Iranian Polymer Journal
20 (2), 2011, 147-159

Available online at: http://journal.ippi.ac.ir

Synthesis and Characterization of pH-Sensitive Pectin/Acrylic Acid Hydrogels for Verapamil Release Study

Nazar M Ranjha, Jahanzeb Mudassir*, and Zuhair Zubair Sheikh

Faculty of Pharmacy, Bahauddin Zakariya University, Multan-60800, Pakistan

Received 17 September 2010; accepted 22 January 2011

A B S T R A C T

The objective of the present work was to synthesize the novel hybrid based polymeric networks of pectin and acrylic acid (AA) showing pH-sensitive swelling performance in relation to acrylic acid component of the gel. A series of hydrogels were prepared using pectin and acrylic acid (AA) in the presence of *N,N*-methylene bisacrylamide (MBAAm) as cross-linker and benzoyl peroxide as initiator. FTIR confirms the formation of network. The prepared hydrogels were evaluated for swelling, sol-gel fraction and porosity. Furthermore, the values of equilibrium water content (EWC), diffusion coefficient (D) and volume fraction of the polymer within hydrogels (Φ_2) were calculated. Hydrogels were characterized for surface morphology using scanning electron microscopy (SEM). Swelling data were fitted into Peppas model for evaluating the swelling mechanism. Hydrogels showed pH and monomeric composition-dependent swelling behaviour. Selected samples were loaded with verapamil as a model drug. Drug release was performed in USP phosphate buffers of pH 1.2 and 7.5. Drug release data were fitted into various kinetic models like zero order, first order, Higuchi and Peppas models for investigating the optimum composition suitable for controlled drug delivery. A significant difference in drug release kinetics was observed by varying the composition of pectin/AA and degree of cross-linking.

Key Words:

hydrogels;
pectin;
acrylic acid;
verapamil;
drug release.

INTRODUCTION

Verapamil hydrochloride is a calcium channel antagonist and used in the treatment of hypertension, arrhythmia and angina pectoris [1]. Verapamil is usually formulated in conventional dosage forms. However, limited work has been done on the release of verapamil from advanced polymeric carriers. This drug shows higher pharmacokinetics variability mainly due to its extensive first pass effect. Therefore, controlled release formulations of verapamil are preferred. Controlled release of verapamil due to novel hydrogel improves patient compliance by reducing the dosing frequency because verapamil is used for chronic disease. Moreover, the side effects and therapeutic effects are also improved. For this purpose Ranjha et al. [2] prepared hybrid pH-sensitive chitosan-*co*-acrylic acid hydrogels. They used verapamil as model drug. They reported

(*) To whom correspondence to be addressed.
E-mail: jahanzebmudassir@hotmail.com

that verapamil release from hydrogels was mainly based on non-Fickian diffusion. In another work Kostova et al. [3] prepared super-macroporous polymer hydrogels using cryoptropic gelation also named as cryogels. They used verapamil as model drug and reported that these gels showed sustained verapamil release profile. In another study Kulkarni et al. [4] used anti-hypertensive drug diltiazem for controlled release application by their prepared interpenetrating polymer network (IPN) microspheres of gellan gum and egg albumin. They reported that IPN prepared from higher concentration of cross-linker released the drug with slower rate. Hydrogels are gaining importance in controlled drug delivery and are used for a variety of drugs. These systems remain intact in dry state while swell due to the penetration of biological fluids. Entrapped drug within the polymeric networks dissolves and diffuses through the swollen network into surrounding media [5]. Ranjha et al. [6] showed that swelling characteristics of cross-linked hydrogels could be modified to desired extent by varying the monomeric compositions and degree of cross-linking.

Pectin is heterogeneous, hydrophilic polysaccharide containing linear chains of poly(α-1-4 galacturonic acid) residues, with varying degrees of methylation of carboxylic acid residues [7]. Polysaccharides are generally, non-toxic, biocompatible and biodegradable. Therefore, pectin is widely used as potential carrier for colon specific drug delivery [8,9]. At neutral pH, pectin aggregates tend to dissociate and expand and are digested by large number of colonic microflora. To overcome the problem of high dissolution of pectin in the upper gastrointestinal tract, pectin has been combined with other polymers. In addition, pectin-based, colon-specific drug delivery vehicles have been developed using a chemically modified pectin polymer [10,11]. Mishra et al. [12] successfully prepared pectin/poly(vinyl pyrrolidone)-based hydrogel membrane. It was reported that these novel hydrogel membranes were pH sensitive to an extant that they could be used as potential carrier for controlled drug delivery.

The objective of present study is to prepare hybrid polymeric network of pectin/acrylic acid (AA). This novel hydrogel is expected to modify drug release, improve pH-sensitivity due to AA and retain colon specific characteristics due to pectin. Hydrogels were characterized by dynamic and equilibrium swelling. In this respect the equilibrium water content (EWC), diffusion coefficient (D) and volume fraction of polymer within the hydrogels (Φ_2) were determined. Anti-hypertensive drug verapamil was selected as a model drug to explore in-vitro drug release mechanism in pectin/AA hydrogel systems. Drug release was studied as a function of pH, amounts of MBAAm as a cross-linker, pectin and AA in the network. Finally, release data were analyzed using various kinetics models. The experimental results indicated that dominant mechanism for drug release was based on non-Fickian model.

EXPERIMENTAL

Materials

Acrylic acid (AA) and pectin (M_w=30,000-100,000) were purchased from Fluka, Switzerland. *N,N*, Methylene bisacrylamide (MBAAm) as the cross-linking agent and benzoyl peroxide as the initiator were purchased from Merck, Germany. All the solvents used were of analytical grades.

Synthesis of Pectin/Acrylic Acid Hybrid Polymeric Network

Structures of polymer, monomers and cross-linker used in the preparations of hydrogels are given in the Table 1. In the present work three series of samples were synthesized and their compositions are given in Table 2. Procedure for the preparation of hydrogels

Table 1. Monomers and cross-linkers for preparation of hydrogels.

