Chemical Engineering Communications
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454788

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To cite this Article Vadalkar, V. S. , Premnath, V. , Kulkarni, M. G. and Mashelkar, R. A.(1996) 'DIFFUSION LIMITATIONS IN ENZYME MIMICING POLYMER MEDIATED REACTIONS', Chemical Engineering Communications, 152: 1, 139 — 160
To link to this Article: DOI: 10.1080/00986449608936559
URL: http://dx.doi.org/10.1080/00986449608936559

Chem. Eng. Comm., 1996, Vols. 152–153, pp. 139–160
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DIFFUSION LIMITATIONS IN ENZYME MIMICING POLYMER MEDIATED REACTIONS

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(Received March 20, 1995; in final form December 27, 1995)

Diffusional effects in reactions involving macromolecular substrates and reactants in heterogeneous media lead to unique features. We report investigations on the hydrolysis of 2-hydroxy ethyl methacrylate copolymers catalyzed by imidazole and poly (*N*-vinyl imidazole) selected as an enzyme mimicing polymer. This process has been mathematically modelled, and the predictions compared with experimental findings. The model framework is general for diffusion-reaction problems of this class. The work has also pragmatic relevance in the design of drug delivery systems, since the reaction takes place in pH ranges of physiological significance.

KEYWORDS Diffusion limitations Enzyme mimicing Reaction engineering

INTRODUCTION

Functionalization of polymers and their applications in fields such as ion exchange resins, affinity separations, enzyme immobilization, temporal and site specific drug delivery systems involves reactions, in which the substrate as well as the reactant, or either of the two, could be macromolecular in nature (Takemoto *et al.*, 1987; Akelah and Moet 1990). Further complexities arise depending on whether the reactions take place in homogeneous or heterogeneous phases (Kadoma *et al.*, 1982).

Hydrolysis in homogeneous solutions involving polymeric/monomeric nitrophenyl esters or anilides as substrates and enzymes as well as enzyme mimicing polymers has been extensively investigated and reviewed to elucidate the mechanism of enzyme action and to design polymers which could serve as carriers for targeted drug delivery systems (Overberger and Maki, 1970; Overberger *et al.*, 1971; Kunitake and Okahata, 1976; Fu and Morawetz, 1978; Duncan and Kopecek, 1984). Polymers and copolymers of imidazole, pyridine etc. as well as low molecular weight enzyme analogues have been extensively investigated as enzyme mimics (D' souza *et al.*, 1985). One of the limitations of such systems is that at higher levels of conjugation, the macromolecules in solution undergo conformational changes as a result of which the enzymatic attack on the

[1]NCL Communication No. 5635, Phone: 91-212-336151, Fax: 91-212-330233.

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linkage is no longer possible (Ulbrich *et al.*, 1987). Moreover, insoluble carriers offer a number of advantages over soluble polymers.

Reactions involving low molecular weight reactants and immobilized enzymes have been extensively studied (Colowick and Kaplan, 1987; 1988). In contrast, reactions involving enzymes and low molecular weight substrates linked to polymer matrices have not received much attention. Jakube and Lange (1974) demonstrated chymotrypsin catalyzed hydrolysis of sepharose bound L- phenylalanine-4-nitroanilide. Lapicque and Dellacherie (1986) linked pholcodine covalently to a cellulose derivative through spacer groups and investigated the kinetics of hydrolysis of the linkage by chymotrypsin under simulated intestinal conditions. Subr *et al.* (1990) showed that hydroxy propyl methacrylamide hydrogels comprising covalently linked daunomycin through various spacer groups were hydrolyzed by tritosomes.

Investigations involving diffusion reaction problems in polymers, which undergo structural changes as a result of chemical reactions or interaction with the solvent have been undertaken by us to evolve newer strategies to develop systems in which the diffusional flux remains constant (Shah *et al.*, 1990a, 1990b; Vyavahare *et al.*, 1990; Vadalkar *et al.*, 1994; Kulkarni *et al.*, 1992). The results could find pragmatic applications, if such changes occur under physiologically relevant conditions.

The objective of our subsequent efforts has been to investigate enzymatic hydrolysis of polymer conjugates from hydrogels. As a first step in this direction, we report in this paper, the results of imidazole catalyzed hydrolysis of *p*-nitrophenyl *p*-vinyl benzoate from poly (2-hydroxy ethyl methacrylate *p*-nitrophenyl *p*-vinyl benzoate), P(HEMA-PNPVB). It is shown that the hydrolysis is brought about under conditions of physiological significance. Although the kinetics of hydrolysis is identical to that in the solution, the rate of release of *p*-nitrophenol is constant with respect to time. A model incorporating diffusion of the catalyst, hydrolysis and desorption of *p*-nitrophenol is proposed. The model predictions based on the values of the parameters determined from independent experiments are in reasonable agreement with the experimental results.

EXPERIMENTAL SECTION

Synthesis

Materials

2-hydroxy ethyl methacrylate (HEMA) imidazole, *t*-butyl hydroperoxide and ammonium persulphate initiators and *N*,*N*,*N*′,*N*′ tetramethyl ethylene diamine (TEMED) activator, were obtained from Aldrich Chem. Co. (USA). *p*-Nitrophenol, triphenyl phosphene, *p*-toluic acid, *N*-vinyl imidazole, *N*-bromo succinimide, thionyl chloride and triethyl amine were obtained from local suppliers. These chemicals were purified by standard procedures.

Synthesis of Poly (N-vinyl imidazole)

Poly (*N*-vinyl imidazole) was synthesized by solution polymerization using Azobisiso butyronitrile as the initiator.

Synthesis of P-Nitrophenyl P-Vinyl Benzoate

p-vinyl benzoic acid (PVB) was synthesized in the laboratory by the bromination of *p*-toluic acid followed by a Wittig reaction (Broos *et al.* (1978). *p*-Nitrophenyl *p*-vinyl benzoate (PNPVB) was prepared by the reaction of *p*-vinyl benzoyl chloride and *p*-nitrophenol in presence of triethyl amine. The crude monomer was purified by recrystallization from acetone/pet ether. The purity was confirmed by spectral and elemental analysis, (m.p. 103 °C).

Synthesis of P-Nitrophenyl P-Vinyl Benzamidocaproate

p-vinyl benzamidocaproic acid was prepared by the Schotten Baumann reaction from *p*-vinyl benzoyl chloride and 6-amino caproic acid. The raw product was purified from ethanol water mixture (7:5) (m.p. 112°C). *p*-nitrophenyl *p*-vinyl benzamido caproate (4CS-6ACA-PNP) was prepared by esterification of *p*-vinyl benzamidocaproicacid with *p*-nitrophenol using dicyclohexyl carbodiimide and purified by recrystallization from methylene chloride/petroleum ether (m.p. 130 °C).

Dense Polymers

HEMA and PNPVB were taken in the desired weight ratio in a glass tube (15 mm ID). t-butyl hydroperoxide 0.8% by weight, was added as an initiator. Polymerization was carried out at 60°C for 12 hrs. The polymer was isolated in the form of a cylinder by breaking the tube. 0.09–0.11 cm thick discs were prepared by cutting the cylinder on the lathe. The thickness/diameter ratio for the discs was thus ~ 16. The release of *p*-nitrophenol from the slab could thus be treated as release from semiinfinite parallel slab. The absence of the free monomer was confirmed by the absence of the characteristic peak of the monomer in the extracts of the postpolymerized discs.

