Amphoteric Hydrogels for Immobilization of Enzymes Using Template Polymerization Technique

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Received 18 December 2006; accepted 1 June 2007
DOI 10.1002/app.26931
Published online 28 August 2007 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: Novel ionizable amphoteric hydrogels were prepared from poly(acrylic acid) and dimethylaminoethyl methacrylate monomer, employing template polymerization technique. The mode of interaction, as proved by FTIR, was multiple H-bonding between the tertiary amino group of the monomer and the carboxylic groups of the polymer. The impact of varying equal polymer–monomer feed ratios from 0.1 to 1.1 on the swelling dynamics was examined. Penetrant sorption experiments demonstrated that the swelling behavior depends strongly on the polymer complex composition. The polymer complex of feed ratio 0.5 : 0.5 (polymer : monomer) showed maximum swelling percentage. The mechanism of the polymer complexes swelling was probably a non-Fickian with n values approaching Fickian behavior. The hydrogels showed maximum swelling efficiencies of 27 folds and 13.5 folds in drastic acidic and basic medium, respectively, using polymer complex of 0.5 : 0.5 feed ratio. Because of reversibility and rapidity of swelling, the gel could be considered as a mechanochemical system. The prepared hydrogel successfully immobilized the industrially used β-galactosidase as an acidic model enzyme. The novel immobilized enzyme showed a remarkable improvement in its activity (13.8 μmol min^{-1} mg^{-1}) compared to the free enzyme (3.2 μmol min^{-1} mg^{-1}). The optimum pH values for free and immobilized enzyme were 4.5–5 and 4, respectively. J Appl Polym Sci 106: 3571–3580, 2007

Key words: template polymerization; polyacrylic acid; amphoteric hydrogels; non-Fickian mechanism; β-galactosidase immobilization

INTRODUCTION

Seventy percent of all current chemical industries involves catalysts with the majority using nonbiological catalysts. Few exceptions, for example, penicillinase (used for production of semisynthetic antibiotics[1]), lipase, β-galactosidase, chymosin, and pectinase (used for food production) have been used industrially in the immobilized form; since it is more economic.[2] However, leakage of enzyme, substrate/product diffusion limitation, and low reaction rate limit the application of these supports. There are two ways to overcome diffusion limitation and speed-up the reaction rate of the immobilized enzyme either by increasing the surface area to volume ratio of the particle or by reducing the quantity of immobilized enzyme within the particle. Both approaches lessen the diffusion limitation within the immobilized enzyme particle, thus allowing the entire enzyme to come into contact with the maximum concentration of substrate and consequently, the reaction rate increases.

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Journal of Applied Polymer Science, Vol. 106, 3571–3580 (2007)

The unique and interesting properties of hydrogels such as perm-selectivity and permeability, which dominate their surface properties,[3,4] make them good candidates for enzyme immobilization. Hydrogels may also contain functional groups that interact with the external environment (e.g., temperature, ionic strength, and pH). In addition, their response to environmental conditions may lead to an increase or a decrease of the hydrogel mesh size.[5]

Hydrogels enjoy the above interesting properties, but the prepared ones, using the traditional chemical crosslinking techniques, have lower water contents than those of thermoplastic ones[6] and are usually mechanically weak because they are rendered insoluble due to chemical bonds.[7] Although, pH-sensitive interpolymer complexes of poly(acrylic acid) and poly(dimethylaminoethyl methacrylate) were synthesized for controlled drug-delivery systems applications,[8,9] it was proved that the polycomplexes obtained by template polymerization had a high number of active centers for water absorption than that obtained by mixing the corresponding polymers.[10]

''Template polymerization'' is a novel alternative for the traditional crosslinking techniques. It is a method of polymer synthesis in which specific interactions, between performed macromolecule (template) and a growing chain, are utilized. These interactions affect the structure of the polymerization

product (daughter polymer) and the kinetics of the process.[11] The influences of the template on the process and the product are usually the enhancement of the reaction rate, the similarity of the degree of polymerization of the daughter polymer with that of the template used, in the ideal case and the daughter polymer can have the structure complementary to the structure of the template used.[12]

Two types of polymer materials can be obtained by template polymerization. The first one is polymers with a ladder-type structure in which, monomer units are connected by covalent bonds within the frame of the template and the second one is polycomplexes in which, small molecules (monomers) are absorbed on the large molecule (template) by means of ionic or charge transfer interactions or by hydrogen bonding.[13]

According to the best of our knowledge, no one used hydrogels for the purpose of enzyme immobilization using template polymerization technique; consequently, this was the aim of the present study. Specifically, the objectives of this research were (i) to synthesize hydrogels of poly(acrylic acid)/poly(dimethylaminoethyl methacrylate) polycomplexes via template polymerization technique, (ii) to investigate the dynamic swelling behavior of the hydrogels, (iii) to study the swelling response of the hydrogels to pH and ionic strength, and (iv) to test the hydrogel, showing high swollen tendency in acidic medium, for immobilization of β–galactosidase (used in milk industries for hydrolysis of lactose to glucose) as an acidic model enzyme.

MATERIALS

Acrylic acid and potassium persulfate were supplied by Merck-Schuchard (Muchen, Germany). 2-Dimethylaminoethyl methacrylate (DMAEMA) was purchased from Fluka (Buchs, Switzerland). β-Galactosidase (EC 3.2.1.23) from *Aspergillus oryzae*, 11.8 U/mg, was obtained from Sigma (Steinheim, Germany). All chemicals were used without any further purification.

