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Studies on α-amylase induced degradation of binary polymeric blends of crosslinked starch and pectin

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Abstract A blend matrix of crosslinked starch and pectin was prepared and characterized by infra-red (IR) spectroscopy, differential scanning calorimetry (DSC), and scanning electron microscopy (SEM). The prepared blends were investigated kinetically for water sorption studies and α-amylase induced degradation adopting a gravimetric procedure. Based on the experimental findings, a plausible mechanism including both diffusion and surface enhanced degradation was suggested and degradation profiles were interpreted. The influence of various factors such as chemical architecture of the blend, pH and temperature of α-amylase solution were examined for the swelling and degradation kinetics of crosslinked starch–pectin blends. The effect of concentration of enzyme solution was also studied on the degradation profile of the blends. A correlation was established between the extent of degradation and water imbibing capacity of the degrading blends.

1 Introduction

Degradation of polymers continues to remain the emerging area of interest in pharmacological research endeavors to explore polymer degradation in the production and use of implantable and other medical devices. During the last two decades, several clinical issues related to degradation behaviour of biomaterials became relevant [1, 2]. Polymeric based systems aimed at being degraded (in a controlled way) under service conditions have been developed. The understanding of how to balance the relevant degradation mechanisms, in order to tailor an implant material to degrade and transfer stress, at the appropriate rate to the surrounding healing tissues is a major challenge now-a-days. The mechanism of degradation, which can be applied, is mainly hydrolysis and enzymatic degradation [3]. For many applications, enzymatic degradation is advantageous because of ubiquitous presence of enzymes in the body. Many researchers indicate the role of enzymes in the process and degradation of biodegradable polymers [4–6].

Biodegradable polymers are highly desirable as they degrade within the body as a result of natural biological processes to biologically inert and compatible molecules, or they can participate in and control the rate of drug release by polymer hydration and degradation [7]. Furthermore, if employed as a drug delivery system they essentially need to be removed [8]. However, the major drawback of biodegradable polymers is the eventual toxicity following absorption of degraded products.

Thus, realizing the need for degradation products to be non-toxic, and cause no deteterious effects to the body and environment, attention has been focussed on employing natural polymers such as cellulose [9], gelatin [10], chitosan [11], sodium alginate [12], etc. to compose hydrogels with a specific response to a biological environment. But, still the most widely investigated natural polymer, achieving a great significance are starch-based

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polymers, which can be tailored to provide optimum performance in pharmaceutical applications. The major reasons are their reputation as safe suture materials and their degradation to products that are normal metabolites.

A thorough survey of literature cites numerous studies on starch-based polymers employed as hydrogels, but, yet, only a few attempts have been made to use starch-based polymers in drug delivery applications [13]; despite being well known that they are biodegradable, they have been proposed in several works to be used as biomaterials [14]. Starch acrylic polymeric hydrogels have also been used as drug delivery systems for biomedical applications [15]. The authors also performed their degradation studies in simulated physiological solutions, and noticed that these systems performed in vivo are expected to be resorbed and degraded faster. In another study [16], a starch matrix implant was fabricated and in vitro release of ciprofloxacin was investigated for varying composition of matrices. An attempt was made [17] to study the effect of crosslinked cationic starch matrix on the slow release of carboxylic group containing herbicides. An original degradation model of composites of starch–(EVOH) reinforced with hydroxyapatite for temporary biomedical implants has been proposed [18]. Recently, biodegradation of starch and acrylic grafted starch by *Aspergillus niger* was also examined [19]. The authors observed that temperature of maximum decomposition of starch decreased as enzymatic degradation proceeded. The degradation of starch films by amylase enzymes was monitored, and their use as membranes in bioreactors was proposed [20]. Biodegradation of polyacryl starch microspheres was investigated using radiolabelling technique [21]. The authors also observed the influence of various factors on the distribution and degradation of microspheres. The long term degradation behaviour of blends of starch with poly(ethylene-vinyl alcohol) copolymer by α-amylase was analysed [22] and it was noticed that biodegradation rate decreased as a function of time, probably due to structure and porosity of the material.

Thus, being motivated by the challenges coming across in enzyme induced degradation of starch in biomedical fields, the objectives of the present study include designing polymeric blends of starch and pectin, which could exhibit controlled degradation, when induced by α-amylase. It has been the matter of interest to choose pectin as one of the constituent polymers because of its non-toxicity, pH-sensitivity and gelling activity [23] and its frequent use in bio-encapsulation technologies [24]. Primarily, pectin is a heteropolysaccharide consisting of partially esterified linear polymer of α (1 → 4) bond polygalacturonic acid [25]. Pectin has been used as thickener, stabilizer, cosmetics, adhesives, as an excipient for pharmaceutical purposes, viz. the popular medicine for diarrhoea called Kaopectate. It is also effective at binding toxic and radioactive metals and has been applied to environmental cleanup and detoxification in humans [26].

2 Experimental

2.1 Materials

Soluble starch was obtained from S. Merck (India) and used as received. Pectin (EP) was purchased from Research Lab, Bombay, India. Epichlorohydrin employed as a crosslinker was obtained from Loba Chemie, Mumbai, India and used without any pretreatment. α-amylase (fungal source) was obtained in powdered form, from Research laboratory, Mumbai with an activity of 1300 IU/g and always stored in refrigerator. Other chemicals required for the study were of standard quality (AR Grade) and double distilled water was used for preparing the solutions, throughout the experiments.

3 Methods

3.1 Preparation of blends

Blends of crosslinked starch and pectin were prepared by cast solution technique. In brief, the adopted procedure for preparation of blend is given below.

In a typical experiment, 0.5 g of pectin was added into 25 mL of distilled water, and to it was further added 3 mL of 1M NaOH solution to prepare a clear solution. To this clear solution, 3 g starch powder was added with heating and continuous stirring, followed by addition of 0.5 mL epichlorohydrin (2.5% solution v/v) (0.15 mM). The reaction mixture was then homogenized by manual mixing, and placed in a petridish (diam. 4″ Corning) at 35°C for 48 h, so that the entire mass converted into a thick, yellowish disc. The blend so prepared was equilibrated with bidistilled water for 48 h to ensure complete removal of unreacted chemicals and impurities. Now, the blend was cut into circular buttons, which were dried at room temperature for 24 h and stored in airtight polyethylene bags.