Porous Polymers

Porous hydrogels were prepared by carrying out the polymerization in the presence of porosigen such as PEG 9000. An aqueous solution of PEG (50%) was prepared. To this, the monomer mixture was added. Polymerization was carried out at 37°C using 0.5% ammonium persulfate as the initiator and TEMED as the activator. The unreacted monomer and PEG 9000 were extracted from the discs in dry ethanol.

Hydrolysis of P-Nitrophenol Esters from Polymer Discs

The hydrolysis of the copolymer discs containing covalently linked p-nitrophenol in the pendent chain was carried out in a jacketed vessel at 37°C and followed by monitoring the absorbance of the medium at $\lambda = 400$ nm on a Shimadzu 240 UV vis spectrophotometer. *p*-Nitrophenol formed at time t (M_t), was determined from the appropriate calibration curves. The total amount of *p*-nitrophenol initially present in the disc was taken as M_∞. The fraction of *p*-nitrophenol in the bulk solution at time t was expressed as (M_t/M_∞) was used to monitor the reaction kinetics. In all the cases,

discs swollen to equilibrium in water were used. The media in which the release kinetics was investigated were a) 0.01 N NaOH b) imidazole solutions (0.01 – 0.15M), c) Poly (*N*-vinyl imidazole) solution (0.1 – 1.0g/dl) d) phosphate buffer (pH 8 – 8.6) e) tris buffer (pH 7.9).

Determination of Model Parameters

Diffusion Coefficient of P-Nitrophenol and Imidazole

The copolymer discs containing 5% PNPVB were hydrolyzed for different time intervals in 1% imidazole solution at 37°C. Hydrolyzed *p*-nitrophenol and imidazole present in the disc were extracted in acetone. Complete removal of *p*-nitrophenol and imidazole from the discs was confirmed by following UV spectra of the extracts of the disc. The discs were dried and reloaded with *p*-nitrophenol from 1% solution of *p*-nitrophenol in phosphate buffer (pH = 7.4) at 4°C. The discs were equilibrated at 37 °C prior to the experiment. The desorption runs were carried out in phosphate buffer (pH = 7.4). The time for which the desorption experiment was carried out was very small (< 1hr) compared to the time required for complete hydrolysis of the polymeric ester (> 250 hrs.)

Diffusion coefficients of *p*-nitrophenol and imidazole from P(HEMA-PNPVB) discs prepared as above were determined experimentally by the desorption technique reported by Shah *et al.* (1990b).

Determination of Hydrolysis Constants

The copolymer P(HEMA-PNPVB) was crushed into fine powder. The unpolymerized monomer, if any, was removed during extraction in acetone. The polymer in the powder form was then dried at 50°C and sieved.

Hydrolysis of *p*-nitrophenyl *p*-vinyl benzoate was investigated from the microparticles in the range 50–250μ. The rate constants for various concentrations of imidazole were obtained under conditions, wherein diffusional limitations were eliminated. For first order reaction, fractional conversion X_A, is given by

$$\ln (1 - X_A) = -K't \tag{1}$$

where K' denotes the rate constant. In the present case, $X_A = M_t/M_\infty$ where M_t is the amount of *p*-nitrophenol released at time t and M_∞ denotes the amount of *p*-nitrophenol linked chemically, which is eventually released at time $t \to \infty$. First order rate constant were therefore calculated from equation

$$\frac{M_t}{M_\infty} = 1 - \exp(-K't)$$

which correlates the release kinetics when the catalyst concentration is constant and reaction is first order with respect to the substrate concentration.

RESULTS AND DISCUSSION

The rationale behind the investigation of imidazole catalyzed hydrolysis of polymer bound esters was outlined earlier. Copolymers of *p*-nitrophenyl *p*-vinyl benzoate were

selected since hydrolysis of its monomeric structural analogues and copolymers with acrylic acid by imidazole and poly (*N*-vinyl imidazole) in solution has been reported earlier. Rates of hydrolysis of this copolymer by poly (*N*-vinyl imidazole) are significantly influenced by the pH of the medium (Letsinger and Klaus 1965). While the maximum in the pH-activity curve for chymotrypsin catalyzed hydrolysis of *p*-nitroanilide esters copolymerized with acrylamide occurred at pH = 7.7, it was shifted to pH = 6.3 when a copolymer with acrylic acid was used as the substrate. Since such effects are not desirable in the intended applications of the copolymers in enzyme assisted hydrolysis, a neutral monomer viz., HEMA was copolymerized with PNPVB. This also avoided the possibility of intramolecular catalysis due to anions, if any, and swelling of the polymer following hydrolysis. Moreover, HEMA is biocompatible and is widely used in several biomedical formulations and devices.

Alkaline Hydrolysis of P(HEMA-PNPVB)

Shah *et al.* (1990b) investigated the kinetics of alkaline hydrolysis of copolymers of 2-methacryloyl ethyl benzoates and 2-hydroxy ethyl methacrylate (HEMA). Depending upon the reactivity of the ester link as governed by the nature of the substituent on benzoic acid and the diffusivity of the substituted benzoic acid in the polymer, the observed release kinetics was controlled by the rate of the hydrolysis step or by the diffusivity of substituted benzoic acid molecule. These results illustrate that heterogeneous reactions offer a means of manipulating the release kinetics, which could be exploited for applications of polymers in areas such as drug delivery systems.

Bender and Turnquest (1957, 1959) investigated the hydrolysis of *p*-nitrophenyl acetate in aqueous media. It was shown that the rate constant for hydrolysis by hydroxyl ions (510 l/mol sec) was almost thousand folds higher than that for hydrolysis by bases such as imidazole (0.475 l/mol sec). In comparison to the esters such as *p*-nitrophenyl acetate and 2-methacryloyl ethyl 2,4 dinitro benzoate, hydrolysis of *p*-nitropheny *p*-vinyl benzoate is far more facile as the ester link is flanked by two benzene rings. Thus the scission of the ester link in P(HEMA-PNPVB) to yield *p*-nitrophenol would be very rapid. Hence the observed reaction kinetics, as monitored by the rate of appearance of *p*-nitrophenol in the solution, is expected to be controlled by the diffusion of *p*-nitrophenol.

The observed kinetics of hydrolysis of P(HEMA-PNPVB) is shown in Figure 1.

The observed release kinetics in such situations is often described by the empirical relationship

$$M_t/M_\infty = Kt^n \tag{2}$$

where (M_t/M_∞) denotes the fraction of the solute released upto time t. K is a constant and n denotes the release index (Ritger and Peppas 1987). When the solute release is controlled by its diffusion from the polymer matrix and diffusion is fickian, the release index $n = 0.5$. Higher values are observed when non fickian diffusion results due to diffusion-relaxation coupling in glassy polymers (Vadalkar *et al.*, 1994; Korsmeyer *et al.*, 1986) and diffusion-reaction coupling in heterogeneous systems (Shah *et al.*, 1990a; Vyavahare *et al.*, 1990). The value of n ($n = 0.59$) obtained by fitting the data for the release of *p*-nitrophenol, further confirms that the observed kinetics is governed by the diffusion of *p*-nitrophenol.