EXPERIMENTAL TECHNIQUES

Synthesis of hydrogels and elucidation of the reaction mechanism

The template polymerization of DMAEMA with lab-prepared PAA of MW ≈ 100,000, based on the intrinsic viscosity measurements, was carried out in an aqueous solution. The polymerization initiator was potassium persulfate (PPS). The molar ratio of polymer to monomer was kept constant at 1 : 1. The study parameter was the polymer to monomer molar concentration (feed ratio), which varied from 0.1 : 0.1 to 1.1 : 1.1, using $3.5 \times 10^{-3}M$ of PPS. The polymer–monomer solutions were transferred into polymerization tubes, thoroughly degassed, and flushed with nitrogen gas. The tubes were then sealed and kept at a constant temperature bath (25°C). After the required time for gel formation (time of gel formation varied according to the feed ratios), the polymer complexes were isolated and cut into disks with dimensions of 7 mm diameter and 3 mm thick. The hydrogels were then dried in a vacuum oven at 45°C until a constant weight was achieved.

FTIR spectra were recorded using a Nicolet 520 spectrometer with a resolution of 4 cm^{-1} and averaged over 32 scans. Samples were thoroughly ground with KBr and pellets were prepared by compression under vacuum.

Swelling studies of hydrogels

The swelling behaviour of dried hydrogels (7 mm diameter, 3 mm thick) was determined by immersion in doubly distilled water at room temperature in a water bath. The water absorbed was calculated by weighing the samples after wiping, at various time intervals. Swollen gels were weighed by an electronic balance (Sartorius, BP 210S, $d = 0.1$ mg).

Percentage swelling (%S), the most important parameter in swelling studies, was calculated from the following equation:

$$\%S = \frac{(M_t - M_o)}{M_o} \times 100 \quad (1)$$

where M_t is the mass uptake of the swelling medium at time t, and M_o is the mass of dry hydrogel.

For extensive swelling of hydrogels, the following second-order kinetics relation was used:

$$\frac{t}{S_{(t)}} = A + Bt \quad (2)$$

where $S_{(t)}$ is swelling at time (t), $B = (1/S_{\text{eq}})$ is the inverse of the maximum or equilibrium swelling, $A = (1/k_s S_{\text{eq}}^2)$ is the reciprocal of the initial swelling rate $(dS/dt)_o$ of the hydrogel, and k_s is the swelling rate constant.

The swelling mechanism could be analyzed for polymeric systems by the following equation:

$$\frac{M_t}{M_{\text{eq}}} = Kt^n \quad (3)$$

where M_{eq} is mass of hydrogel at equilibrium. K (the swelling constant) and n (the swelling exponent) are characteristic parameters of the specific (bioactive agent/dissolution medium) system. By taking natural log of eq. (3):

$$\ln \frac{M_t}{M_{\text{eq}}} = \ln K + n \ln t \quad (4)$$

The values of n and K were calculated from the slope and intercept of the plot of $\ln \frac{M_t}{M_{eq}}$ against ln (t), respectively.

This equation is applied to the initial stages of swelling and plots of eq. (4) yield straight lines up to almost 60% increases in the mass of the hydrogel.

To obtain a better model after 60%, we assume that for long periods the penetrant sorption was mainly dominated by relaxation of the polymer network and that the sorption process of the polymer by relaxation was first-order. Then, the *Berens-Hopfenberg* differentiation equation for the relaxation process could be written as follows.[6]

$$\frac{dM_t}{dt} = -k_2(M_{eq} - M_t) \quad (5)$$

by integrating eq. (5)

$$\frac{M_t}{M_{eq}} = 1 - A\exp(-k_2 t) \quad (6)$$

To study the swelling behavior of the hydrogels in medium of different pH, three pieces of preweighed hydrogels (7 mm diameter, 3 mm thick) were placed in buffer solutions of the required pH and allowed to equilibrate. In each case, the final weight swelling equilibrium ratio (q) was determined for the three pieces of hydrogels. Good agreement was found in the degree of swelling for the pieces, each had the same volume and history, the average of three determinations being used for calculations. q was calculated as follows:

$$q = \frac{M_{eq}}{M_o} \quad (7)$$

The total ionic strength of the swelling medium was kept constant by addition of KCl powder.

The same procedure was carried out in the study of effect of ionic strength on the hydrogels swelling. KCl salt was used with different concentrations in doubly distilled water at room temperature.

To study the reversibility of the swelling process, the dry samples were allowed to swell in water and then placed in 2*M* KCl solution, which caused the gel to swell further. The reversibility of swelling and deswelling was determined, using the same samples for consecutive swelling and deswelling experiments. The duration of each cycle was 35 min. The degree of swelling had been expressed as weight swelling equilibrium ratio (q).

The experiments of the ionic strength of the swelling medium and the reversibility of the hydrogel swelling were performed on 0.5 : 0.5 PAA/PDMAEMA complex, using doubly distilled water at 30°C.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} \text{Glucolactone} + H_2O_2$$

$$\text{hydroxybenzoate-Na-4-aminoantipyrine} + H_2O_2 \xrightarrow{\text{POD}} \text{quinon complex} + H_2O$$

Scheme 1 GOD-Perid test for enzymatic determination of glucose.

All experiments were performed in triplicate and the average was taken. SD in all measurements was close to zero.