Materials	Formula	Abbreviations
Acrylic acid	CH_2CHOOH	AA
Benzoyl peroxide	$[C_6H_5C(O)]_2O_2$	BPO
N,N-Methylenebisacrylamide	$[H_2C=CCH_3COOCH_2]_2$	MBAAm

Table 2. Feed composition for the preparation of hybrid polymeric networks of pectin/AA hydrogels.

Sample	Pectin content (g/100 g solution)	AA content (g/100 g solution)	Pectin/AA (wt%)	MBAA content (g/100 g solution)
S_1	0.15	37.50	0.39/99.60	0.30
S_2	0.30	37.50	0.79/99.20	0.30
S_3	0.60	37.50	1.57/98.40	0.30
S_4	0.60	18.75	3.10/96.89	0.30
S_5	0.60	25.00	2.34/97.65	0.30
S_6	0.60	31.25	1.88/98.11	0.30
S_7	0.60	31.25	1.88/98.11	0.05
S_8	0.60	31.25	1.88/98.11	0.10
S_9	0.60	31.25	1.88/98.11	0.15

was used after modification of previously reported method [6]. A weighed amount of pectin was dissolved in water in 50 mL-round bottom flask at room temperature under constant stirring. Varying amount of MBAAm and benzyl-peroxide were dissolved in AA, as well. After mixing thoroughly, final solution was introduced into several glass tubes (Pyrex). Each tube was bubbled with nitrogen for 10-20 min and then tightly fitted with lid. These tubes were placed in water bath. Temperature was gradually increased in all samples to avoid auto-acceleration and bubble formation. Reaction temperatures of solution polymerization were set at 45°C for 1 h, 50°C for 2 h, 55°C for 3 h, 60°C for 4 h, and 65°C for 24 h. After this period, tubes were cooled to room temperature and cylinders were removed from the tubes. In all trials, cylinders were cut into small disks (8 mm in length). These disks were washed with 50 %v/v ethanol/water solution for complete removal of catalyst and unreacted monomers and to accomplish this procedure the solvent was changed daily. The washing of the gels was completed until the pH values of the washing solution and freshly prepared solution were the same. After the washing stage the disks were dried at first at room temperature and then in vacuum oven at 40-45°C, for one week.

Swelling Studies

Swelling experiments were carried out in 100 mL solution at 37°C. Dry discs were weighed and immersed in USP phosphate buffer solutions of varying pH, i.e., 1.2, 5.5, 6.5, and 7.5. Concentration of the buffering agent was 0.2 M. Samples were taken out at regular time intervals and weighed after removing the excess surface water by blotting using laboratory tissue. After the completion of dynamic swelling for 8 h, samples were remained in the same solutions and used for equilibrium swelling. Swelling was considered at equilibrium after reaching constant weight. The swelling coefficient (q) was calculated as given by Peppas et al. [5]:

$$q = \frac{W_s}{W_d} \tag{1}$$

where, W_s is the weight of swollen gel and W_d is the weight of dry gel.

Percentage equilibrium water content of the swollen hydrogels was calculated using the following equation:

$$EWC(\%) = \frac{m_{eq} - m_0}{m_{eq}} \times 100 \tag{2}$$

where, m_{eq} is the mass of the gel at equilibrium while m_0 is the mass of dry gel.

The volume fraction of the hydrogel was calculated using the following equation:

$$\phi_2 = \left(\frac{D_0}{D} \right)^3 \tag{3}$$

where, D_0 and D are the diameters of the dry and swollen gels, respectively.

Verapamil Loading and Release

Hydrogels were loaded with model drug using

Table 3. Porosity and amount of drug loaded in selected samples.

Sample code	Porosity (%)	Verapamil loaded gels (g/g of dry gel)	
		By swelling	By extraction
S_1	9.59	-	-
S_2	17.32	-	-
S_3	36.18	-	-
S_4	20.05	0.043	0.040
S_5	31.60	0.044	0.041
S_6	64.00	0.048	0.043
S_7	85.34	0.053	0.050
S_8	42.54	0.048	0.046
S_9	34.66	0.047	0.043

absorption method after removing unreacted monomer by extensive washing and drying. Various selected samples for drug loading are given in Table 3. A solution of 1 %w/v verapamil was used for drug loading. Selection of the solvent was based on swelling capacity of the polymer in the solvent. Therefore, 50/50 %v/v ethanol/water mixtures were used as solvent. Samples were loaded with model drug by immersing each small disk in the drug solution. In this solvent, polymer showed maximum swelling, without breaking. These disks remained in drug solution until they attained equilibrium swelling. Then, disks were removed from the solution and dried at room temperature and then placed in an oven of 40-45°C until constant weights were reached. For determination of the percentage of loaded drug in hydrogels, a weighed quantity of polymer was extracted repeatedly with the same solvent used for drug loading until there was no drug in the extracting solution. Each drug containing solution was processed separately. Finally, drug concentration was determined spectrophotometrically at λ_{max} = 271 nm. Drug contents were calculated from the weight ratio of the drug and copolymer used [6].

Verapamil release was studied in 0.2 M USP phosphate buffer solutions of pH 1.2 and pH 7.5. Therefore, weighed polymer disks were immersed in 500 mL dissolution medium and stirred at the rate of 100 rpm. Dissolution media were maintained at 37°C. Amount of verapamil release was observed at λ_{max} = 271 nm with readings recorded to 12 h.

Kinetics of Drug Release

In order to characterize the drug release mechanism, the obtained release data were subjected to different drug release models given as follows:

Zero-order kinetics [13]:

$$M_t = M_0 + K_0 t \quad (4)$$

where, M_t represents the fraction of drug release in time t and K_0 is the apparent rate constant of zero-order release constant.

First-order kinetics [14]:

$$\ln M_t = \ln M_0 + K_1 t \quad (5)$$

where, K_1 is the first-order release constant.