3.2 Swelling experiments

The progress of the swelling process was monitored by the general gravimetric procedure as described elsewhere [27]. In a typical experiment, preweighed pieces of blends (0.1 g) were immersed in bidistilled water, and the extent of blend swelling was monitored by recording weights of swollen blends at desired time intervals. The swelling process was quantified by the parameter given below:

$$\text{Swelling ratio (SR)} = \frac{\text{Weight of swollen blend}}{\text{Weight of dry blend}}. \quad (1)$$

3.3 Degradation studies

In vitro, degradation studies were performed by incubating polymeric blends into α-amylase solutions at constant pH (6.8) and temperature (25 ± 0.2°C).

In brief, preweighed pieces of blends were added into a 20 mL of enzyme solution containing 6.5 IU/ mL of α-amylase. As a result of the degradation of starch content of the blend, a weight loss of the blend occurs, which was periodically recorded at desired time intervals by a sensitive balance (Denver instrument, APX-203). The extent of degradation may be calculated by the final equation:

$$\%\ \text{Degradation} = \frac{m_o - m_d}{m_o} \times 100, \quad (2)$$

where m_o and m_d are the initial and final weights (before degradation and after degradation respectively) of dry polymer blends.

3.4 Effect of pH

The effect of pH on the swelling and degradation of the blends was studied by varying the pH of the respective solutions by adding 0.1 M HCl and/or 0.1 NaOH to bring the pH to the desired value. The pH was determined on a Digital pH meter (Systronics, MK VI, Ahmedabad, India) before and after each experiment and almost no change in pH was recorded.

4 Characterization of blends

4.1 FTIR spectra

The FTIR spectra of the polymer blend were recorded on FTIR spectrophotometer (Perkin Elmer, 1000 Paragon). The blend samples were prepared by using KBr pellets, and spectra were run with four scans at a resolution of 4.0 cm^{-1}.

4.2 DSC analysis

Thermal properties of prepared blend were evaluated by recording DSC thermograms between 25°C and 600°C under N_2 atmosphere and at a heating rate of 10°C/min on a Differential Scanning Calorimeter (2100 Du Pont).

4.3 Scanning Electron Micrograph

Morphological studies of polymeric blends were made on a Scanning Electron Micrograph (STEREO SCAN, 430, Leica SEM, USA).

5 Results and discussion

5.1 FTIR spectral analysis

The blends of crosslinked starch and pectin were examined by FTIR spectral analysis. The FTIR spectra of the blend are shown in Fig. 1, which clearly confirms the presence of both crosslinked starch and pectin as discussed below.

The evidence of starch comes from a slightly broad band occurring around 3500 cm^{-1} which is typical of H-bonded (O–H stretch) starch and pectin. The bands appeared at 3751 cm^{-1}, 3805 cm^{-1}, 3906 cm^{-1}, all due to O–H stretching [28] are observed at slightly shifted wave numbers which confirm the blend nature of the material as reported elsewhere.

The spectra clearly show two prominent peaks for pectin, a strong asymmetrical stretching band at 1634 cm^{-1} and a weak symmetrical stretching band at 1388 cm^{-1} due to COO^- groups of pectin [29]. Moreover, a sharp band is observed at 2936 cm^{-1} which may be assigned to CH_2 groups of pectin [30]. As can be seen in the spectra, a sharp band occurs at 2363 cm^{-1} which could be assigned to the sodium salt of pectin [31] in addition to the two characteristic carbonyl absorption bands. The presence of epichlorohydrin is also indicated by a weak CH_2 wagging band for CH_2Cl group at 1153 cm^{-1} [31].

5.2 DSC analysis

The thermal analysis of a polymer is important not only in evaluating thermal parameters of the polymer

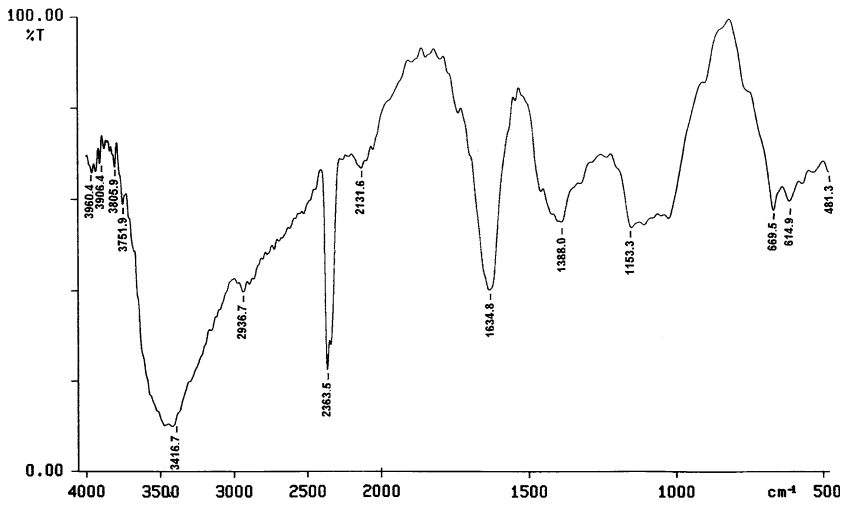

Fig. 1 The IR spectra of crosslinked starch–pectin blend

but in also exploring their molecular structure. In the present study, the DSC curves were constructed for both crosslinked starch and starch–pectin blend and the respective thermograms are shown in Fig. 2a and b, respectively.

The crosslinked starch displays a broad endotherm ranging from room temperature to 150°C as shown in the curve (2a). This frequently reported broad endotherm [32] has been assigned to the gelatinization of the material resulting from the disrupture of hydrogen bonds. The thermogram also depicts a minor exotherm at 200°C implying for crystallization of starch. The minor crystallites melt at 247°C and undergo thermal decomposition beyond 280°C, characterized by a broad exotherm as shown in the curve (2a). Some authors, however, have reported only a broad endothermic peak from 163°C to 230°C as in the dry powder of native starch [33]. Thus, the process at 280–350°C may be attributed to the degradation of starch components.