Immobilization and evaluation of β-galactosidase activity

β-Galactosidase was immobilized on 0.5 : 0.5 PAA/PDMAEMA complex using *p*-phenylene diamine and glutaraldehyde. The dry gel disks, 0.8 g, were immersed in 200 mL of 2% (w/v) *p*-phenylene diamine prepared in carbonate buffer, pH 10, for 45 min. The gel disks were washed thoroughly using doubly distilled water, then treated with 200 mL of 1.25% (v/v) glutaraldehyde aqueous solution for 1 h. The gel disks were washed thoroughly with doubly distilled water, then soaked for 16 h in a solution of 50 mL of 5 U/mL β-galactosidase prepared in 100 m*M* citrate-phosphate buffer, pH 4.5. At the end of this step, the immobilized enzyme was washed thoroughly with the buffer solution to remove any unbound enzyme. The immobilized enzyme was stored at 4°C for enzyme catalytic activity measurements and pH studies.

The soluble protein content was determined using Lowry assay. The catalytic activity of the free and immobilized enzyme was determined by measuring the glucose concentration by the GOD-Perid test (Borheringer GmbH, Mannheim, Germany). This test uses a coupled enzyme reaction of glucose oxidase (GOD) and peroxidase (POD) as represented in Scheme 1.

The obtained colored solution (quinine complex) was measured spectrophotometrically at 510 nm; since the absorbance is proportional to glucose concentration, according to the well-known Beer–Lambert law.

The kinetic parameters, K_M and V_{max}, were determined by Hanes–Woolf plot method for 7 U of free and immobilized enzyme using substrate concentration of 20–300 m*M* at 35°C and pH 4.0.

The pH-activity profile for 7 U of free and immobilized enzyme was measured at pH 2.5–6.5 and 35°C using 100 m*M* citrate-phosphate buffer solution of 200 m*M* lactose.

RESULTS AND DISCUSSION

Synthesis of hydrogels synthesis and elucidation of the reaction mechanism

Some methods of polypeptide synthesis *in vitro* include aspects of template-type interaction, for

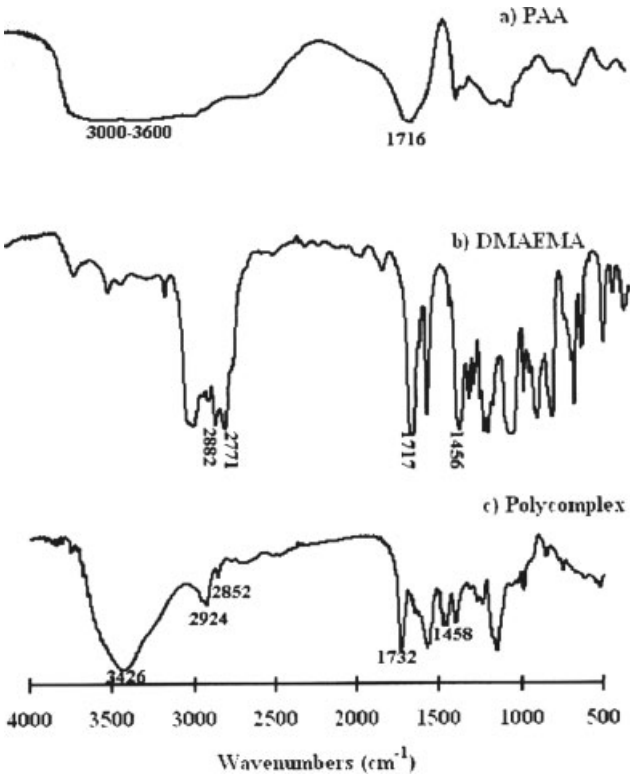

Figure 1 FTIR spectra of (a) PAA, (b) DMAEMA, and (c) PAA/PDMAEMA complex.

instance, in enzymatic polypeptide synthesis. The unusual selectivity and high efficiency of such template reactions suggest that this type of reaction can be regarded as a catalyzed reaction. The template plays a role of a polymeric catalyst.[14]

Figure 1(a–c) shows the IR spectrograms of the prepared PAA, DMAEMA, and PAA/PDMAEMA complex of feed ratio 0.5 : 0.5, respectively. The most characteristic bands are given in Table I.

On comparing the IR spectra of pure components with that obtained for the polycomplex, distinct shifts had been observed in —C=O, R—N$(CH_3)_2$, and —OH frequencies. This may be due to the involvement of these functional groups in the complex formation. In fact, on performing the template polymerization reaction, a precipitate was formed immediately. When this precipitate was pressed out and dried, a clear complex, water-insoluble, homogeneous sheet was obtained with properties quite different from those, which could be predicted based on the properties of either polymer alone. Thus, the precipitation was not the result of incompatibility of the polymers, but rather the result of a high degree of intermolecular association resulting from a multiplicity of hydrogen bonds between the nitrogen atom in PDMAEMA chains and the carboxyl groups of PAA.[15]

TABLE I
Analysis of FTIR Spectra of PAA, DMAEMA, and the Prepared PAA/PDMAEMA Complex

Functional group	Wavenumbers (cm^{-1})		
	PAA	DMAEMA	Polycomplex
—C=O	1716	1717	1732
R—N$(CH_3)_2$	–	2822, 2771, 1456	2924, 2852, 1458
—OH	3000–3600	–	3426