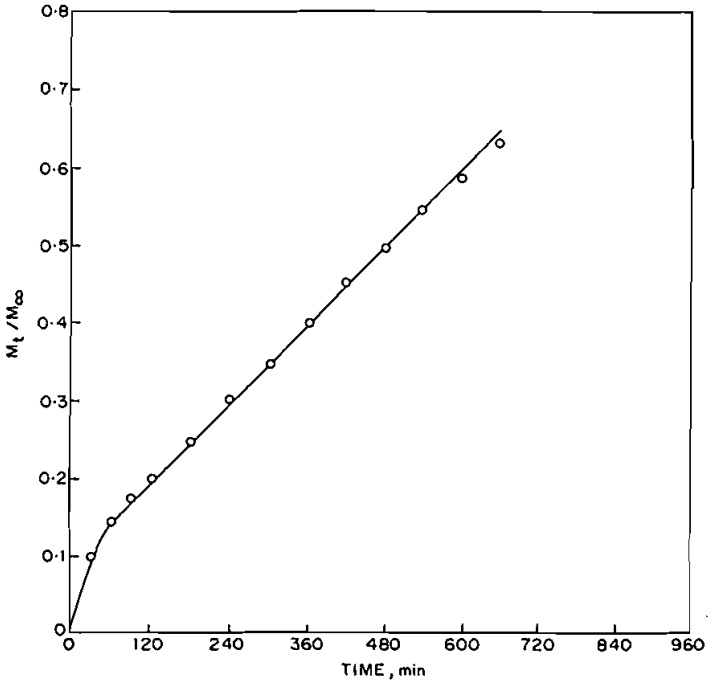

FIGURE 1 Alkaline hydrolysis of ester copolymers (o) P(HEMA-PNPVB).

In summary, alkaline hydrolysis of P(HEMA-PNPVB) is controlled by the diffusion of *p*-nitrophenol from the polymer matrix. Since the diffusion of the hydroxyl ion through the swollen polymer and subsequent hydrolysis are relatively rapid these steps do not influence the observed kinetics. In subsequent sections we illustrate situations under which all these factors can play an important role.

Hydrolysis in Phosphate Buffer

In order to establish catalytic activity of imidazole, hydrolysis of the polymeric esters in phosphate buffer in the same pH range as the imidazole solutions (viz 8.0 – 8.6) was investigated. The results are shown in Figure 2. It is clear that even in the absence of a catalyst, the hydrolysis of *p*-nitrophenyl *p*-vinyl benzoate proceeds at a finite rate since the ester link being attacked is activated by the presence of two phenyl rings. Since the hydroxyl ion concentration at this pH is very low, the rate of the hydrolysis step is very low and is not influenced significantly by the pH of the medium in the range investigated. Interestingly, the observed reaction kinetics shows that the fraction of *p*-nitrophenol released is linear with time.

In Figure 3 the effect of porosity on the release kinetics of *p*-nitrophenol is illustrated. It is seen that the observed release rate increases with increasing porosity of the matrix. The release index in the present case tends to unity unlike $n = 0.5$ for strictly diffusion controlled release. This could be attributed to the changes in the polymer structure

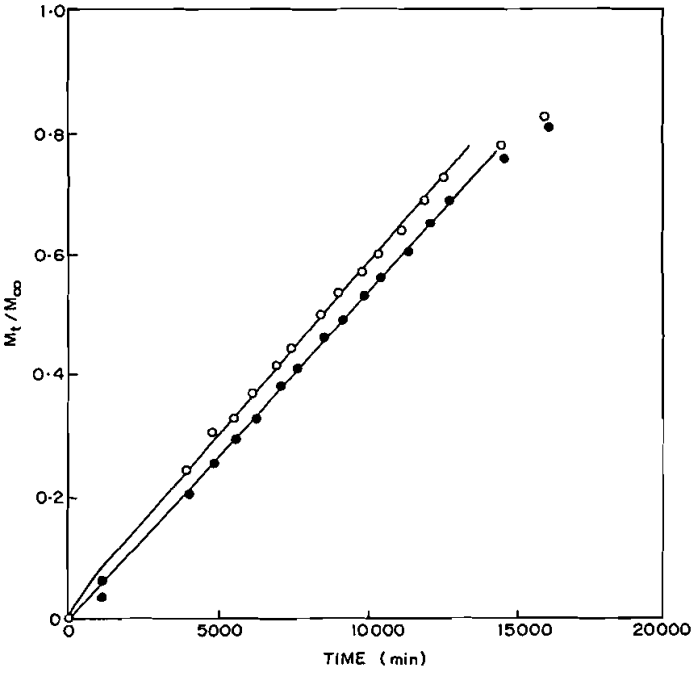

FIGURE 2 Hydrolysis of P(HEMA-PNPVB) discs in phosphate buffers (●) pH = 8.0, (○) pH = 8.6.

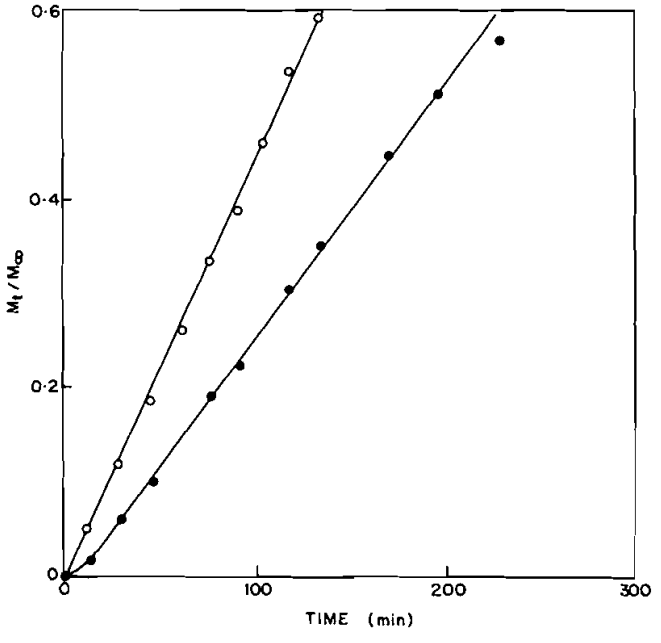

FIGURE 3 Effect of porosity on the hydrolysis of P(HEMA-PNPVB) in phosphate buffers. (●) Dense, (o) Porous.

which lead to modification of diffusional characteristics of the molecule diffusing out (Shah *et al.*, 1990a).

Imidazole Catalyzed Hydrolysis from P(HEMA-PNPVB) Discs

Experimental Results

Imidazole catalyzed hydrolysis of *p*-nitrophenyl esters in solution has been investigated by Bender and Turnquest (1957, 1959). The hydrolysis follows first order kinetics with respect to the ester and imidazole. The reaction involves attack of imidazole on the ester to form an acyl imidazole intermediate and release of *p*-nitrophenol. The intermediate then reacts with water to yield the acid and the catalyst.

Results of hydrolysis of P(HEMA-PNPVB) in phosphate buffer (pH = 8.6) and in imidazole solutions of varying concentration are shown in Figure 4. The catalytic effect of imidazole is obvious. It has been shown in the preceding section that in this range, pH of the medium does not influence the rate of the reaction. The rate constant for the hydrolysis of *p*-nitrophenyl acetate has also been shown to be independent of the primary salt effect (Bender and Turnquest 1957). Imidazole catalyzed hydrolysis of *p*-nitrophenyl hydrogen terephthalate, *p*-nitrophenyl *p*-isopropyl benzoate and copolymers of acrylic acid and *p*-nitrophenyl *p*-vinyl benzoate has been shown to follow first order kinetics (Letsinger and Klaus 1965). However, in the present case the