The thermogram of the starch–pectin blend shown in the curve (2b) depicts a broad endotherm from room temperature to 300°C containing a point of inflexion at 203°C, which could be assigned to the glass transition temperature of the blend. The thermogram also displays a sharp exotherm commencing at 420°C with a large enthalpy change of 4.96 kJ/g indicating the phenomenon of premelt crystallization. The observed large value of enthalpy suggests for the formation of highly crystalline domains induced by the possible arrangements of charged pectin chains. The later portion of the thermogram, i.e., beyond 420°C consists of melting endotherm and exotherm indicating of melting and subsequent decomposition of the constituent biopolymers.

5.3 SEM analysis

The Scanning Electron Micrographs of undegraded and degraded blend surfaces are shown in Fig. 3(a) and 3(b), respectively, which clearly distinguish their surface morphology. As shown in the micrograph (a), the blend presents a smooth surface with small hairy cracks developed probably during the preparation and processing of the blend.

On the other hand, the micrograph shown in photograph (b) clearly depicts a fractured and heterogeneous surface of the degraded blend containing well-defined cavities and cracks. This obviously provides an evidence for the enzymatic degradation of the blend.

6 Water sorption studies

6.1 Effect of chemical composition

In majority of the experiments, the dynamics of water sorption process has been investigated either by monitoring the change in physical dimension of the swelling hydrogel or by knowing the amount of water imbibed by hydrogel at various time periods. The swelling behaviour of a polymeric system has a great importance when it is applied in the biomedical field as its hydration degree significantly influences the surface properties and mobility, mechanical properties and the type of solute transport mechanism through the hydrogels [34].

In the present study, the influence of chemical composition of the blend on the swelling ratio (SR) has been investigated by varying amounts of starch, pectin

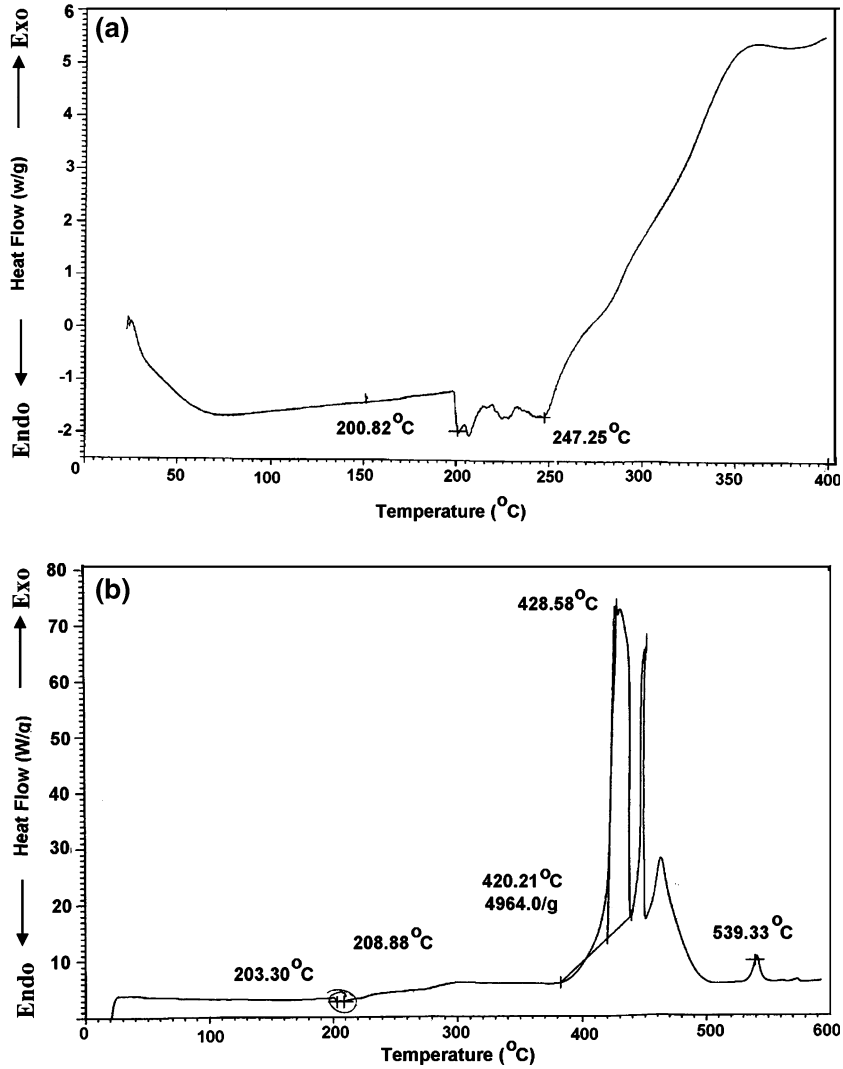

Fig. 2 The DSC thermograms of (**a**) crosslinked starch, (**b**) polymer blend

and epichlorohydrin in the feed mixture of blends. The results summarized in Table 1 may be explained as below.

Starch is a hydrophilic polymer and its increasing amount will obviously increase the hydrophilicity of the blend. Thus, an increase in swelling ratio is expected. However, the results obtained in Table 1 are quite opposite, and indicate a constant decrease in swelling ratio with increasing starch content in the studied range (3–6 g). The observed decrease in the

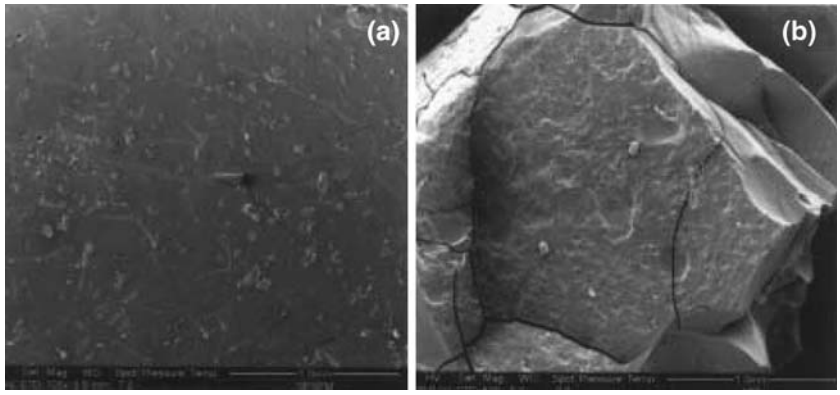

Fig. 3 The Scanning Electron Micrographs (SEMs) of (**a**) polymer blend before degradation, (**b**) polymer blend after degradation