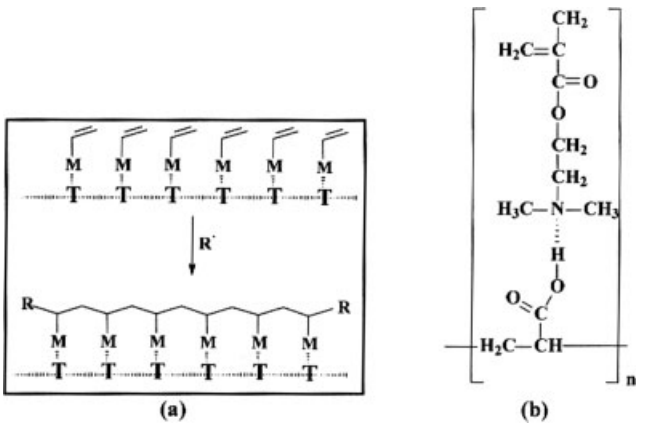

Figure 2 Template polymerization mechanism: (a) General mechanism, where -T-, -M-, and R represent template unit, monomer, and initiator radical, respectively; (b) Hydrogen bond interaction between DMAEMA and PAA.

Consequently, in the chain template polymerization of 2-DMAEMA and PAA, the monomer is preadsorbed by, or complexed with, template macromolecules. Initiation, propagation, and perhaps mostly termination take place on the template. The mechanism can be represented by the scheme given in Figure 2. On the template unit, -T-, monomer, -M-, having double bond, is adsorbed. Radical $R^{\bullet}$ initiates propagation process, which proceeds along the template, and eventually a complex of the template and

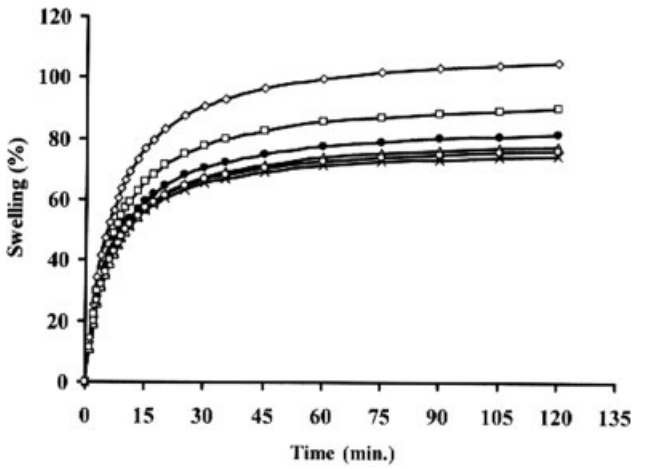

Figure 3 The dynamic swelling behavior of PAA/PDMAEMA complex with different feed ratios: 0.1 : 0.1 (△), 0.3 : 0.3 (□), 0.5 : 0.5 (◇), 0.7 : 0.7 (●), 0.9 : 0.9 (×), and 1.1 : 1.1 (○), at pH 5.4 and 30°C.

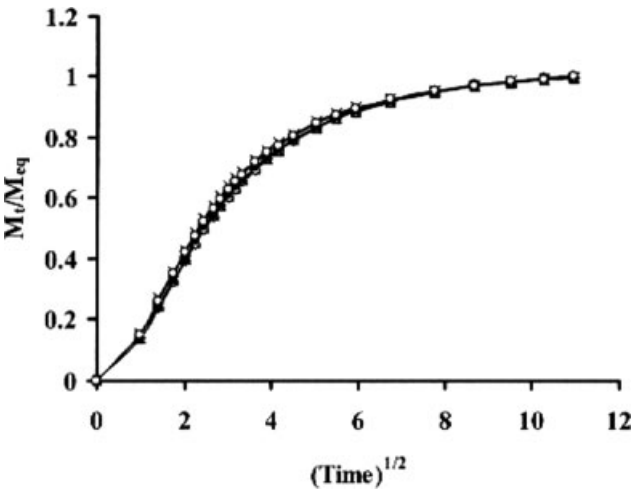

Figure 4 M_t/M_{eq} as a function of the square root of time for PAA/PDMAEMA complex with different feed ratios: 0.1 : 0.1 (△), 0.3 : 0.3 (□), 0.5 : 0.5 (◇), 0.7 : 0.7 (●), 0.9 : 0.9 (×), and 1.1 : 1.1 (○), at pH 5.4 and 30°C.

the daughter polymer, consisting of *M* units, is created.[11]

Hydrogels swelling

When a glassy hydrogel is brought into contact with water, the water diffuses into the hydrogel and the hydrogel swells. Diffusion involves migration of water into pre-existing or dynamically formed spaces between hydrogel chains. Swelling of the hydrogel involves larger scale segmental motion resulting, ultimately, in an increased distance of separation between hydrogel chains.[16]

Figure 3 illustrates the dynamic swelling behavior of PAA/PDMAEMA complex with different feed ratios at pH 5.4 and 30°C.

As shown from Figure 3, maximum swelling percentage was observed for the moderate feed ratios, 0.5 : 0.5 and 0.3 : 0.3, whereas at very low (0.1 : 0.1) and high (0.7 : 0.7, 0.9 : 0.9 & 1.1 : 1.1) feed ratios, the swelling percentage decreased with no significant difference.

The gradual increase of the swelling percentage from 0.1 : 0.1–0.5 feed ratios may be attributed to the addition of a more ionic groups, which hinders the aggregation of the polymer chains and acts to expand the collapsed structure.[17] The maximum swelling percentage at 0.5 : 0.5 feed ratio started to decrease by increasing the polymer/monomer concentration due to two reasons: a decrease in the degree of dilution of the matrix[18] and an increase in the medium viscosity causing a heterogeneity in the system.