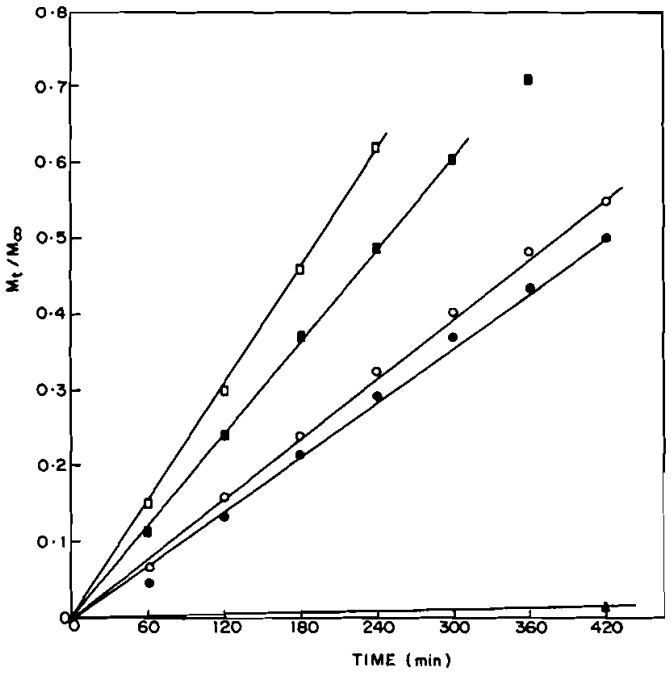

FIGURE 4 Imidazole catalyzed hydrolysis of P(HEMA-PNPVB) (▲) Phosphate buffer, (□) 0.15 M Imidazole, (■) 0.09 M Imidazole, (o) 0.06 M Imidazole, (●) 0.03 M Imidazole.

rate of appearance of *p*-nitrophenol is linear with time. The release of *p*-nitrophenol is diffusion controlled, as indicated by the effect of porosity on the release kinetics. Yet the release profiles are not fickian ($n \rightarrow 1$ rather than $n = 0.5$) since the diffusion in this case is non-fickian.

In order to verify if this was a consistent feature of hydrolysis in heterogeneous media, the effect of imidazole concentration was investigated. From the results summarized in Figure 4, it is clear that this is indeed the case. Further, based on the results of alkaline hydrolysis, one would suspect that in this case too, the observed kinetics is modified by the diffusional effects. This was confirmed on the basis of reactions carried out in porous polymers, wherein it was noticed that the observed reaction rate increased with the porosity of the polymer matrix (Fig. 5).

Evaluation of Intrinsic Kinetics

From the results summarized in the preceding section it is apparent that the observed hydrolysis of P(HEMA-PNPVB) from the discs does not follow first order kinetics as observed in the case of monomeric analogues of PNPVB and copolymers of PNPVB with acrylic acid in solution.The concentration of *p*-nitrophenol in the medium increases linearly with time. Similar observations have been reported in the past.

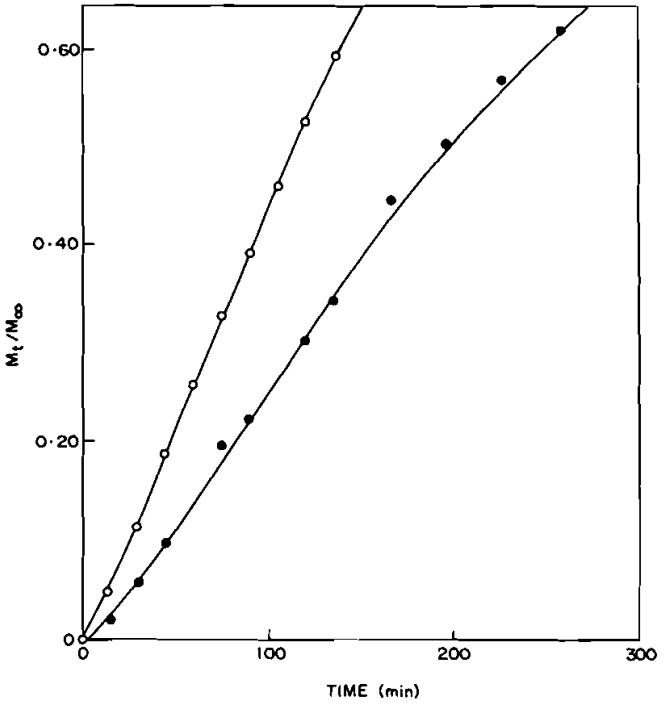

FIGURE 5 Effect of porosity on imidazole catalyzed hydrolysis of P(HEMA-PNPVB) [0.15 M Imidazole] (●) Dense, (○) Porous.

Chaves and Arranz (1988) reported that during hydrolysis of nicotinic acid ester of amylose from polymeric discs, the amount of nicotinic acid in the medium increased linearly with time. Yean *et al.* (1990) linked chloramphenicol to methacryloyl chloride via *m*-hydroxy benzoic acid as the spacer. In this case too, the conversion to chloramphenicol increased linearly with time. The pseudo zero order kinetics observed was attributed to very low value of rate constant and to the fact that the reaction was studied upto very low levels of conversion. For example, in ten days the conversion was only 1%. Reactions of polymeric substrates even in homogeneous solutions show anomalous kinetics (Letsinger and Klaus, 1965). Although imidazole catalyzed hydrolysis of the copolymer of acrylic acid and *p*-nitrophenyl *p*-vinyl benzoate followed first order kinetics, hydrolysis of the copolymer containing 2,4-dinitrophenyl *p*-vinyl benzoate could be correlated only in terms of a second order rate equation. This was attributed to change in the reactivity of the ester group with conversion.

These examples illustrate that before we attempt to elucidate the results reported herein, it is important to confirm that the reaction follows first order kinetics when investigated in solution or under conditions when no diffusional constraints prevail.

Hence, a general rate equation

$$-\frac{\partial C_S}{\partial t} = k C_S^s C_I^i \tag{3}$$

was used to determine k, s and i. Here, C_S and C_I correspond to the concentration of the substrate S and catalyst I, respectively. If hydrolysis were the only determining factor, then the catalyst concentration would reach its equilibrium value ($K_I C_{Ib}$) very rapidly. K_I denotes the partition coefficient of imidazole at the polymer-medium interface and C_{Ib}, the concentration of imidazole in the medium. Thus, Equation (3) simplifies to

$$-\frac{dC_s}{dt} = k(K_I C_{Ib})^i C_S^s = k' C_S^s \tag{4}$$

Hence, for $s = 1$ we would expect the following relation to hold.

$$\frac{C_{So} - C_S}{C_{So}} = \frac{M_t}{M_\infty} = 1 - \exp(-k't) \tag{5}$$

Further, if $i = 1$ the plot of k' versus C_{Ib} would be linear. The slope of the line divided by K_I would yield k.

In order to establish if this was so, the polymer was obtained in a fine particle form (50–250μ diameter) so as to eliminate diffusional limitations and the kinetics of hydrolysis in imidazole solution was followed. A typical plot shown in Figure 6 illustrates that the reaction indeed follows first order kinetics. The experimental results fit Equation (5) well thus confirming $s = 1$. The fit also yields the value of k'. In Figure 6 are also shown the results for the hydrolysis of the polymer in the particle size range 50–150μ and 150–210μ. The fact that the results superimpose, further confirms that the diffusional limitations are no more present. From a comparison of the order of magnitudes of the diffusion times and reaction time using the values of diffusion coefficients of imidazole (2.8×10^{-7}cm^2/sec) and *p*-nitrophenol (1.97×10^{-7}cm^2/sec) and the observed rate constant of reaction for 0.15 M imidazole, it can be seen that the