Table 1 Data showing the variation of swelling ratio of the blends with varying experimental conditions

Starch (g)	Pectin (g)	Epichlorohydrin (mM)	pH	Temp. (°C)	Swelling ratio
3.0	0.5	0.15	6.8	25	1.89
4.0	0.5	0.15	6.8	25	1.79
5.0	0.5	0.15	6.8	25	1.69
6.0	0.5	0.15	6.8	25	1.57
3.0	0.5	0.15	6.8	25	1.89
3.0	1.0	0.15	6.8	25	1.68
3.0	1.5	0.15	6.8	25	1.59
3.0	2.0	0.15	6.8	25	1.48
3.0	0.5	0.062	6.8	25	1.52
3.0	0.5	0.15	6.8	25	1.89
3.0	0.5	0.31	6.8	25	1.72
3.0	0.5	0.46	6.8	25	1.64
3.0	0.5	0.15	2.10	25	1.78
3.0	0.5	0.15	4.08	25	1.83
3.0	0.5	0.15	6.80	25	1.89
3.0	0.5	0.15	9.10	25	1.80
3.0	0.5	0.15	11.08	25	1.72
3.0	0.5	0.15	6.8	15	1.34
3.0	0.5	0.15	6.8	25	1.89
3.0	0.5	0.15	6.8	45	1.40

swelling ratio may be attributed to the fact that starch, being hydrophilic, will produce stronger interactions with other macromolecular chains, when present in increasing amounts in the blend. This will, consequently, result in an increased compactness and reduced mesh sizes of the blend. In this way, a smaller number of penetrant water molecules will be allowed to enter and swell the blend, thus, bringing about a fall in the swelling ratio.

Pectin is an anionic biopolymeric component of the blend, with the ultimate property to bind with other polymers, either naturally occurring or synthetic, and create useful new properties. In the present work, an attempt has been made to combine pectin with starch so that the prepared polymeric blend may retain the properties of their parent polymers. The effect of variation of pectin on the swelling ratio of the blend has been studied by varying its amount in the range 0.5–2.0 g in the feed composition. The results are displayed in Table 1, which clearly reveal that as the amount of pectin increases in the studied range in the blend, swelling ratio is significantly suppressed.

The results may be explained by the fact that with increasing content of pectin in the blend, the H-bonding forces become operative between the pectin chains and starch molecules. This clearly enhances the network density of the blend, which results in a lowering of swelling ratio. Another reason may be that with increasing amount of pectin in the blend, the polymer volume fraction increases due to which invading water molecules will have to travel a longer path within the blend. This will obviously, result in a fall in the swelling ratio.

The swelling characteristics of a hydrogel could be suitably modified by employing varying concentration of crosslinking agent [35] and experimental protocol. It has been frequently observed [36] that increasing the percentage of crosslinker in the hydrogel, reduces the degree of swelling, while affecting the swelling kinetics in a complex way. In the present study, the concentration of crosslinker (epichlorohydrin), has been varied in the feed mixture in the range 0.062–0.460 mM. The results are depicted in Table 1, which adequately imply that swelling ratio of the blend increases with increasing concentration of epichlorohydrin from 0.062–0.15 mM, while beyond 0.15 mM, a significant fall in swelling ratio is noticed. The observed results may be well understood by the explanation mentioned below.

Epichlorohydrin, a low molecular weight crosslinking agent of starch reacts with hydroxyls of starch at its two terminals and crosslinks them. Thus, a crosslinked starch network could be imagined as an aggregated starch molecule, that generates wide pore sizes in the whole network and, therefore, possess abnormal capacity of accommodating water into the network (Fig. 4). This will obviously result in a greater water sorption by the polymer blend.

However, beyond a certain concentration of the crosslinker in the network, the blend network contains greater number of crosslinked points, which results in a reduction in mesh sizes of the voids. This obviously leads to a slow diffusion of water molecules into the network and restricted relaxation of polymeric network chains, slowing down the swelling ratio and swelling rate.

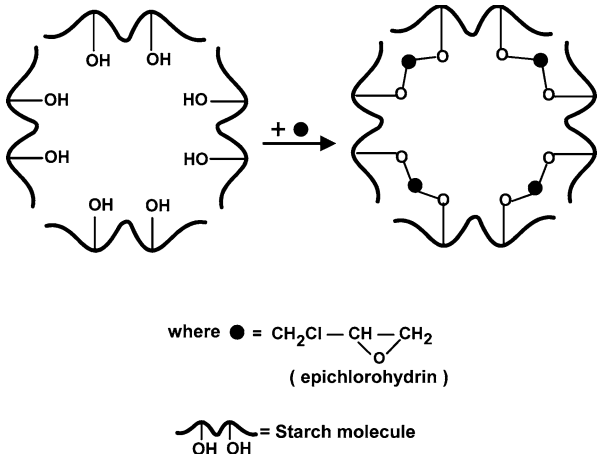

Fig. 4 A hypothetical model depicting the formation of larger starch network due to the crosslinking of starch by epichlorohydrin

Another reason, as noticed by some other workers [37] may be that an increased crosslink density of the blend results in an increase in glass transition temperature (Tg) of the polymer, which because of glassy nature of the matrix does not permit loosening of the macromolecular chains, and therefore, results in a lower water sorption.

6.2 Effect of pH

The role of pH in regulating swelling behaviour of hydrogels is of great practical significance, particularly when hydrogel incorporates components of polyelectrolyte nature [38]. The change in pH of the swelling medium often results in a fluctuation in free volumes accessible to penetrant water molecules, which, in turn affects the swelling characteristic of hydrogel.

In the present investigation, the polymer blend contains a non-ionic component (starch) and an anionic component (pectin). To modulate the swelling behaviour with varying pH, the blends were swollen in different pH media till equilibrium (ranging from pH 2.10 to 11.08). The results are shown in Table 1, which reflects that swelling ratio constantly increases up to pH 6.8 while beyond 6.8, a substantial fall in the swelling ratio is observed. The obtained results may be explained as below.

