sequence of metal ions toward free jack bean urease was found to be $Hg^{2+} > Ag^{+} > Cu^{2+} > Ni^{2+} > Cd^{2+} > Zn^{2+} > C0^{2+} > Pb^{2+} > Mn^{2+}$. He found that the stability of jack bean urease against metal

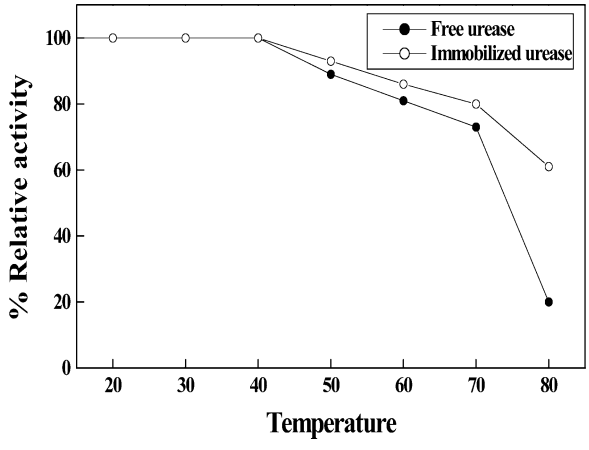

Fig. 6. Effect of temperature on the thermal stability of free and immobilized urease.

Table 3. Effect of metal ions on free and immobilized *C. vulgaris* urease

	% Relative activity	
Metal ions	*Free Urease*	*Immobilized Urease*
Control	100	100
Hg^{2+}	0	25
Cu^{2+}	7	39
Zn^{2+}	13.3	36
Ni^{2+}	15	41
Cd^{2+}	17.8	58
Co^{2+}	20	40
Mn^{2+}	2	14

*The enzyme was pre-incubated for 15 min with 1 mM of listed ions as a final concentration prior to substrate addition. Each value represents the average of two experiments.

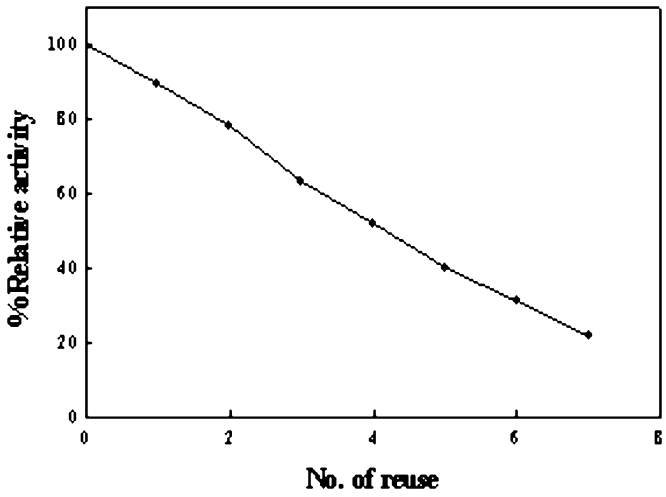

Fig. 7. Effect of reuse number on immobilized C. *vulgaris* urease activity on HEMA/IA (92.5/7.5) at 6 kGy.

ion inactivation was considerably improved after immobilization, whereas Ni^{2+}, Co^{2+}, Fe^{3+}, Pb^{2+} and Mn^{2+} did not inactivate the immobilized urease in the range of concentrations at which free urease was thoroughly inactivated; Hg^{2+}, Ag^{+}, Cu^{2+}, Zn^{2+} and Cd^{2+} inactivated the immobilized enzyme at considerably higher concentrations. In contrast, Dumitriu *et al.* (1989) (39) reported that, although the stability of the free urease at a concentration of 10^{-6}M Cu^{2+} ion was higher than that of the immobilized urease on carboxymethyl-cellulose with 95 and 83% relative activities, respectively, the stability of the immobilized enzyme was higher upon increasing Cu^{2+} concentration.

3.10. *Reusability*

The reusability was examined because of its importance for repeated applications in a batch reactor. Reusability of immobilized C. *vulgaris* urease on HEMA/IA (92.5/7.5) at 6 kGy activity of the immobilized urease was determined for successive times at 37°C in 120 mM sodium phosphate buffer, pH 7.5, containing 1 mM EDTA and 10 mM urea. The matrix was subjected to several washes by 0.05 M sodium hosphate buffer, pH 7.5, after each assay. The maximum activity in the range of 100% was obtained at the beginning of the reusability experiments. After 4 cycles of reuse, a decrease of 47% on relative activity was recorded (Fig. 7). Immobilization of urease on polyvinyl alcohol-g-butyl acrylate membrane was reused over 100 times (40) (Hsiue et al., 1987). The reusability of crab chitosan bound jack bean urease was determined after 30 times and caused no appreciable loss. Immobilization of urease on Vermiculite using glutaraldehyde resulted in 65% after five times reuses (41); immobilization on chitosan membrane resulted in complete loss after nine reuses (8); on Porous silicon (42).

4. Conclusions

Poly(HEMA/IA) was synthesized by γ-radiation-induced copolymerization and crosslinking. It has been found that the immobilization of hydrogels increases with increasing MAA content in the gel system. This has been explained as being due to the incorporation of more specific acidic groups into the network and almost higher swelling capacity of the gels. Urease immobilized on HEMA/IA (92.5/7.5) at 6 kGy membrane showed suitable yield of immobilization, and it was less sensitive to higher pH, temperature and metal ions. In the future, this immobilized enzyme preparation could be exploited for several industrial and environmental purposes.

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