Plotting the fractional sorption data as functions of the square root of time (Fig. 4) provides valuable information for distinguishing between Fickian and Case II transport mechanisms, because the Fickian diffusion curve exhibits a monotonic inflection-free approach to equilibrium, whereas the Case II curves are clearly sigmoidal.[19]

Figure 4 indicates that the swelling mechanism of these polymer complexes was closer to Fickian transport mechanism.

Swelling kinetics studies

The swelling kinetics parameters shown in Table II had been calculated using eq. (2). The values of the theoretical equilibrium swelling were parallel to the results of the experimental ones. The maximum swelling rate constant was clearly observed for the polymer complex of 0.5 : 0.5 feed ratio.

Swelling power and mechanism

A critical analysis of the swelling process reveals that there are two underlying molecular process: penetration of the solvent molecules into the void spaces in the network and subsequent stretching or relaxation of the network segments. Equation (3) defines the following three situations:

1. $n = 0.5$: When the rate of solvent penetration is the rate-limiting step; the diffusion mechanism is Fickian.
2. $n = 1$: When the penetration velocity is far greater than the chain relaxation rate, the solvent uptake is proportional to time; the diffusion mechanism is relaxation-controlled, Case III transport.

TABLE II
Swelling Rate Parameters of PAA/PDMAEMA Complex

Swelling kinetics constants	Values of swelling kinetics constants					
	0.1 : 0.1[a]	0.3 : 0.3[a]	0.5 : 0.5[a]	0.7 : 0.7[a]	0.9 : 0.9[a]	1.1 : 1.1[a]
A	0.081	0.070	0.061	0.077	0.074	0.075
B	0.012	0.011	0.009	0.012	0.013	0.013
Initial swelling rate (g_{water}/g_{gel} min)	12.407	14.306	16.502	13.004	13.495	13.298
Theoretical equilibrium swelling (g_{water}/g_{gel})	81.967	95.238	111.111	86.207	78.125	80.000
Experimental equilibrium swelling (g_{water}/g_{gel})	77.717	90.017	105.108	81.507	74.417	76.181
Swelling rate constant (K_s) (10^3) (g_{gel}/g_{water} min)	83.357	129.761	203.724	96.640	82.368	85.106

[a] Feed ratio.

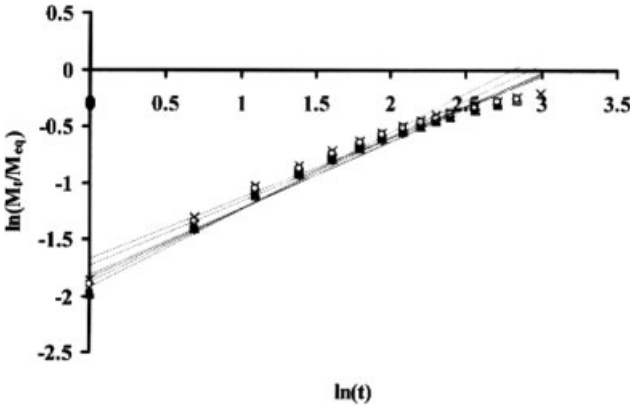

Figure 5 Relation between $\ln(M_t/M_{eq})$ and ln(time) for PAA/PDMAEMA complex (swelling % $\leq$ 60%) with different feed ratios: 0.1 : 0.1 (△), 0.3 : 0.3 (□), 0.5 : 0.5 (◇), 0.7 : 0.7 (●), 0.9 : 0.9 (×), and 1.1 : 1.1 (○), at pH 5.4 and 30°C.

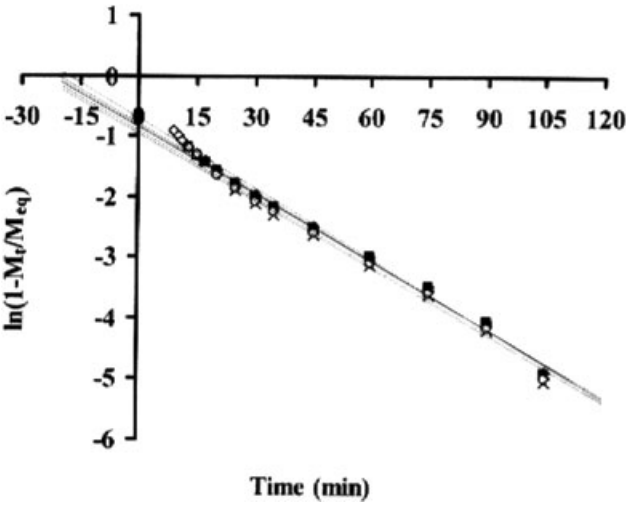

Figure 6 Relation between $\ln(1 - M_t/M_{eq})$ and the time for PAA/PDMAEMA complex (swelling % > 60%) with different feed ratios: 0.1 : 0.1 (△), 0.3 : 0.3 (□), 0.5 : 0.5 (◇), 0.7 : 0.7 (●), 0.9 : 0.9 (×), and 1.1 : 1.1 (○), at pH 5.4 and 30°C.

3. $0.5 < n < 1$: When both the diffusion and polymer relaxation control the overall rate of water uptake; the diffusion mechanism is non-Fickian.[20]

The portion of the water absorption curve, with a fractional water uptake less than 60%, was analyzed with equation (3). The values of n and K were calculated using Figure 5 as shown in Table III.