Higuchi model [15]:

$$M_t = K_2 t^{1/2} \quad (6)$$

where, K_2 is the Higuchi constant.

Peppas model [16]:

$$\frac{M_t}{M_\infty} = k_3 t^n \quad (7)$$

where, K_3 is a constant incorporating the structural and geometric characteristics of the gels and n is the release exponent. When n = 0.45 order of release is Fickian and n = 0.89 corresponds to case II transport, while $0.45 < n > 0.89$ shows the diffusion mechanism is non-Fickian. No kinetic data or n values were calculated when swelling and drug release values were not significant.

Diffusion Coefficient

Diffusion coefficient for selected samples of swollen hydrogels was determined using deswelling phenomena. Swollen gels were dried gradually at room temperature and weighed after 15 min until a constant weight was achieved. Diffusion coefficients of the hydrogels were calculated using eqn (8) [17]:

$$D = \left(\frac{\delta m_t}{\delta m_\infty}\right)^2 \left(\frac{l^2}{16t}\right) \quad (8)$$

where, δm_t is the weight loss at time t, δm_∞ is the weight loss at infinity, l is the thickness of the dried hydrogels, and t is the time of diffusion of water from the hydrogels during drying.

Sol-gel Fraction

Hydrogel were cut into pieces of 3-4 mm in length. They were first dried in vacuum oven at 45°C until constant weight was achieved and then subjected to soxhelt extraction with de-ionized water as solvent. The extraction process resulted in the removal of uncross-linked polymers from the gel structure. Extracted gels were dried again in vacuum oven at 45°C until constant weight was obtained. Gel fraction was calculated using initial weight of dry gel and weight of extracted dry gel using following equations [18]:

$$Sol\ fraction\ (\%) = \frac{m_o - m_e}{m_o} \times 100 \quad (9)$$

$$Gel\ fraction\ (\%) = 100 - Sol\ fraction \quad (10)$$

where, m_0 represents the dry weight of the hydrogel and m_e represents the weight of the hydrogel which is dried after the extraction process.

Porosity Measurement

For porosity measurement, solvent replacement method was used. Dried hydrogels were immersed in absolute ethanol overnight and weighed after excess ethanol was blotted from the surface. Porosity was calculated from the equation given by Yin et al. [18]:

$$Porosity = \frac{M_1 - M_2}{\rho V} \quad (11)$$

where, M_1 and M_2 are the mass of hydrogel before and after immersion in ethanol, respectively. Where ρ is the density of absolute ethanol and V is the final volume of hydrogel.

Scanning Electron Microscopy

Surface morphology of the synthesized hydrogel samples was investigated using a Hitachi scanning electron microscope S3400-N (Japan). Hydrogel samples were scanned at different magnifications.

Fourier Transform Infrared Spectroscopy

FTIR spectrum of hydrogels were recorded using a Shimadzu FTIR 8400 S, Japan. The adopted procedure was as follows. Hydrogel sample was crushed with pestle in an agate mortar. Crushed material was mixed with potassium bromide (Merck, IR spectroscopy grade) in 1/100 proportions and dried at 40°C. The mixture was compressed to 12 mm diameter semi-transparent disk by applying a pressure of 65 kN (pressure gauge, Shimadzu, Japan) for 2 min. The FTIR spectrum was recorded over the wavelength range 4000-400 cm^{-1}.

Statistical Analysis

A two-factor with three-level factorial design was used to investigate the effect of selected independent variables on the swelling behaviour of pectin/AA hydrogels. Two independent variables selected for present work were X_1, cross-linker concentration and X_2, AA or pectin content. Selected response variable (Y) was the equilibrium degree of swelling obtained in USP phosphate buffer solutions of pH 1.2 and 5.5. However, results at pH values of 6.5 and 7.5 could not obtain as most of the sample fragmented at that pH due to the excessive swelling. Multiple linear regressions were applied to the experimental results to calculate the regression coefficients (b_0-b_5) of the mathematical model which includes the linear and quadratic terms of the investigated factors, as well as the interaction factor, given in eqn (12) as follows:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_1^2 + b_4X_2^2 + b_5X_1X_2 \quad (12)$$

RESULTS AND DISCUSSION

pH and Pectin Contents in Relation to Swelling and Drug Release Values

For studying the effect of pectin on swelling and on drug release, a series of three samples (S_1 to S_3) were prepared. In this series the amount of pectin varied from 0.15 g per 100 g of solution to 0.60 g per 100 g of solution, at fixed AA and MBAAm concentrations (Table 2). Results presented in Figure 1 show the effect of variable pectin content on dynamic swelling after 8 h period as a function of varying pH. It is observed that swelling of the gels increases on

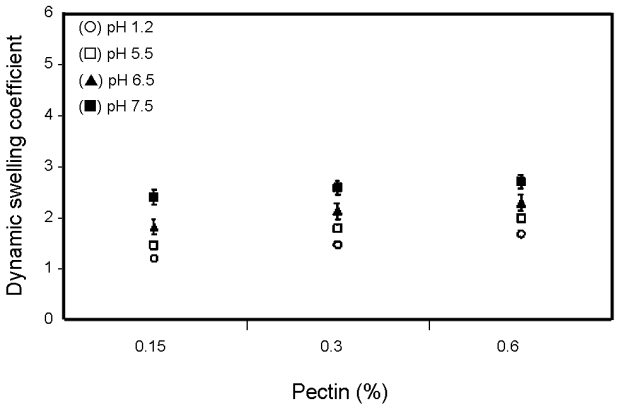

Figure 1. Dynamic swelling after 8 h of pectin/AA hydrogels having different pectin concentrations, i.e., 0.15 %w/w, 0.3 %w/w, 0.6 %w/w with 0.30% MBAAm as cross-linking agent, in various pH solutions at 37°C.