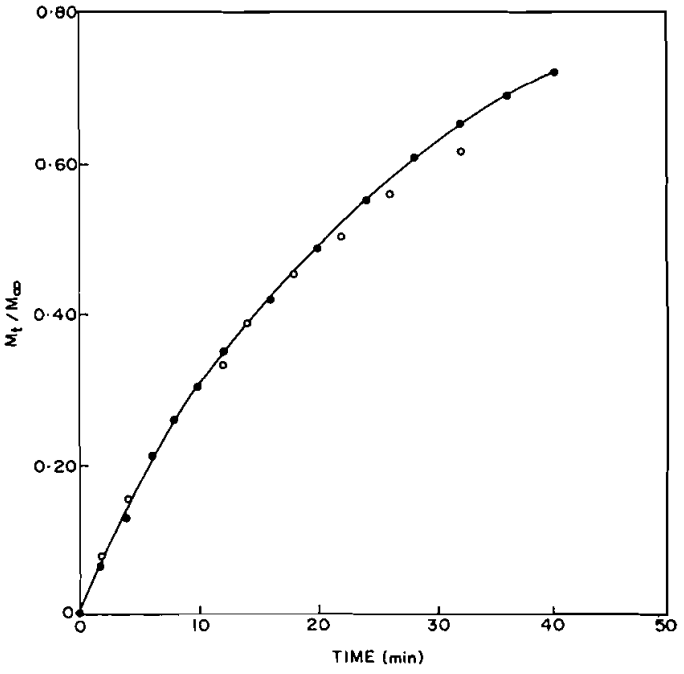

FIGURE 6 Hydrolysis of P(HEMA-PNPVB) from microparticles [Imidazole concentration 0.15 M] (o) 50–150μ (•) 200–250μ.

observed kinetics under these conditions is controlled only by the rate of the hydrolysis step. The effect of imidazole concentration was investigated and the observed rate constant was plotted as a function of imidazole concentration (Fig. 7). It is clear that the hydrolysis follows first order kinetics with respect to imidazole (i.e., $i = 1$) as reported in the case of hydrolysis in solution. The value of the rate constant, predicted using the value of k calculated by us ($k = 0.547$ 1/mol min for $K_I = 0.389$), for the reaction at imidazole concentration 5×10^{-4}M is 1.0×10^{-4} min^{-1}, which compares reasonably well with the value 1.4×10^{-4} min^{-1} reported by Letsinger and Klaus (1965) for the hydrolysis of *p*-nitrophenyl *p*-isopropyl benzoate.

Kinetics of Heterogeneous Hydrolysis: A Reaction Model

We now show how these results can be explained on the basis of the role of various factors which influence the observed reaction kinetics from the polymer discs.

The observed rate of reaction as monitored by the concentration of *p*-nitrophenol in the reaction medium will depend upon the contribution of various steps which are enumerated below:(1) diffusion of imidazole from the surrounding medium into a thin layer viz., the boundary layer on the polymer surface, (2) partitioning of imidazole between the external medium and the polymer matrix, (3) diffusion of imidazole within the polymer matrix, (4) hydrolysis of the ester link and generation of *p*-nitrophenol, (5)

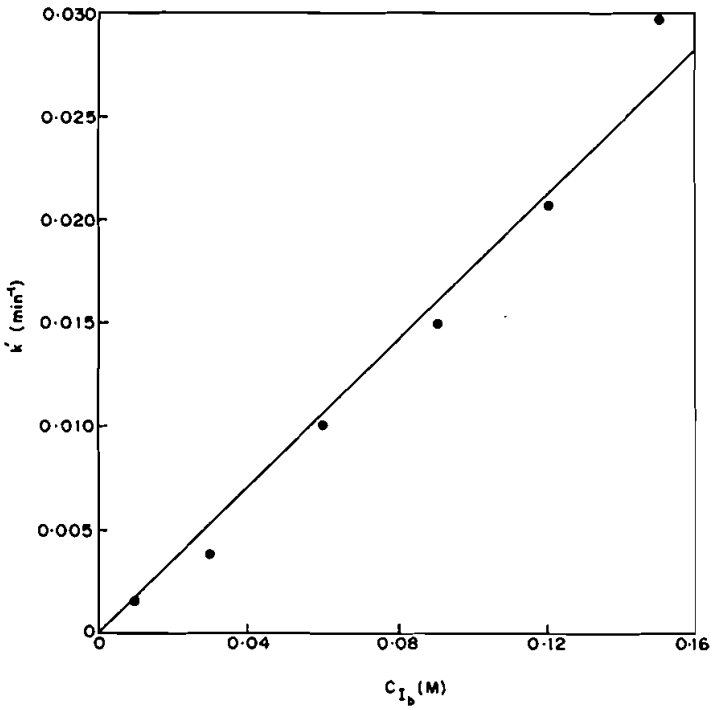

FIGURE 7 Effect of imidazole concentration on the observed rate constant.

diffusion of *p*-nitrophenol from the matrix to the surface, (6) partitioning of *p*-nitrophenol between the polymer matrix and the medium and (7) diffusion of *p*-nitrophenol from the boundary layer into the reaction medium.

Evidently when the hydrolysis of PNPVB from the polymer is studied in solution or from microparticles as discussed in the preceding section, the role of all factors except the hydrolysis reaction becomes insignificant. The observed kinetics in this case is therefore identical to that observed in solution. However, these factors need to be accounted for in the analysis of reactions from polymers in the disc form.

The analysis is based on the following assumptions. Diffusion of imidazole as well as *p*-nitrophenol is assumed to occur only in the direction perpendicular to the plane of the disc. This is justified since the ratio of thickness to diameter is 1/16. The role of diffusion within the boundary layer is ignored, since the experiments were carried out under well stirred conditions. Concentration of *p*-nitrophenol in the reaction medium is considered to be negligible i.e., perfect sink conditions are assumed to prevail. It is also assumed that the concentration of *p*-nitrophenol never reaches its solubility in the medium. Diffusivities of imidazole as well as *p*-nitro phenol are expressed as a function of the degree of hydrolysis of the polymer and are assumed to be of the form

$$D = D_i + (D_\infty - D_i)[1 - \exp(-X_s)] \tag{6}$$

where, χ_s denotes the conversion of the substrate. D_i and D_∞ denote the initial and final diffusivities of the mobile species, respectively. Thiis relationship is analogous to the one proposed by Lee *et al.* (1987) viz.

$$D_t = D_i + (D_\infty - D_i)\,(1 - \exp(-K_t))$$

to take into account the time dependent, penetrant induced polymer relaxation, in glassy polymers. The terms D_i,D_∞ and D_t have the same significance as in Equation (6). The constant K denotes the average relaxation constant controlling the approach to equilibrium. In the present case, the matrix is a swollen rubbery polymer and complications due to glass transition temperature do not arise. However, the diffusivity of the solute would vary with the fractional conversion which in turn is a function of time. K'' then denotes constant related to the rate of hydrolysis reaction.

Under these conditions, the balance equation for imidazole can be written as

$$\frac{\partial C_I}{\partial t} = \frac{\partial}{\partial x}\left(D_I \frac{\partial C_I}{\partial x}\right) - r_I \tag{7}$$

where C_I denotes the concentration of imidazole. D_I denotes the conversion dependent diffusion coefficients of imidazole and r_I denotes the rate of consumption of imidazole. x and t denote position and time coordinates, respectively.

When the origin of the position coordinate is fixed at the centre of the disc and 2L denotes the thickness of the disc, the initial and boundary conditions for the above equation are given by

$$C_I = 0 \text{ at } t = 0 \text{ for} - L \leqslant x \leqslant L \tag{8}$$

$$C_I = K_I C_{Ib} \text{ at } x = \pm L \quad \text{for } t > 0 \tag{9}$$

K_I denotes the partition coefficient of imidazole between the polymer and the medium.