By observing the n values, the mechanism of the polymer complexes swelling was probably a combined non-Fickian [anamolous transport with n values approaching Fickian (diffusion controlled) behavior]. The mechanism of transport mainly depended on diffusion of water rather than polymer relaxation. The swelling mechanism showed little dependence on the polymer–monomer concentration. Our results coincided with that obtained by Satish et al.,[21] who found that the water transport mechanism of cationic hydrogels of poly(methyl methacrylate-*co*-dimethylaminoethyl methacrylate) is non-Fickian in acidic pH.

To obtain a suitable model after 60% swelling, eq. (6) could be applied as shown in Figure 6. The relaxation constants and constant A are tabulated in Table IV.

The relaxation constants of the synthesized hydrogels were approximately equal. The complex relaxation may be independent on the polymer–monomer concentration.

Effect of the swelling medium pH on hydrogels swelling

In general, pH-dependent hydrogels have ionizable functional groups on the polymer backbone or the side chains. The pH-dependent swelling behavior results from the ionization or deionization of the functional groups responding to the external environmental pH changes.[22]

The role of pH on the extent of water sorption of polymeric gels is of great importance, since a change in pH of the swelling medium often causes a fluctuation in free volumes accessible to penetrating water molecules, it affects swelling properties of gels.[23]

The equilibrium-swelling ratio, q, describes the amount of water within the hydrogel at equilibrium and is a function of the network structure, hydrophilicity, and the degree of ionization of the functional groups. Therefore, investigation of the equilibrium-swelling ratio can elucidate the network structure

TABLE III
Swelling Exponents and Swelling Constants of PAA/PDMAEMA Complex

Feed ratio	n	K
0.1 : 0.1	0.586	1.811
0.3 : 0.3	0.64	1.876
0.5 : 0.5	0.687	1.927
0.7 : 0.7	0.603	1.834
0.9 : 0.9	0.536	1.672
1.1 : 1.1	0.565	1.731

TABLE IV
Relaxation Constant and Constant A of PAA/PDMAEMA Complex

Feed ratio	K_2 (10^3)	A
0.1 : 0.1	37	0.848
0.3 : 0.3	38	0.791
0.5 : 0.5	39	0.714
0.7 : 0.7	37	0.833
0.9 : 0.9	37	0.960
1.1 : 1.1	37	0.920

TABLE V
Weight Equilibrium Swelling Ratio (q) of PAA/PDMAEMA Complex at Different pH

	Weight equilibrium swelling ratio (q)					
pH	0.1 : 0.1[a]	0.3 : 0.3[a]	0.5 : 0.5[a]	0.7 : 0.7[a]	0.9 : 0.9[a]	1.1 : 1.1[a]
1	10.587	17.836	26.889	Sticky	Sticky	Sticky
7	2.310	2.375	2.279	2.343	2.663	2.230
10.5	9.563	10.238	13.491	Sticky	Sticky	Sticky

[a] Feed ratio.

and the degree of ionization of the functional groups that interact with the external environment.[5,22]

Table V describes the q values of the polymer complexes at selected pH values. The q values of the complexes increased from 0.1 to 0.5 at both extreme pHs, whereas at neutral pH 7 there was no significant effect. It is well known that PDMAEMA homopolymer undergoes an abrupt precipitation above its $pK_b \approx 7.5$ due to deprotonation of the amino groups followed by hydrophobic interactions.[24] The high swelling observed at pH 1 was due to the protonation of the tertiary amino group of the PDMAEMA. The protonated species causes electrostatic repulsion; thus leading to high swelling. The protonation thus creates a change in gel ionization leading to a change in the swelling capacity.[25] On the other side, an increase in the degree of ionization of carboxylic groups of PAA contributes to electrostatic repulsion between charged groups and, therefore, swells the gels to a high degree at pH 10.5.[19]

For the complexes concentration of feed ratio 0.7 : 0.7 to 1.1 : 1.1 at extreme pHs, the gels were very sticky and fragile. This behavior may be due to a significant decomplexation.

Effect of the ionic strength on hydrogels swelling

Figure 7 represents the effect of the ionic strength of the swelling solution on the swelling behavior (q) of 0.5 : 0.5 PAA/PDMAEMA complex. The direct proportionality between ''q'' and the ionic strength may be explained as follows:

The kinetic swelling behavior of an ionic hydrogel depends upon mass transfer limitations, Donnan equilibrium considerations, ion exchange, and ionic interactions. When such an ionic hydrogel is immersed in a high dielectric constant medium, these ionic moieties will dissociate and create an overall charge density along the chains, as well as a high concentration of mobile ions in the gel. As compared to the nonionic gel behavior, this ionic character will introduce two ''new player'' forces in the system: the osmotic pressure resulting from difference in ion concentrations between the swollen gel and the external solution (for ''macroscopic'' electroneutrality reasons, mobile ions belonging to the gel cannot leave it and hence minimization of the osmotic pressure can only be achieved through dilution of the network charge density i.e., swelling) and the net charge density along the chain will generate some electrostatic repulsion (i.e., coulombic forces) between chain segments. The resulting expansion of the network will contribute to the overall swelling behavior.[20]

In fact, this behavior does not contradict with Donnan equilibrium; based on the counter ion concentration in the external swelling medium, which reduces the concentration difference across the hydrogel and the swelling medium leading to a decrease in Donnan potential and consequently in osmotic swelling pressure with an increase in the ionic concentration.[26,27] This is because the present swelling studies were performed on dry samples not on pre-equilibrated ones.