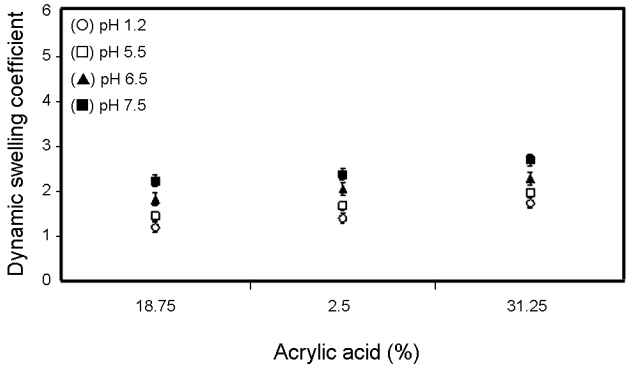

Figure 2. Dynamic swelling after 8 h of pectin/AA hydrogels with different AA concentrations i.e., 18.75 %w/w, 25 %w/w, 31.25 %w/w with 0.30 %w/w MBAAm as cross-linking agent, in various pH solutions at 37°C.

increasing the pH and amount of pectin in the gel. However, the increase in swelling due to increase in the concentration of pectin was not significant, therefore these samples were not considered for drug loading and release studies.

The increase in swelling was due to ionization of carboxymethyl groups (-$COOCH_3$) of the pectin at high pH. Our results can be correlated with Sutar et al. [19]. They prepared pH-sensitive polyacrylamide grafted pectin hydrogels and allowed the gels to swell in pH media of 1.4, 5.4, 7.4 and 9.4. They reported that hydrogels swelled significantly at pH 7.4 which accounted for large swelling forces created by the electrostatic repulsion between the ionized acid groups. In another study Mishra et al. [12] prepared pectin/poly (vinyl pyrrolidone) membranes and studied the swelling behaviour of the membrane in buffer solutions with pH values of 1.4, 5.5, 7.4 and 9.4. They suggested that pK_a values of the pectin ranges from 3.55 to 4.10. Hence at pH values lower than the pK_a value, the carboxymethyl groups (-$COOCH_3$) of pectin completely collapse which result in low swelling.

Acrylic Acid Role on Swelling and Drug Release

In order to study the effect of AA content on swelling and drug release, a second sample series (S_4 to S_6) were synthesized. For these samples, the amount of AA was varied from 18.75 g per 100 g of solution to 31.25 g per 100 g of solution. However, other parameters like pectin and cross-linker contents were kept constant. Figure 2 shows the effect of varying AA contents on dynamic swelling behaviour of the gels over 8 h periods. It is observed that swelling of the polymer increases on increasing the concentration of AA which is due to ionization of COOH groups in AA. This causes the expansion of the coiled chains and results in greater swelling of the gels. Ranjha et al. [20] prepared poly(vinyl acetate-*co*-acrylic acid) hydrogels and reported similar increase in swelling upon increases in AA concentration.

Since samples S_4 to S_6 showed significant swelling at higher pH values, these compositions were selected for drug loading and release studies. Table 3 shows the amount of verapamil loaded in the gel samples (S_4 to S_9). Two methods were used for determining the amount of drug loaded in the gels. The entrapped drug molecules in the gels were related to the system's swelling; as with higher percentage of drug entrapped in the gels there was higher swelling.

Verapamil release studies were performed to a maximum period of 12 h in USP phosphate buffer solutions of pH 1.2 and 7.5. Results presented in Figure 3 show verapamil released from a gel containing 31.25 %w/w AA at fixed pectin and cross-linker contents. It is observed that maximum 20% of the total loaded drug was released after 12 h at pH 1.2. However, 75% to 90% of the total drug loaded was released at pH 7.5 in 12 h period of time. Similar findings are reported by Sutar et al. [19].

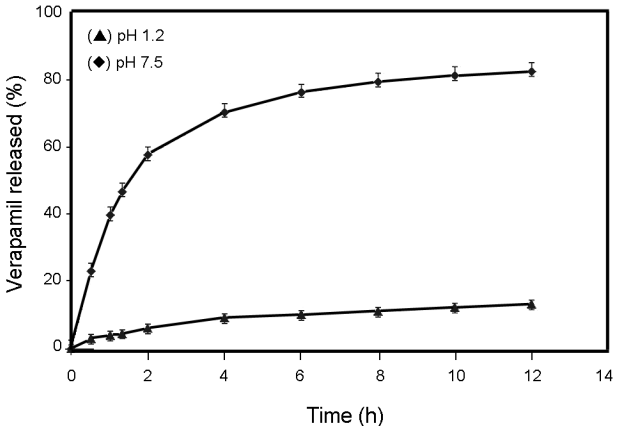

Figure 3. Verapamil released after 12 h from pectin/AA hydrogels having 31.25 %w/w AA using 0.30 %w/w MBAAm as a cross-linking agent, in solutions of pH 1.2 and pH 7.5. [loading: S6 = 5.3 %].

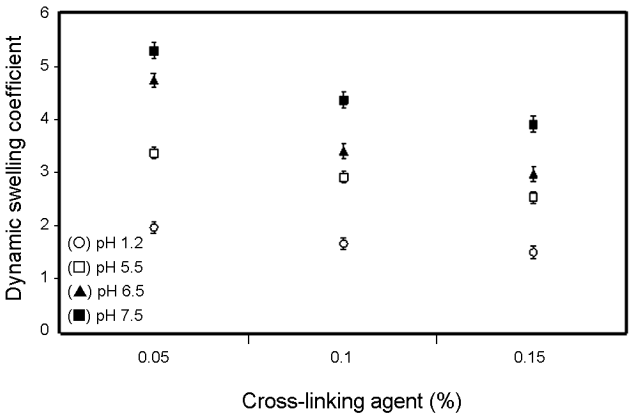

Figure 4. Dynamic swelling after 8 h of pectin/AA hydrogels with different cross-linking agent (MBAAm) concentrations, i.e., 0.05 %w/w, 0.1 %w/w, 0.15 %w/w with pectin and AA concentrations constant in various pH solutions at the pH values are 37°C: pH 1.2, pH 5.5, pH 6.5, and pH 7.5.