Similarly the diffusioin of *p*-nitrophenol is described by

$$\frac{\partial C_P}{\partial t} = \frac{\partial}{\partial x}\left(D_P \frac{\partial C_P}{\partial x}\right) + r_P \tag{10}$$

where C_P denotes the concentration of *p*-nitrophenol, D_P denotes the conversion dependent diffusivity and r_P the rate of generation of *p*-nitrophenol due to hydrolysis of the ester. The associated initial and boundary conditions are

$$C_P = 0 \text{ at } t = 0 \text{ for } -L \leqslant x \leqslant L \tag{11}$$

$$C_P = 0 \text{ at } x = \pm L \text{ for } t > 0 \tag{12}$$

Equation (12) represents the perfect sink condition.

For the polymer substrate we have

$$\frac{\partial C_S}{\partial t} = -r_S \tag{13}$$

Here, C_S is the concentration of the substrate in the polymer matrix and r_S is the rate of conversion of *p*-nitrophenyl *p*-vinyl benzoate to *p*-nitrophenol.

The associated initial condition is

$$C_S = C_{S0} \text{ at } t = 0 \text{ for } -L \leqslant x \leqslant L \tag{14}$$

r_I, r_P and r_S are related by the stoichiometry of the reaction. The diffusion coefficients in Equation (7) and (10) are written as

$$D_I = D_{Ii} + (D_{I\infty} - D_{Ii})\,[1 - \exp(-X_S)] \tag{15}$$

and

$$D_P = D_{Pi} + (D_{P\infty} - D_{Pi})\,[1 - \exp(-X_S)] \tag{16}$$

where, X_S denotes fractional conversion of substrate, S.

The fraction of p-nitrophenol released in the medium at time t is then given by

$$\frac{M_t}{M_\infty} = 1 - \left[\frac{\int_0^L C_s dx + \int_0^L C_P dx}{C_{S0} L}\right] \tag{17}$$

when one mole of substrate S leads to one mole of product P. This is valid in the present case since one mole of p-nitrophenyl p-vinyl benzoate leads to one mole of p-nitrophenol.

The validity of the rate equation

$$r = kC_I C_S \tag{18}$$

was demonstrated in the preceding section. The above equations were then nondimensionlized using

$$\bar{C}_P = \frac{C_P}{C_{So}},\ \bar{C}_S = \frac{C_S}{C_{So}},\ \bar{C}_I = \frac{C_I}{K_I C_{Ib}},\ \theta = \frac{tD_{Ii}}{L^2},\ \eta = \frac{x}{L} \tag{19}$$

to yield

$$\frac{\partial \bar{C}_I}{\partial \theta} = \frac{\partial}{\partial \eta}\left[\left(\frac{1}{\beta_I} + \left(1 - \frac{1}{\beta_I}\right)(1 - \exp(-X_S))\right)\frac{\partial \bar{C}_I}{\partial \eta}\right] - \phi^2 \Psi \bar{C}_S \bar{C}_I \tag{20}$$

$$\bar{C}_I = 0 \text{ at } \theta = 0 \text{ for } -1 \leqslant \eta \leqslant +1 \tag{21}$$

$$\bar{C}_I = 1 \text{ at } \eta = \pm 1 \text{ for } \theta > 0 \tag{22}$$

and

$$\frac{\partial \bar{C}_P}{\partial \theta} = \mu \frac{\partial}{\partial \eta}\left[\left(\frac{1}{\beta_P} + \left(1 - \frac{1}{\beta_P}\right)(1 - \exp(-X_S))\right)\frac{\partial \bar{C}_P}{\partial \eta}\right] + \phi^2 \bar{C}_S \bar{C}_I \tag{23}$$

$$\bar{C}_P = 0 \text{ at } \theta = 0 \text{ for } -1 \leqslant \eta \leqslant +1 \tag{24}$$

$$\bar{C}_P = 0 \text{ at } \eta = \pm 1 \text{ for } \theta > 0 \tag{25}$$

and

$$\frac{\partial \bar{C}_S}{\partial \theta} = -\phi^2 \bar{C}_S \bar{C}_P \tag{26}$$

$$\bar{C}_S = 1 \text{ at } \theta = 0 \text{ for } -1 \leqslant \eta \leqslant +1 \tag{27}$$

$$\frac{\partial \bar{C}_S}{\partial \theta} = -\phi^2 \bar{C}_S \text{ at } \eta = \pm 1 \text{ for } \theta > 0 \tag{28}$$

Here, a number of dimensionless groups featuring the physics of this diffusion-reaction process have been defined. viz.

$$\phi^2 = L^2 k \frac{(K_I C_{Ib})}{D_{Ii}}$$

$$\mu = \frac{D_{Pi}}{D_{Ii}}$$

$$\Psi = \frac{C_{So}}{K_I C_{Ib}}$$

$$\beta_X = \frac{D_{xi}}{D_{X\infty}}$$

The value β_X was determined experimentally by finding out the diffusivity of *p*-nitrophenol from the polymer disc before and after the complete hydrolysis of *p*-nitrophenyl *p*-vinyl benzoate. It was not possible to determine D_{Ii} experimentally since imidazole catalyzed the hydrolysis of the polymer. We, therefore, assumed $\beta_I = \beta_P = \beta$ which is justified in view of the similar sizes of the two mobile species. The above equations were then solved numerically using a finite difference method. This allowed us to predict the effect of dimensionless parameters ϕ, β, μ and ψ on the observed kinetics and provide guidelines for tailoring polymer systems so that the desired release profiles could be achieved. The model predictions and their implications towards this end are discussed below.

Model Predictions

The effect of Thiele modulus, on the release profile as a function of time, is shown in Figure 8. These are typically sigmoidal and show linearities within specific time intervals. However, with increasing ϕ the release profiles tend to become linear and tend to show negative deviations from linearity at higher conversions. The conversion levels at which the deviations become apparent increase with increasing ϕ. From the definition it is apparent that the value of ϕ could be enhanced as a result of an increase in the rate of reaction and/ or a decrease in the diffusivity of the catalyst. Use of substituted imidazole containing the electron donating groups would lead to higher reaction rates as well as lower diffusivity, thus leading to higher values of ϕ. Alternatively, the use of enzymes and enzyme mimicing polymers would also lead to the same end result. In these cases, deviations from linearity become apparent at higher levels of

conversion but lower values of ϕ. However, it is to be noted that θ denotes the time nondimensionalized with respect to the diffusion time. Since the diffusion coefficient of such macromolecular catalysts will be atleast two orders of magnitudes lower than those for the low molecular weight catalysts, the durations over which the linearities will be obtained in real time will be longer. Further, the diffusion of macromolecules through the polymers could be facilitated in order to modify the release profiles by incorporating appropriate carriers [Kokufuta and Jinbo (1992)]. The effect of μ on the release profiles is shown in Figure 9. It is seen that the profiles tend to be linear and level off at higher conversion levels and shorter dimensionless times with increasing values of μ, leading to linear release profiles over extended duration in real time especially in the case of macromolecular catalysts for the reasons discussed in the preceding paragraph. It is also noteworthy that the diffusivity of the reaction product will affect μ when low molecular weight catalyst such as imidazole or its derivatives are used since the diffusivities of the two are comparable. However, such changes will not influence macromolecular catalysis since the value of μ will be so high that any further change will be of little consequence.