Swelling reversibility

It was necessary for the swelling process to be reversible to ensure that the release of the solute could be initiated and stopped readily.[19] Hydrogels are capable of undergoing large reversible deformations

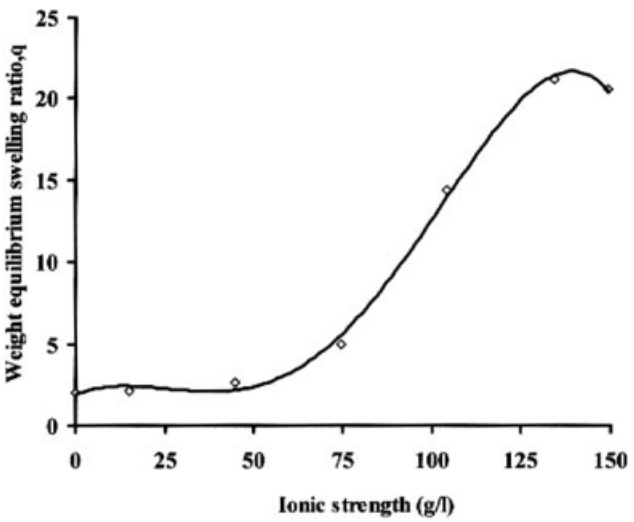

Figure 7 Effect of ionic strength of the swelling medium on the swelling of 0.5 : 0.5 PAA/PDMAEMA complex, at pH 5.4 and 30°C.

in response to changes in several environmental factors.

Ionic gels also swell/deswell due to general tendency of the polymer network to mix with the solution, but typically, the osmotic force is much greater than the mixing force. The osmotic pressure inside the gels is higher than that out of the gel. Equilibrium of the ionic gels occurs when the elastic restoring force of the network balances the osmotic force.[28]

Figure 8 illustrates the swelling reversibility of 0.5 : 0.5 PAA/PDMAEMA complex.

When a dry sample of the hydrogel was placed in doubly distilled water, the swelling occurred to a certain extent. When this swollen gel was placed in 2*M* KCl, the gel swelled largely and expanded due to the osmotic pressure resulting from the difference in ion concentrations between the swollen gel and the external solution. The results were reproducible for three swelling–deswelling cycles and no decrease in sorption capacity was observed. Because of reversibility and rapidity of swelling, the gel could be considered as a mechanochemical system in which chemical ionization energy could be transformed into mechanical energy.[20]

Immobilization of β-galactosidase on 0.5 : 0.5 PAA/PDMAEMA complex

The gel complex of 0.5 : 0.5 PAA/PDMAEMA had been selected for immobilization of one of the most commonly used industrial enzyme, β-galactosidase. This gel complex showed high swollen tendency in acidic medium and thus it was expected to be a suitable support for immobilization of the present acidic enzyme. The enzyme was successfully immobilized and the amount of bound protein was found to be 10 mg/g gel.

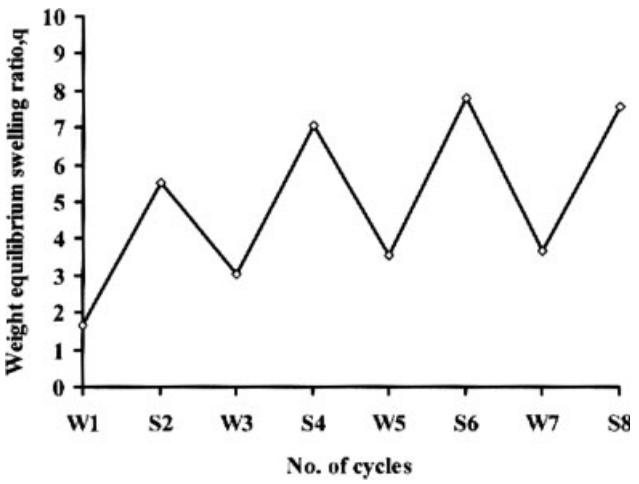

Figure 8 Dynamic swelling/deswelling for 0.5 : 0.5 PAA/PDMAEMA complex, at pH 5.4 and 30°C. W is doubly distilled water, S is 2M KCl, and the numbers represent the cycle numbers. The duration of each cycle was 35 min.

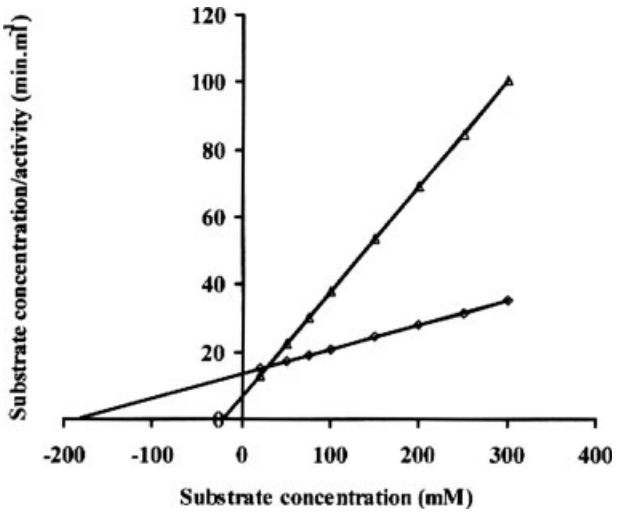

Figure 9 Hanes–Woolf plot of free (△) and immobilized (◇) β-galactosidase on 0.5 : 0.5 PAA/PDMAEMA complex.