Effect of MBAAm Content on Swelling and Drug Release

To study the effects of degree of cross-linking on swelling and drug release, further series of three samples (S_7 to S_9) were prepared. In these samples the amount of cross-linker was varied between 0.05 %w/w to 0.15 %w/w at fixed pectin and AA contents (Table 2). Results presented in Figure 4 show the effect of varying amounts of cross-linker on swelling in different pH solutions. It is observed that swelling of the gels decreases on increasing the concentration of MBAAm as cross-linker.

These results are consistent with those reported by Mudassir et al. [21]. They prepared methylmethacrylate-*co*-itaconic acid hydrogels using MBAAm and EGDMA as cross-linker. They suggested that swelling of the gels decrease on increasing the amount of cross-linker.

Since these samples (S_7 to S_9) showed significant swelling, these compositions were included in drug loading and release studies. Table 3 shows the amount of verapamil loaded by two different methods. It is observed that the amount of cross-linker influences the total amount of drug entrapped in the gels. However, it was observed that there was a slight difference in the amount of drug incorporated in the gels by changing the amount of cross-linker. Drug release studies were performed in USP phosphate buffer solutions with pH 1.2 and 7.5 for 12 h period. It is observed that there is a significant difference in drug release profile by changing the pH of the medium (Figure 5).

Kinetics of Drug Release

Drug release kinetics was investigated using various kinetic models like zero order, first order, Higuchi and Peppas models. Table 4 shows the results obtained from pectin/AA hydrogels at varying content of AA and cross-linker. The release order can be predicted by considering the *r* value close to 1.

The results presented in Table 5 show the values of release exponent *n* indicating the release mechanism

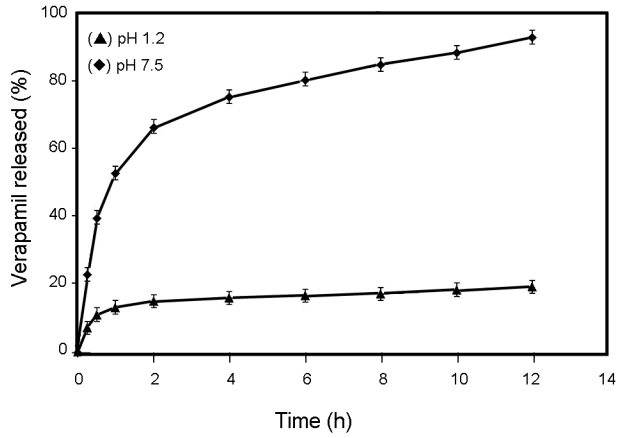

Figure 5. Verapamil released after 12 h from pectin/AA hydrogels using 0.05 %w/w MBAAm as cross-linking agent in solutions with pH 1.2 and pH 7.5. [loading: S7 = 4.8 %w/w].

Table 4. Effect of AA and MBAAm contents on release kinetics (zero order, first order and Higuchi models) from pectin/AA hydrogels solutions of different pH.

Sample		pH	Zero order		First order		Higuchi	
			r	K_0 (h^{-1})	r	K_1 (h^{-1})	r	K_2 ($h^{-1/2}$)
AA content (g/100 g solution)								
	AA content							
S4	18.75	1.2	0.90	0.63	0.80	0.11	0.98	19.100
		7.5	0.83	4.64	0.54	0.16	0.93	19.870
S5	25.00	1.2	0.93	0.68	0.82	0.12	0.98	2.340
		7.5	0.84	4.99	0.57	0.17	0.94	20.680
S6	31.25	1.2	0.95	0.72	0.83	0.14	0.52	2.700
		7.5	0.85	5.30	0.57	0.17	0.95	21.900
MBAAm content (g/100 g solution)								
	MBAAm content							
S7	0.05	1.2	0.78	1.06	0.57	0.11	0.89	4.47
		7.5	0.83	5.86	0.56	0.17	0.94	24.0
S8	0.10	1.2	0.83	1.06	0.54	0.10	0.94	4.30
		7.5	0.89	1.28	0.71	0.14	0.96	5.0
S9	0.15	1.2	0.86	1.06	0.52	0.10	0.97	4.21
		7.5	0.91	0.13	0.93	0.12	0.99	0.97

after fitting the data into Peppas model. It is observed that at almost all compositions and cross-linker contents, hydrogels followed non-Fickian release mechanism.

Volume Fraction of Polymer in Hydrogels (Φ_2) and Diffusion Coefficient (*D*)

Volume fraction of polymer in hydrogels (Φ_2) and diffusion coefficient (*D*) was calculated using eqns (3) and (8), respectively and the results are presented in Table 6. It is observed that as AA content increases in the gels the value of Φ_2 is reduced. Katime et al. [22] have reported similar findings and suggested that the value of Φ_2 is high for hydrogels containing no IA because of low water absorption.

Diffusion involves the migration of solvent into pre-existing or thermodynamically formed spaces between hydrogel chains [23]. Diffusion coefficient was determined using de-swelling phenomena for initial 60% de-swelling. Diffusion of water from the gels determines the release of drug from hydrogels therefore, diffusion coefficient phenomenon has been investigated for selected samples which showed maximum swelling. Densities of polymeric gels and xerogels were calculated and results are given in Table 6. Xerogels are solids formed from drying gels. The density of xerogels ranges between 1.13 and 1.25 g/cm^3.

Composition of Gels and Equilibrium Water Contents

Equilibrium water contents (EWC) were calculated for samples S_4 to S_6. These samples were prepared using AA of various concentrations at fixed pectin and MBAAm concentration. The data obtained for EWC using eqn (2) are given in Table 6. It is indicated that the amount of water retained in the gel samples (S_4 to S_6) increases from 623% to 1344 %. This observation

Table 5. Effect of AA and MBAAm contents on drug release mechanism by applying Peppas model on pectin/AA hydrogels solutions of different pH.