As the polymeric blend is composed of a non-ionic component (starch) and anionic component (pectin), starch molecules almost remain unaffected, while pectin is greatly affected by varying pH of the swelling medium. When pH of the medium is low, the carboxylic groups of pectin are almost in undissociated state and the ionization of carboxylic groups on pectin is suppressed. Thus, the pectin molecules now no longer repel each other over their entire chains and, therefore, can associate tightly via H-bonding forces, resulting in incorporation of less water into interchain entanglements. Hence, swelling ratio is suppressed in the lower pH.

However, when pH of the swelling medium rises up to 6.8, the carboxylic groups of pectin present in the blend gets ionized, and generate carboxylate anions (COO^-) resulting in a cleavage of H-bonds. This facilitates chain relaxation within the blend that ultimately leads to a faster diffusion of water molecules into the network, causing a greater swelling. Another plausible explanation rests upon the possibility that because of mutual repulsion operating between the carboxylate anion groups along the pectin molecule, the network chains of the blend get relaxed, thus widening the free volumes in the blend. This ultimately results in a larger swelling ratio. Similar type of results have been frequently reported in the literature [39].

When the pH exceeds 6.8 and enters alkaline range, the number of carboxylate anions reach at their maximum, and produce greater repulsion within the blend matrix. This obviously results in a much greater relaxation of macromolecular chains of the blend, which causes expulsion of water molecules from within the blend into the swelling medium. This appears justified also as because of reduction in COOH groups of pectin molecules, the water molecules bound to pectin via H-bonds (i.e., bound water) become free and are forced out due to frequent relaxation of network chains. A substantial reduction in swelling ratio is henceforth, observed. Similar type of findings have been observed elsewhere [40].

6.3 Effect of temperature

The temperature of the external medium is another important parameter that largely influences the swelling behaviour of hydrogels. In the present article, the temperature of the swelling medium was varied in the range 15–45°C, and its effect on the swelling ratio of the blend was analysed. The results tabulated in Table 1 precisely, gives information that swelling ratio increases up to 25°C, while beyond it, an appreciable decrease in swelling ratio is observed. The above findings are quite expected and may be interpreted on the basis of explanation given below.

With the rising temperature of the swelling bath in the initial range, both the diffusion of water molecules and segmental mobility of starch and pectin chains increase, which, consequently widen the free volume available in the blend network which may be easily accessible to penetrant water molecules. This, rightly explains the observed increased swelling in the blend. However, in the higher temperature range, i.e., beyond 25°C, swelling ratio decreases which could be attributed to the weakening of binding forces between the water molecules and the polymeric chains of the blend at higher temperature. As a result, the water molecules may detach from the macromolecular chains, showing an appreciable decrease in the swelling ratio. Similar type of results have also been reported elsewhere [41].

7 Degradation studies

7.1 Mechanism of degradation

In vitro degradation of starch induced by α-amylase may be considered to occur through three pathways; either by the erosion of the blend surface, or by the diffusion of enzyme molecules into the blend network or by a

combination of the two. In the surface erosion mechanism, the α-amylase molecules invade the blend surface and cleave α (1–4) linked glucose units of starch whereas in the second pathway the enzyme molecules may diffuse into the blend network and cause degradation. A third possibility is that both the surface erosion and diffusion mechanisms are operative simultaneously.

In the present study, the degradation results (to be discussed later on in detail) show two significant features, first the inclusion of pectin into the starch has been found to enhance the degradation of native starch and second a biphasic curve is obtained when the percent degradation is plotted against time. The biphasic portion of the curve also varies in a regular fashion with the changing composition of the blend. Thus, in order to explain the above two general results, the following scheme of degradation may be presented.

The prepared blends of starch and pectin could be imagined like an intimate network of crosslinked starch and pectin entangled into one another and held with each other via weak electrostatic attractive forces thus forming a large mesh sized networks. Since the hydrodynamic radius of α-amylase (M_r = 56,000) has been estimated to be roughly 3 nm [42], the α-amylase can diffuse only in pores larger than 6 nm in diameter. Moreover, if the possibility of transient adsorption of enzyme on the walls of the pores are considered, the minimal pore diameter for diffusion has been estimated to be 24 nm as reported in the case of α-amylase and amylase–amylopectin gels [43]. Thus, it is most likely that when the starch–pectin gel contacts an aqueous solution of α-amylase, the enzyme molecules diffuse into the gel and cause degradation of starch. Because of anionic nature of pectin chains, they will repel each other generating large voids in the blend through which a facilitated diffusion of α-amylase will cause an enhanced degradation.

Upon an appreciable degradation, the blend disintegrates into smaller fragments and during this time period the degradation nearly remains constant producing a plateau in the degradation curve. However, after the blend has been substantially fragmented, the degradation follows a surface-erosion mechanism, which further accelerates extent of degradation. This obviously explains the biphasic nature of the degradation versus time curve. Similar type of biphasic curves have also been reported by other workers [44].

7.2 Effect of starch

The influence of starch content, in the range 3.0–6.0 g has been investigated on the percent degradation, which can be visualized in Fig. 5.

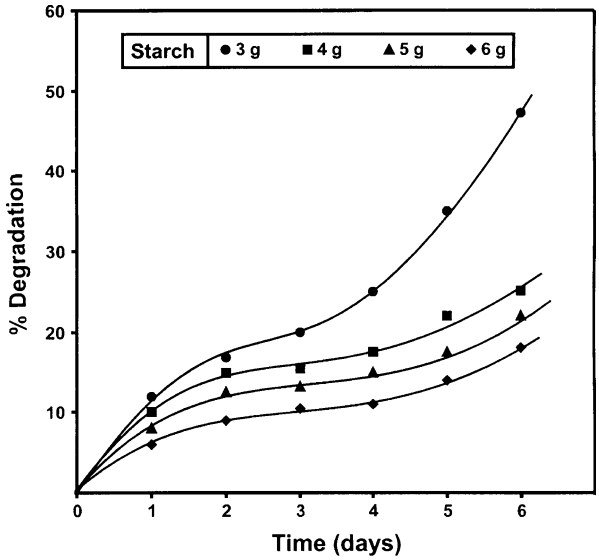

Fig. 5 Effect of varying concentrations of starch on the percent degradation of the blend at definite composition of [pectin] = 0.5 g, [epichlorohydrin] = 0.15 mM, [α-amylase] = 6.5 IU/mL, pH = 6.8, Temp. = 25 ± 0.2°C

The results reveal that the percent degradation constantly decreases with increasing starch content in the blend. This could be explained by the fact that as the amount of starch increases in the blend, the blend acquires sufficient molecular compactness, and the volume fraction of the polymer being increased resulting in a restricted diffusion of α-amylase molecules into the network. This obviously results in a fall in the percent degradation. A close examination of degradation curves clearly indicates that with increasing starch content in the blend, the plateau portion constantly increases and becomes more and more prominent. This observed finding may be explained by the reason that a blend with higher starch content requires greater time period for fragmentation which is further followed by surface-erosion degradation. This obviously explains a larger plateau size of degradation curve at greater starch containing blends.