Kinetic constants of free and immobilized β-galactosidase

Hanes–Woolf plot method was used to calculate the kinetic constants of free and immobilized β-galactosidase as shown in Figure 9. The calculated values are shown in Table VI. The increase of the apparent K_M after immobilization may be attributed to the conformational changes of the enzyme protein molecules during the immobilization process and steric hindrance that usually leads to an increase in K_M value; due to a decrease in the affinity between the enzyme and the substrate.[29]

In literature, the maximum reaction velocity "V_{max}" values for different immobilized enzymes were surprising, e.g., V_{max} for an immobilized glucoamylase was reported to be about 10 times higher than that of the native enzyme and for gel-entrapped β-D-fructofuranosidase, V_{max} value decreased to $\frac{1}{10}$th of that of the free enzyme.[30]

In the present study, the high value of V_{max} of the immobilized β-galactosidase with respect to the free counterpart may be interpreted from an energetic point of view as follows: for catalysis to be efficient, the loss of entropy, arising out of the initial binding of substrate to enzyme to form the enzyme–substrate

TABLE VI
Kinetic Constants of Free and Immobilized β-Galactosidase on 0.5 : 0.5 PAA/PDMAEMA Complex

Enzyme form	K_M (m*M*)	V_{max} (μmol min^{-1} mg^{-1})
Immobilized	187.2	13.8
Free	21.4	3.2

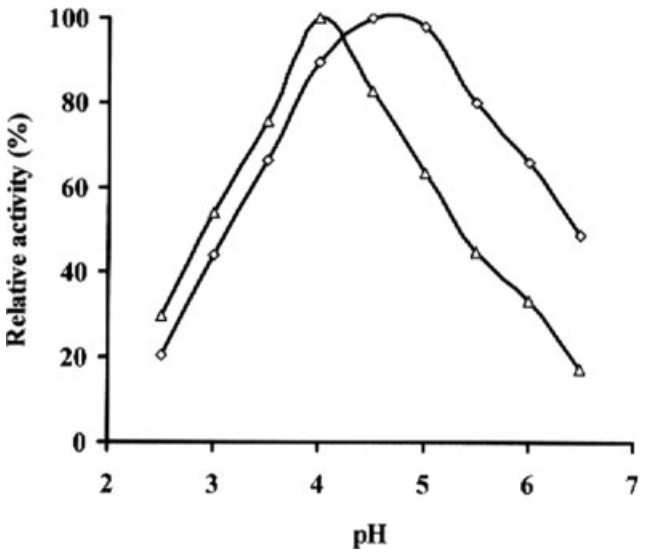

Figure 10 pH-activity profile of free (◇) and immobilized (Δ) β-galactosidase on 0.5 : 0.5 PAA/PDMAEMA complex.

complex, must be paid for by the binding energy released from the favorable interaction between enzyme and substrate. This implies that the loss of entropy, on forming the enzyme–substrate complex, acts to increase its dissociation constant (K_M). Thus, weak binding of substrate to enzyme presents the enormous catalytic advantage of the intermolecular effect. Consequently, a high K_M is in fact an advantage for the maximization of the reaction rate; a low K_M leads to an enzyme–substrate complex with lower free energy (ΔG^*) than that of the uncombined enzyme plus substrate and therefore ΔG^* for the catalytic process is increased, where a high K_M leaves ΔG^* unaffected.[31]

pH-activity profile of immobilized β-galactosidase

The enzymatic activity is markedly affected by environmental conditions, especially the pH of the aqueous medium, due to the protein nature of the enzymes. The changes in optimum pH and pH activity curve depend on the charge of the enzyme protein molecule or of the support.[30] Figure 10 illustrates the pH-activity profile of free and immobilized β-galactosidase. The optimum pH values for free and immobilized enzyme were 4.5–5 and 4, respectively. The shift in optimum pH value indicated that the immobilized enzyme had relatively more resistance toward drastic acidic conditions. In addition, the change in the pH-activity profile may be attributed to the partition effects that were arising from different concentrations of charged species in the microenvironment of the immobilized enzyme and in the domain of the bulk solution, owing to electrostatic interactions with fixed charges on the support.[29,30]

CONCLUSION

In this study, ionic hydrogels were prepared by template-induced polymerization of poly(acrylic acid) and dimethylaminoethyl methacrylate monomer. The mode of interaction, as proved by FTIR, was multiple H-bonding between the tertiary amino group of the monomer and the carboxylic groups of the polymer. The compositional effects on the dynamic swelling behavior were carefully probed, using a series of experiments designed to elucidate differences in swelling mechanism, rate, and relaxation. The gradual increase of the swelling percentage, by increasing the polymer–monomer feed ratio from 0.1 : 0.1 to 0.5 : 0.5, may be attributed to the increase in ionic groups and the counter-ions inside the gel, which acts to expand the collapsed structure. The mechanism of transport mainly depended on diffusion of water rather than polymer relaxation. The swelling mechanism and the complex relaxation showed little dependence on the polymer–monomer concentration. The hydrogels showed an amphoteric behavior with maximum swelling percentages at pH 1 and 10.5. Because of reversibility and rapidity of swelling, the gel could be considered as a mechanochemical system. The prepared hydrogel successfully immobilized the industrially used β-galactosidase as an acidic model enzyme, showing a remarkable improvement in its activity compared to the free one. Also, the immobilized enzyme had a relatively more resistance toward drastic acidic conditions. In conclusion, these hydrogels are good candidates for bioapplications such as biologically active molecules immobilization.

This work was supported by the National Research Center, Polymers and Pigments Department. The authors would like to thank chemist ''Noha Deghiedy'' for her assistance throughout the work.

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