Sample		pH	Release exponent (n)	r	Order of release
AA content (g/100 g solution)					
	AA content				
S4	18.75	1.2	0.45	0.76	non-Fickian
		7.5	0.59	0.52	non-Fickian
S5	25.00	1.2	0.46	0.77	non-Fickian
		7.5	0.56	0.51	non-Fickian
S6	31.25	1.2	0.47	0.77	non-Fickian
		7.5	0.50	0.50	non-Fickian
MBAAm content (g/100 g solution)					
	MBAAm content				
S7	0.05	1.2	0.45	0.51	non-Fickian
		7.5	0.53	0.51	non-Fickian
S8	0.10	1.2	0.48	0.51	non-Fickian
		7.5	0.53	0.68	non-Fickian
S9	0.15	1.2	0.49	0.52	non-Fickian
		7.5	0.54	0.73	non-Fickian

is supported by the fact that AA contains COOH functional groups. Ionization of these groups causes excessive repulsion between the coiled chains which is ultimately responsible for retaining more water. Another fact which can be used to explain the increased value of EWC is the relative ratio of hydrophilicity and hydrophobicity in the gels, as AA is more hydrophilic and confers more hydrophilicity to the gels. It causes the increase in EWC on increasing the amount of AA in the gels. Wang et al. [24] prepared 2-hydroxyethyl methacrylate/epoxy methacrylate copolymer hydrogels and have reported similar findings. They suggested that increasing the EMA in the gel leads to lower hydrophilicity and therefore lower EWC in the gels.

Sol-gel Fraction

Results of gel fraction of different formulations of pectin/AA are presented in Figure 6. It is observed

Table 6. The values of EWC, density of the gels, Φ_2 and D from selected samples of pectin/AA hydrogels.

Sample code	EWC (%)	Density (g/cm^3)		Φ_2	D 10^{-6} (cm^2/s)
		Xerogels	Hydrogels		
S4	623	1.13	0.89	0.07	8.5
S5	717	1.15	0.68	0.07	2.2
S6	1344	1.25	0.67	0.05	5.4

EWC: equilibrium water content, Φ_2 : volume fraction of the polymer within the hydrogel, and D: diffusion coefficient.

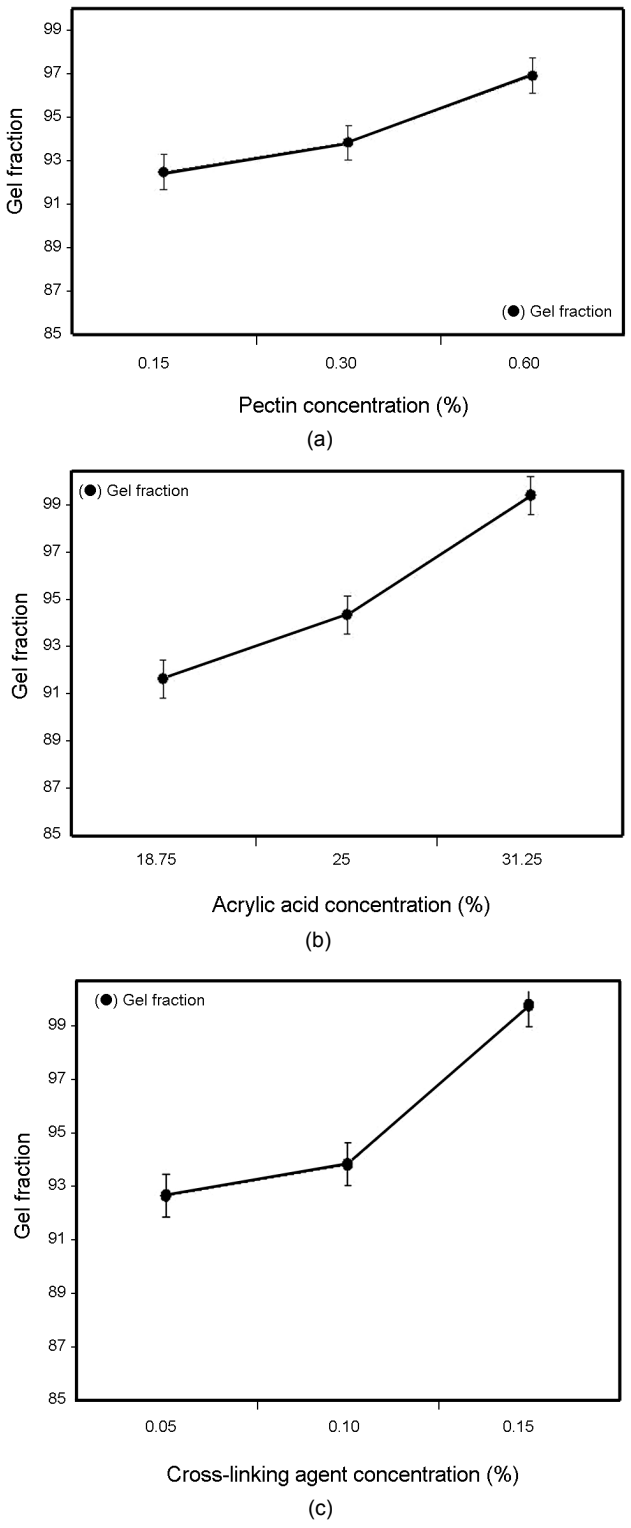

Figure 6. The effect of concentration on gel fraction: (a) pectin, (b) AA, and (c) MBAAm.

that on increasing the concentrations of pectin, AA and cross-linking agent (MBAAm), the gel fraction increases at the same time. Yin et al. [18] have prepared poly(acrylic acid-*co*-acrylamide)/*o*-carboxymethyl chitosan hydrogels and reported similar findings.

Porosity Measurement

Porosity of all the 9 samples (S_1 to S_9) was calculated using eqn (11). Results of porosity measurement are presented in Table 3. It is observed that porosity of the prepared gels increases on increasing the amount of pectin and AA in the gels, while the porosity decreases on increasing the amount of cross-linker in the gels [25].