We have also calculated initial degradation rates (% degradation per h) and the data presented in Table 2 clearly reveal that the degradation rate decreases with increasing starch content. Hence, the kinetic data obtained completely supports the qualitative degradation results.

7.3 Effect of pectin

Variation of pectin in the feed mixture of the blend in the range 0.5–2.0 g brings about a significant increase in the percent degradation as shown in Fig. 6. The

Table 2 Effect of composition of the blend on the degradation kinetics

Starch (g)	Pectin (g)	Epichlorohydrin (mM)	Initial degradation rate (percent per hour)
3.0	0.5	0.15	0.45
4.0	0.5	0.15	0.37
5.0	0.5	0.15	0.33
6.0	0.5	0.15	0.29
3.0	0.5	0.15	0.45
3.0	1.0	0.15	0.83
3.0	1.5	0.15	1.17
3.0	2.0	0.15	1.25
3.0	0.5	0.062	0.25
3.0	0.5	0.15	0.45
3.0	0.5	0.31	0.83
3.0	0.5	0.46	1.04

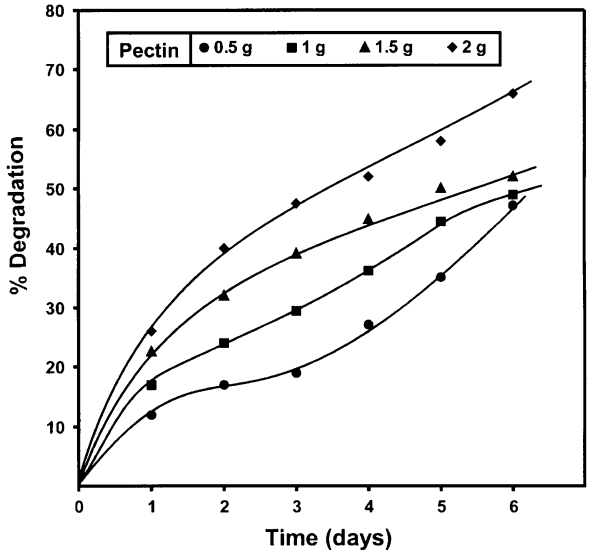

Fig. 6 Influence of varying concentrations of pectin on the percent degradation of blend at definite composition of [starch] = 3.0 g, [epichlorohydrin] = 0.15 mM, [α-amylase] = 6.5 IU/mL, pH = 6.8, Temp. = 25 ± 0.2°C.

observed trend in percent degradation is just opposite to that obtained in swelling studies.

The reason for the observed increase in degradation profiles can be clearly understood by the fact that in case of pectin variation, the degradation is regulated not only by the swelling process, but also by other factors.

It is quite apparent that the blend components, viz. starch and pectin are electrically different from each other, whereas the former is neutral in nature, the later molecules possess a net negative charge (polyelectrolyte). When the concentration of pectin is increased in the feed mixture of the blend, the anionic chains of pectin repel each other, thus widening the mesh size of the blend network. This consequently facilitates diffusion of α-amylase molecules through the blend and, therefore, brings about an increase in the percent degradation. This obviously explains the observed increase in percent degradation with increasing pectin concentration.

The degradation curves shown in Fig. 6 also indicate that with increase in pectin concentration, the plateau portion of the biphasic curve tends to decrease and it finally disappears at highest pectin concentration. The observed results may be explained by the fact that at higher pectin content in the blend because of existing repulsion between the pectin chains, the pores become quite wide and degradation is enhanced. This consequently results in a rapid fragmentation of the blend and, therefore, the rate of degradation constantly increases showing no plateau in the profile.

The degradation results are further supported by the kinetic parameter like initial degradation rate (Table 2), which also suggest for an enhanced degradation with increasing amount of pectin in the prepared blends.

7.4 Effect of crosslinker

The influence of crosslinker on the degradation profile of starch has been investigated by adding epichlorohydrin to the feed composition of the blend in the concentration range 0.062–0.46 mM. The results are presented in Fig. 7 which clearly reveal that whereas in the initial period of degradation process, i.e., at least up to 24 h the rate of degradation constantly increases with increasing concentration of crosslinker, a significant fall is noticed in limiting percent degradation after 6 days. The observed degradation results may be explained as below.

In the initial stage of degradation process, the α-amylase molecules diffuse into the blend network and cause degradation of crosslinked starch molecules. Since with increasing concentration of epichlorohydrin, starch molecules will form a compact network and, therefore, greater number of starch molecules will come across with diffusing α-amylase molecules and as a consequence a fast degradation is noticed. However, after an adequate number of starch molecules are degraded, the blend network begins to disintegrate, thus yielding a plateau portion in the degradation profile. For a blend containing greater crosslinker, disintegration may require greater time which will result in a layer plateau. This clearly explains why plateau portion increases with increasing crosslinker concentration.