The effect of enhancement in the diffusivity of the molecule released during the course of reaction on the release profile is shown in Figure 10. For $\beta = 1$, a typical parabolic profile results. With decreasing β, sigmoidal profiles containing regions over which release is linear with time result. Analysis of similar systems, wherein the molecule diffusing out was physically dissolved in the polymer matrix, showed that

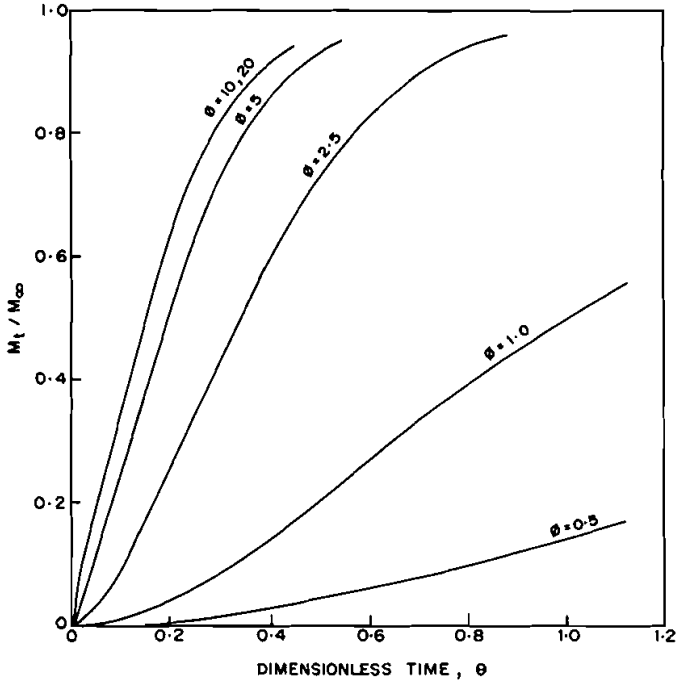

FIGURE 8 Effect of Thiele modulus on the release profiles of *p*-nitrophenol [$\mu = 0.5$, $\psi = 1.0$, $\beta = 0.167$].

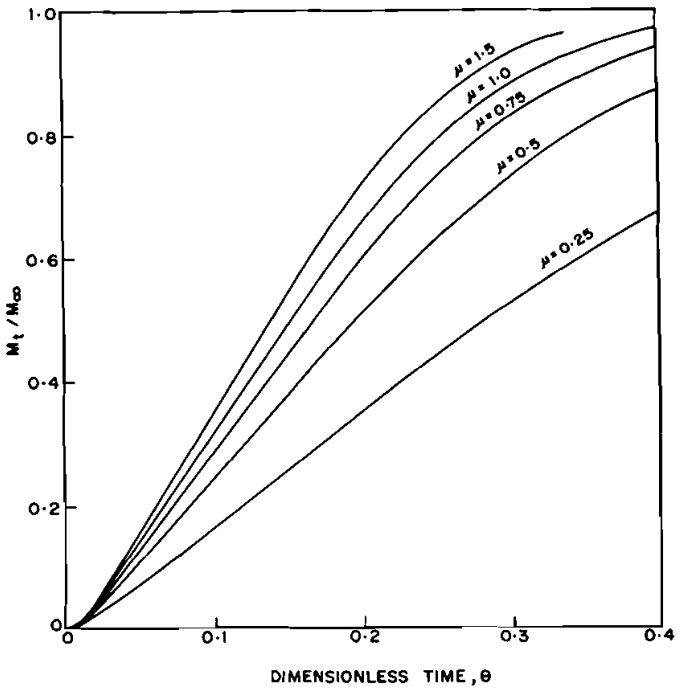

FIGURE 9 Effect of the diffusivity ratio on the release profiles of *p*-nitrophenol [$\phi = 5.0$, $\psi = 1.0$, $\beta = 0.167$].

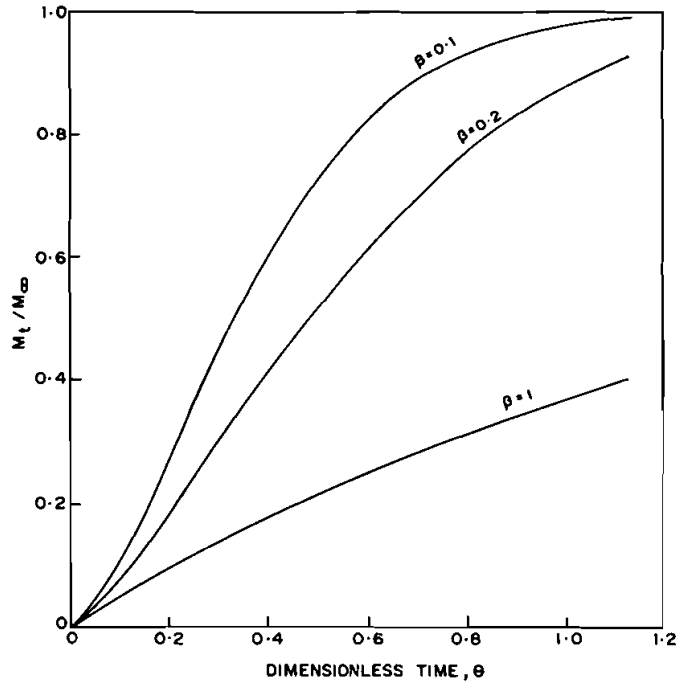

FIGURE 10 Effect of diffusivity enhancement parameter on the release profiles of *p*-nitrophenol ($\varphi = 2.5$, $\mu = 0.5$, $\Psi = 5$).

linear profiles were obtained for very low values of β ($\beta = 0.05$) (Lee, 1987). However, the model prediction for systems involving hydrolysis and diffusion discussed in this work show that linear kinetics will be observed for comparatively high values of β($\beta = 0.113$), which are in agreement with the values experimentally determined in this work and for which linear kinetics is actually observed.

Having thus seen the qualitative predictions which emerge from the model, it will be interesting to compare the model predictions with the experimental results. The values of the model parameters calculated for the systems investigated in this work are summarized in Table 1. The model predictions are compared with the experimental results in Figure 11. It is seen that the model predictions are in good agreement with the experimental results except at very low imidazole concentrations. The reasons for this deviation are being investigated.

TABLE 1

Model Parameters for Various Imidazole Concentrations.

Imidazole concentration (M)	k' (min^{-1})	φ	μ	Ψ	β
0.01	0.00157	1.169	0.6320	47.79	0.113
0.03	0.00550	2.188	0.6320	15.93	0.113
0.06	0.01100	3.095	0.6320	7.965	0.113
0.09	0.01833	3.994	0.6320	5.310	0.113
0.12	0.02200	4.376	0.6320	3.982	0.113
0.15	0.03140	5.228	0.6320	3.186	0.113

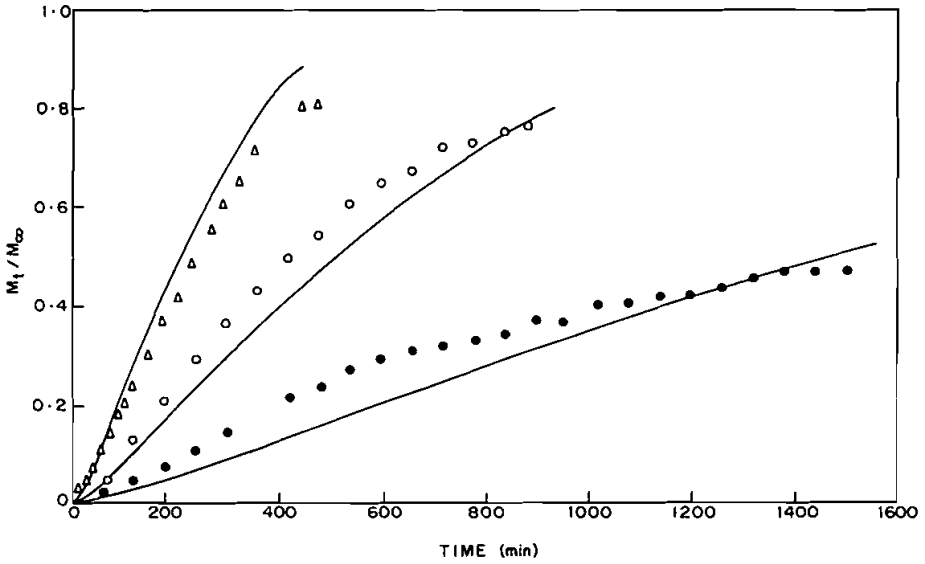

FIGURE 11 Hydrolysis of P(HEMA-PNPVB): Comparison of model predictions (—) with experimental data for imidazole concentrations (Δ) 0.09 M, (o) 0.03 M, (●) 0.01 M.