Scanning Electron Microscopy

Scanning electron microscopy (SEM) was performed to study the morphology of hydrogels. Figure 7 shows fractured surface morphology of loaded and unloaded

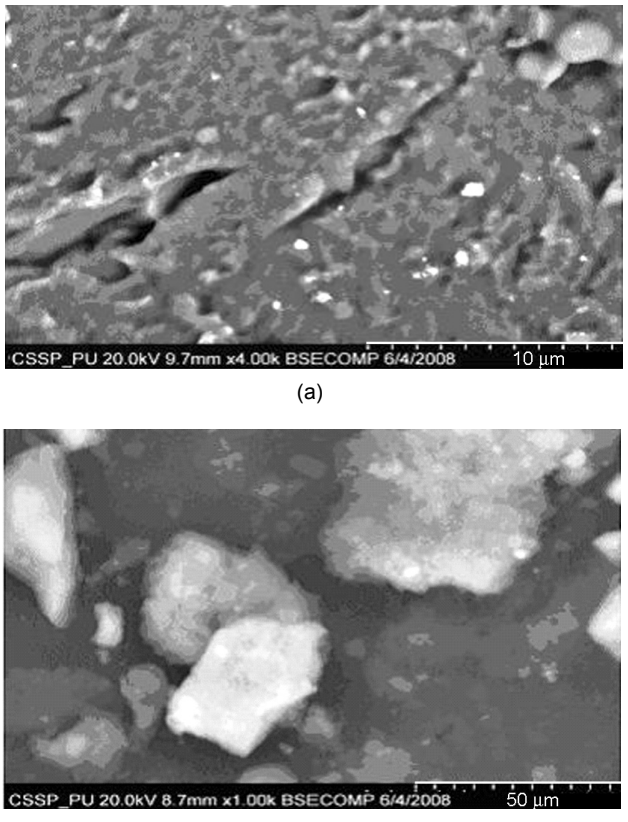

Figure 7. SEM Micrographs of hydrogels: (a) pectin/AA hydrogels in presence of 0.05 %w/w MBAAm as a cross-linking agent and (b) verapamil loaded pectin/AA hydrogels in presence of 0.05 %w/w MBAAm as a cross-linking agent.

pectin/AA hydrogels. From the SEM micrographs it is observed that minute voids are present on the surface that could facilitate the incorporation of the drug. Figure 7b shows the gels loaded with verapamil as model drug, which is adhered on the surface of the matrix.

Fourier Transform Infrared Spectroscopy

Figure 8 represents the FTIR spectra of pectin, pectin/AA hydrogel without drug, pectin/AA hydrogel with drug and poly (acrylic acid). The spectrum of pectin (Figure 8 spectrum a) indicates peak at 3400 cm^{-1} due to stretching of -OH groups. The peaks at 2913 cm^{-1} indicate C-H stretching vibration. The peaks at 1556 cm^{-1} indicate C=O stretching vibrations due to the presence of $COOCH_3$ group. The peaks at 1441 cm^{-1} and 1342 cm^{-1} could be attributed to CH_2 scissoring and -OH bending vibration, respectively. The peak at 1150 cm^{-1} suggested the presence of CH-OH group. The main peaks in FTIR spectrum of pure poly (acrylic acid) (Figure 8 spectrum d) are -OH stretch at 3380 cm^{-1}, -CH stretch at 2922 cm^{-1} and -C=O stretch at 1718.5 cm^{-1}. However, FTIR spectrum of the prepared pectin/AA (Figure 8 spectrum b) hydrogels indicate that the characteristics -OH stretching vibration peak of pectin at 3400 cm^{-1} is shifted to lower frequency. This lowering in frequency of -OH groups indicates the presence of hydrogen bonding in hydrogels. These indications showed -OH groups of pectin have reacted with -COOH groups of acrylic acid.

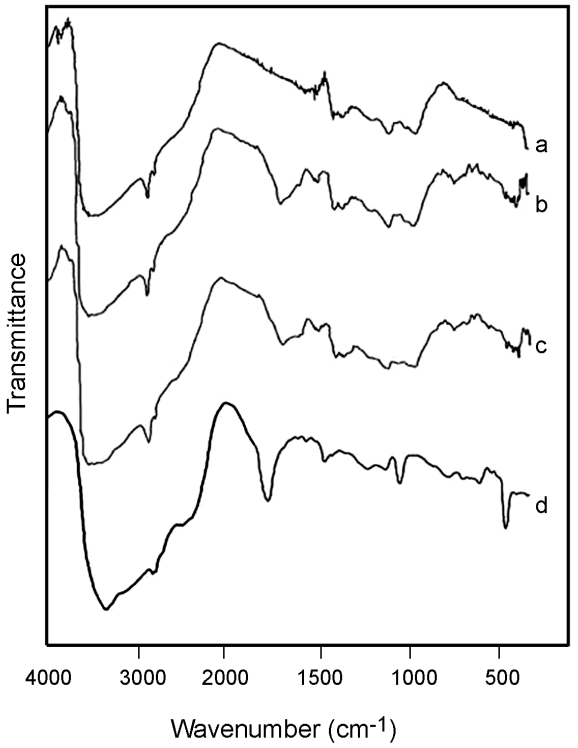

Figure 8. FTIR Spectra of: (a) pectin, (b) pectin/AA hydrogel without drug, (c) pectin/AA hydrogel with drug, and (d) PAA.

Factorial Design Analysis

The equilibrium swelling behaviour of the pectin/AA

Table 7. Pectin/AA hydrogel prepared according to three factorial design and effect of pH.

Sample code	X1	X2	Y		
	MBAAm content (g/100 g solution)	AA content (g/100 g solution)	pH 1.2	pH 5.5	pH 6.5
S1	0.30	37.50	1.42	1.62	[a]
S2	0.30	37.50	1.32	1.90	2.08
S3	0.30	37.50	1.33	1.95	[a]
S4	0.30	18.75	1.40	1.50	1.84
S5	0.30	25.00	1.71	1.87	1.92
S6	0.30	31.25	1.82	1.93	2.01
S7	0.05	31.25	4.34	4.76	[a]
S8	0.10	31.25	1.71	4.02	[a]
S9	0.15	31.25	1.82	3.97	[a]

[(a)] sample broke

Table 8. Summary of the multiple regression analysis.