After the blends have disintegrated to smaller fragments, surface erosion induced mechanism

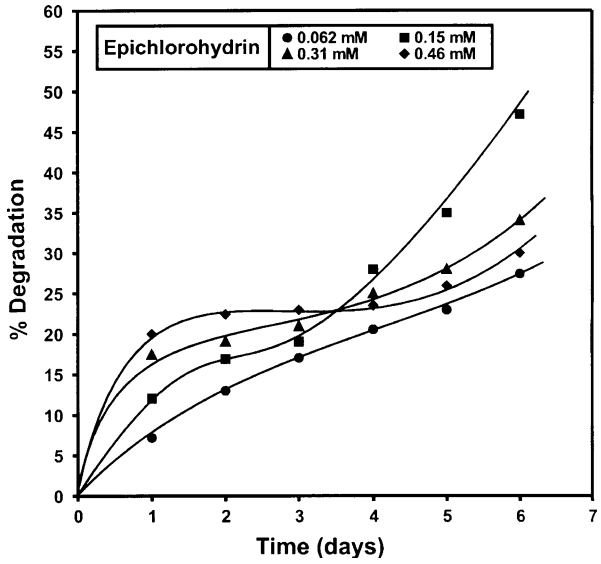

Fig. 7 Effect of varying concentrations of crosslinker (epichlorohydrin) on the percent degradation of the blend at definite composition of [starch] = 3.0 g, [pectin] = 0.5 g, [α-amylase] = 6.5 IU/mL, pH = 6.8, Temp. = 25 ± 0.2°C

becomes operative that leads to an enhanced degradation of the blends. Now, as can be seen from the degradation profiles that limiting percent degradation decreases with increasing crosslinker content in the blend (except for 0.062 mM). This may be explained by the fact that for the blend with low crosslinker content, starch molecules are crosslinked to a lower extent and, this, will result in a greater mobility of starch molecules at the blend surface. This will obviously result in a greater percent degradation. Similarly, for a greater crosslinker content in a blend, restricted mobility of crosslinked starch molecules result in a suppressed percent degradation. It is also interesting to note that limiting percent degradation is swelling dependent also, as a highly swollen blend offers a greater chain relaxation at the surface which, in turn, results in a higher degradation.

Since the swelling results indicate that with 0.062 mM epichlorohydrin a minimum water sorption is obtained, obviously this specific composition will show a minimum percent degradation. The observed results are further supported by the degradation data summarized in Table 2. It is clear from the data that with increasing concentration of epichlorohydrin, the initial degradation constantly increases.

7.5 Effect of pH

pH plays a vital role in regulation of enzymatic processes. In the present study also, the effect of pH variation on the percent degradation of the blend has been examined by changing the pH of the α-amylase solutions in the range 2.10–11.08. The reason for selecting a wide range of pH lies in the fact that enzyme activity is very much susceptible to pH of the solution and, therefore, a broad range of pH is essential. The results obtained are presented in Fig. 8 which notably indicates that optimum degradation is noticed at pH 6.8, while on both the sides of optimum pH, the extent of degradation constantly falls.

The maximum degradation of starch at pH 6.8 could be explained by the fact that α-amylase has optimum activity at this pH, aggreable with many investigations [44]. Hence, as α-amylase molecules experience maximum activity at above-mentioned pH, this will facilitate the invasion of α-amylase into the blend matrix, enhancing the extent of degradation. The lower activity of enzyme on both the sides of optimum pH, may be attributed to the change in the conformation of the α-amylase molecules, suppressing its activity. The suppressed activity of α-amylase, leads to slower rate of diffusion of enzyme molecules, which consequently lowers percent degradation.

7.6 Effect of temperature

The temperature of α-amylase solution remarkably affects enzyme activity. For investigating the effect of temperature on percent degradation, experiments were performed in the range 15–45°C. The results shown in

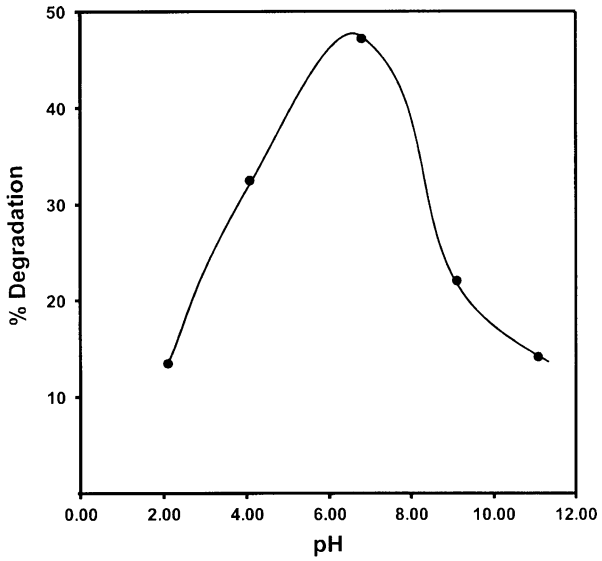

Fig. 8 Effect of pH of the enzyme solution on the percent degradation of the blend at definite composition of [starch] = 3.0 g, [pectin] = 0.5 g, [epichlorohydrin] = 0.15 mM, [α-amylase] = 6.5 IU/mL, Temp. = 25 ± 0.2°C

Fig. 9 reveal a marked increase in percent degradation with increasing temperature of the degradation medium.

The order of degradation rates (R) follow the sequence as under,

$$R_{15^\circ C} < R_{25^\circ C} < R_{45^\circ C}.$$

The observed results can be explained by the fact as the temperature increases, the diffusion of enzyme molecules becomes faster and consequently the percent degradation as well as the rate of degradation increase.

A close examination of the degradation profiles reveal that with increasing temperature, the plateau portion also decreases and eventually disappears at the highest temperature (45°C) of the studied range. The observed results may be attributed to the fact that with increasing temperature, the rate of degradation increases which, in turn, results in a faster fragmentation of the blend. This will obviously result in disappearance of plateau portion due to a faster fragmentation of the blend, which is rapidly followed by surface erosion induced degradation.