Catalysis by Poly (N-vinyl imidazole)

Imidazole and Poly (*N*-vinyl imidazole) catalyzed hydrolysis of monomeric as well as polymeric substrates containing mono and dinitrphenyl esters in solutions was investigated by Letsinger and Klaus (1965). The catalytic activity of poly (*N*-vinyl imidazole) was lower than that of imidazole. For the hydrolysis of poly (acrylic acid-*p*-nitrophenyl *p*-vinyl benzoate), the activity was reversed since acrylic acid provided binding sites for imidazole. The catalysis by poly (*N*-vinyl imidazole) exhibits features similar to enzyme catalysed reactions. Since there are no anionic binding sites on the polymer poly (2-hydroxy ethyl methacrylate *p*-nitrophenyl benzoate), the catalytic mechanism of hydrolysis by imidazole and poly (*N*-vinyl imidazole) would be identical, but for the macromolecular nature of the latter.

The observed rate of release of *p*-nitrophenol from dense poly(HEMA-PNPVB) hydrogels in poly (*N*-vinyl imidazole) solutions was extremely slow. The hydrogels were therefore synthesized in the presence of PEG-9000 as the porosigen to enhance the effective diffusivity of poly (*N*-vinyl imidazole) into the matrix. The results indicate that poly (*N*-vinyl imidazole) catalyses hydrolysis of the ester from the hydrogel (Fig. 12).

In enzyme catalysed hydrolysis of pendent chain linked *p*-nitrophenyl esters in solution, the rate of hydrolysis decreases as the length of the spacer between the ester link and the polymer backbone decreases. For instance, poly (*N*-vinyl pyrolidone-co-maleic acid) esters were not hydrolysed by chymotrypsin in solution (Mora and Pato 1992). It therefore appears surprising that the ester link in the present case is hydrolysed when the spacer is a phenyl ring which is rigid in comparison to the flexible aliphatic spacers used in enzyme catalysed reactions in solution. This is because the hydrolysis in the present case is catalysed by the imidazole group in the repeat unit. In contrast, the ester link undergoing chymotrypsin catalysed hydrolysis, has to have enough mobility as to be accessible to the active site of the enzyme involving the catalytic triad.

The substrate in the present case is a nonionic polymer. The rate constant for poly (*N*-vinyl imidazole) catalysed hydrolysis would be lower than that for imidazole

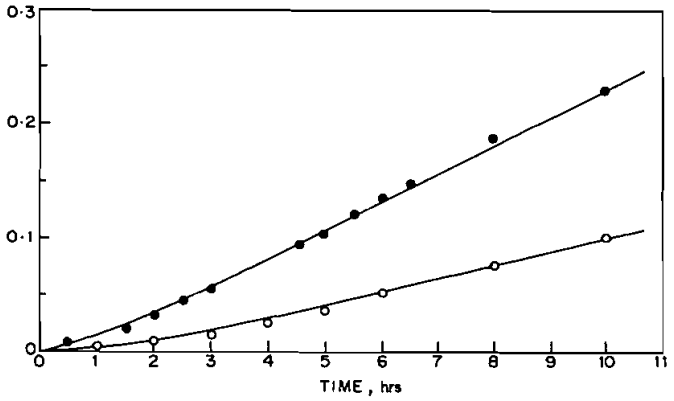

FIGURE 12 Hydrolysis of P(HEMA-PNPVB) (●) in poly (*N*-vinyl imidazole) solution and in (o) tris buffer.

catalysed reaction by a factor of about 2. (Letsinger and Klaus 1965). The molecular weight of poly (*N*-vinyl imidazole) is much higher than that of imidazole. Based on the effective size, the diffusion coefficient of the catalyst would be several magnitudes lower than that of imidazole. However, it is the segmental diffusion rather than the diffusion of the molecule as a whole, which governs the process (Fu and Morawetz, 1976). In the case of poly (*N*-vinyl imidazole) catalysed reaction, therefore ϕ is enhanced three folds while μ is enhanced about four folds. From Figures 8 and 9 it is apparent that increasing ψ and μ lead to linear release profiles after extended time periods in real time. The observed release profiles are thus in qualitative agreement with the model predictions.

Poly (*N*-vinyl imidazole) thus brings about the hydrolysis of immobilized substrate under physiological conditions, when the substrate strucutre is modified as to provide binding sites, this system exhibits features similar to enzyme catalysed reactions (Vadalkar and Kulkarni, 1995).

CONCLUSIONS

In this paper, results of imidazole catalyzed heterogeneous hydrolysis of P(HEMA-PNPVB) have been reported. It has been shown that although the kinetics and mechanism of hydrolysis is the same as in the solution, the hydrolysis of the polymers in the disc form leads to the release of *p*-nitrophenol at a constant rate. A model for heterogeneous reactions incorporating diffusion of imidazole, kinetics of the hydrolysis step and diffusion of *p*-nitrophenol from the polymer matrix has been developed. The values of the model parameters have been obtained from independent experimental measurements. The framework can be applied to the analysis of any heterogeneous reaction by substituting the relevant rate equations and should therefore be useful for interpreting the results of enzyme assisted hydrolysis or hydrolysis catalyzed by enzyme mimicing polymers. Since the hydrolysis takes place in the range of physiological significance, systems of this type could find applications in drug delivery systems of practical significance.

ACKNOWLEDGEMENT

V. S. Vadalkar would like to acknowledge the financial support received from the Council of Scientific and Industrial Research (CSIR).

V. Premnath would like to thank the Jawaharlal Nehru Centre for Advanced Scientific Research for the award of a Summer Research Fellowship.

NOMENCLATURE

C_I Concentration of catalyst I.
C_{Ib} Concentration of imidazole in the medium
C_p Concentration of *p*-nitrophenol

C_s Concentration of substrate in the polymer matrix
D_i Diffusivity of the solute through the matrix of the commencement of the reaction
$D_{(t)}$ Diffusivity of the solute through the matrix at time t
D_∞ Diffusivity of the solute through the matrix when the conversion is complete
K Averaged relaxation constant
K_I Partition coefficient of imidazole at the polymer medium interface
K' Rate constant for hydrolysis
M_t Amount of *p*-nitrophenol released at time t
M_∞ Total amount of *p*-nitro phenol released
n Release index
rp Rate of generation of *p*-nitro phenol due to hydrolysis of the ester
rs Rate of conversion of *p*-nitro phenyl *p*-vinyl benzoate to *p*-nitro phenol
t Time in seconds
X Fractional conversion

Greek Symbols

β_x Ratio of diffusivities of *p*-nitrophenol from polymer disc before and after complete hydrolysis
φ Thiele modulus
θ Dimensionless time
μ Ratio of the initial product diffusivity to the catalyst
Ψ Ratio of the initial substrate concentration to the maximum attainable catalyst concentration in the disc.

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