It is to be noted here that the trends observed in degradation results are opposite to that of swelling pattern. The analysis of the swelling process showed that the swelling ratio increases in the following order,

$$(SR)^{\circ}_{15}C < (SR)^{\circ}_{45}C < (SR)^{\circ}_{25}C$$

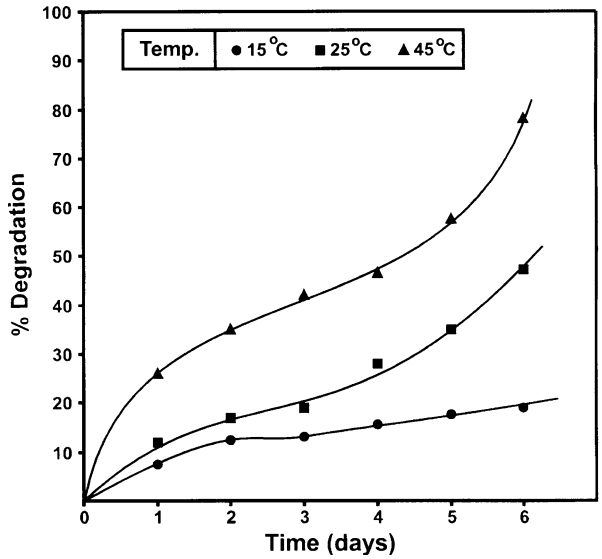

Fig. 9 Effect of temperature on the percent degradation of the blend at definite composition of [starch] = 3.0 g, [pectin] = 0.5 g, [epichlorohydrin] = 0.15 mM, [α-amylase] = 6.5 IU/mL, pH = 6.8

It is quite unexpected that maximum degradation occurs at the highest temperature of the studied range, i.e., 45°C, while the swelling ratio at this temperature appears in the middle of sequence, as is reflected by the above equation. On the other hand, a minimum degradation is shown at 15°C, which is consistent with the swelling results indicating a minimum swelling ratio at 15°C.

The observed anomaly could be explained by the fact that with increasing temperature, the mobility of α-amylase molecules increases, which, in turn, enhances the activity of enzyme. Thus, at 45°C, when swelling is less, activity of enzyme is higher, and in turn, the percent degradation will be maximum. On the contrary, at 25°C, the blend shows an optimum swelling, but due to a decreased activity of enzyme, the percent degradation is reduced.

7.7 Effect of enzyme concentration

Enzymes are mostly involved in the chemical mode of polymer degradation. α-Amylase breaks down starch into sugars, by recognizing starch molecules and binding to them, thus, initiating their chemical decomposition.

In the present study, the effect of concentration of α-amylase on the percent degradation has been elucidated by varying concentration of the enzyme solution in the range 2.6–6.5 IU/mL.

It is an important observation from the degradation profiles visible in Fig. 10 that the percent degradation of starch is increased, when higher concentration of α-amylase (6.5 IU/mL) is used. The observed results are quite usual and may be explained by the fact that at higher concentration of enzyme solution, a large number of α-amylase molecules approach the blend surface and bind less tightly to the blend matrix. Such loosely bound enzyme molecules will not undergo greater structural deformation, thus resulting in an enhanced activity, which ultimately leads to an increase in the percent degradation.

Another reason may be that larger number of enzyme molecules invading the blend surface and/or diffusing across the blend matrix will interact with greater number of starch molecules, thus increasing their degradation. On the contrary, at lower concentration of enzyme solution (2.6 IU/mL), the enzymes molecules may be approaching the blend surface in lesser number, so, they may be strongly bound to the blend matrix. The strongly bound enzymes may not undergo greater deformation in structural conformation and, therefore, show lower activity, thus resulting in a suppressed percent degradation.

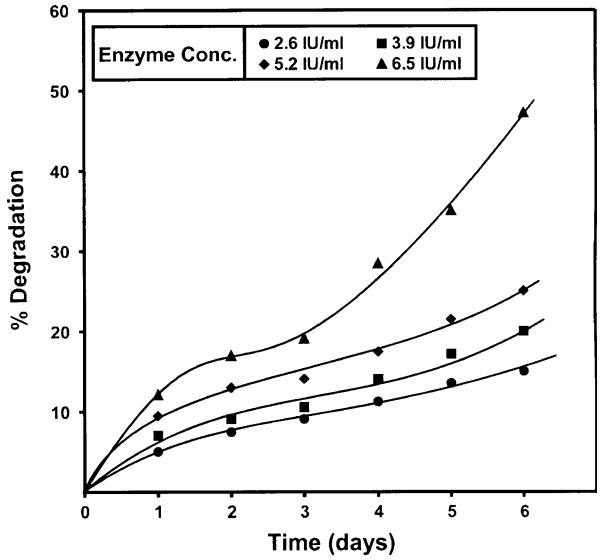

Fig. 10 Influence of concentration of enzyme solution on the percent degradation of the blend at definite composition of [starch] = 3.0 g, [pectin] = 0.5 g, [epichlorohydrin] = 0.15 mM, pH = 6.8, Temp. = 25 ± 0.2°C

8 Conclusions

Inclusion of pectin into crosslinked starch results in a blend having fair water intake capacity and depicting enhanced α-amylase induced degradation. The IR spectral study presents clear evidences of blend formation depicting characteristic absorption bands of both starch and pectin. The thermal characterization of the blend by differential scanning calorimetry (DSC) indicates the formation of crystalline domains within the blend induced by the possible regular arrangement of negatively charged pectin macromolecules. The thermogram of the prepared blend also presents a broad endotherm up to 300°C containing a inflexion point at 203°C which may be assigned to glass transition temperature of the blend. Similarly the phenomenon of premelt crystallization at 420°C is also shown by the blend. The SEM images of the undegraded and degraded blends provide a strong evidence of degradation resulting in a heterogeneous and fractured surface.

The swelling investigations of the blend clearly reveal that the chemical architecture of the blend and swelling conditions greatly influence the degree of water sorption. It is found that on increasing the concentration of starch (3.0–6.0 g) and pectin (0.5–2.0 g), the swelling ratio decreases whereas in the case of epichlorohydrin, the swelling ratio increases from 0.062 to 0.15 mM and it decreases afterwards. An optimum swelling is noticed at pH 6.8 while it decreases on both the sides. A variation of temperature from 15°C to 25°C results in an increased water sorption while at higher temperature (45°C) the swelling ratio falls.

The enzymatic degradation of blend presents quite interesting results. The biphasic shape of degradation profiles reveals that in the initial course whereas degradation is diffusion induced while after some period surface erosion mechanism becomes also operative which accelerates extent of degradation.

The degradation is significantly influenced by composition of the blend, whereas with increasing starch content in the blend, percent degradation constantly decreases, an increase is observed with increasing pectin concentration. In the case of crosslinker, the initial rate of degradation increases with increasing crosslinker concentration, just opposite trend is obtained for limiting percent degradation.

In the case of pH variation, an optimum degradation is obtained at pH 6.8. The degradation also increases with increasing temperature and concentration of enzyme solution. The degradation data, viz., initial degradation rate fully supports our qualitative experimental findings.

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