Regression coefficient	pH of the solution		
	1.2	5.5	6.5
b_0	2.138	1.998	4.59
b_1	-51.02	-5.89	-5.89
b_2	0.3011	0.1933	0.0096
b_3	119.78	-14.88	-13.36
b_4	-0.0053	-003136	0.0001
B_5	-	-	-
R_2	0.8890	0.9870	0.9250

hydrogel was investigated in USP phosphate buffer solutions at pH 1.2, 5.5, 6.5 and 7.5. However, gel starts to fragment in the solutions with high pH values of 6.5 and 7.5 due to excessive swelling. Swelling of hydrogels was investigated by using 32 factorial designs at three pH values selected as response variables which are given in Table 7. Results obtained from multiple regression analysis indicated that both the cross-linking agent concentration and monomer ratio significantly affect the swelling of hydrogel. The summary of the multiple regression analysis is given in Table 8.

CONCLUSION

Pectin/AA hydrogels have been synthesized in the presence of MBAAm as cross-linkers. Water uptake through these gels was significantly dependent on pH of the swelling medium especially at high pH, i.e., higher than the pK_a values of COOH groups of AA and $COOCH_3$ groups of pectin. It was concluded that gel fraction and porosity increase on increasing the concentration of the pectin or AA. However, increasing the amount of cross-linker decreases the porosity and increases the gel fraction. From drug release studies it is suggested that the release of verapamil from pectin/AA depends on the hydrogel composition. It was observed that drug release increase on increasing the AA contents. It was concluded that samples containing higher amount of AA (S_6) and lower amount of cross-linker (S_7) showed optimum results and these compositions could be successfully used as carrier for controlled drug delivery systems.

REFERENCES

1. Kirsten R, Nelson K, Kirsten D, Heintz B, Clinical pharmacokinetics of vasodilators: Part I, *Clin Pharmacokinet*, **34**, 457-482, 1998.
2. Ranjha NM, Ayub G, Naseem S, Ansari TA, Preparation and characterization of hybrid pH-sensitive hydrogels of chitosan-*co*-acrylic acid for controlled release of verapamil, *J Mater Sci Mater Med*, **21**, 2805-2816, 2010.
3. Kostova B, Momekova D, Petrov P, Momekov G, Toncheva-Moncheva N, Tsvetanov CB, Lambov N, Poly(ethoxytriethyleneglycol acrylate) cryogels as novel sustained drug release system for oral application, *Polymer*, doi:10.1016/j.polymer.2011.01.049, 2011.
4. Kulkarni RV, Mangond BS, Mutalik S, Sa B, Interpenetrating polymer network microspheres of gellan gum and egg albumin entapped with diltiazem-resin complex for controlled release application, *Carbohyd Polym*, **83**, 1001-1007, 2011.
5. Pappas NA, Barr-Howell, Characterization of the crosslinked structures of hydrogels. In: *Hydrogels in medicine and pharmacy*, Pappas NA (ed) CRC, vol 1, Fundamentals, CRC Press, Boca Raton, FL, 27-57, 1986.
6. Ranjha NM, Doelker E, pH-sensitive non-crosslinked poly(vinyl alcohol-*co*-acrylic acid) hydrogels for site specific drug delivery, *Saudi*

Pharm J, **7**, 137-143, 1999.
7. Grant GT, Morris ER, Rees DA, Smith PJC, Thom D, Biological interactions between polysaccharides and divalent cations: the egg based model, *FEBS Letters*, **32**, 195-198, 1973.
8. Ashford M, Fell J, Attwood D, Sharma H, Woodhead P, An evaluation of pectin as a carrier for drug targeting to the colon, *J Control Release*, **26**, 213-220, 1993.
9. Warkley Z, Fell JT, Attwood D, Parkins D, Studies on drug release from Pectin/ethyl cellulose film-coated tablets: a potential colonic delivery system, *Int J Pharm*, **153**, 219-224, 1997.
10. Al-Ruqaie IM, Kasapis S, Abeysekera R, Structural properties of pectin-gelatin gels. Part II: effect of sucrose/glucose syrup, *Carbohyd Polym*, **34**, 309-321, 1998.
11. Desai KGH, Preparation and Characteristics of high-amylose corn starch/pectin blend microparticles, *AAPS Pharm Sci Tech*, **6**, 136-701, 2005.
12. Mishra RK, Datt M, Banthia AK, Synthesis and characterization of pectin/PVP hydrogel membranes for drug delivery system, *AAPS Pharm Sci Tech*, **9(2)**, 395-403, 2008.
13. Najib N, Suleiman M, The kinetics of drug release from ethyl cellulose solid dispersions, *Drug Dev Ind Pharm*, **11**, 2169-2189, 1985.
14. Desai SJ, Singh P, Simonelli AP, Higuchi WI, Investigation of factors influencing release of solid drug dispersed in wax matices III: quantitative studies involving polyethylene plastic matrix, *J Pharm Sci*, **55**, 1230-1234, 1966.
15. Higuchi T, Mechanism of sustained action medication, theoretical analysis of rate of release of solid drugs dispersed in solid matrices, *J Pharm Sci*, **52**, 1145-1149, 1963.
16. Peppas NA, Analysis of fickian and non-fickian drug release from polymers, *Pharm Acta Helv*, **60**, 110-111, 1985.
17. Mackie JS, Meares P, The diffusion of electrolytes in a cation-exchange resin membrane, *Proc Royal Soc London Series A*, **232**, 498-509, 1955.
18. Yin L, Fei L, Cui F, Tang C, Yin C, Superporous hydrogels containing poly (acrylic acid-*co*-acrylamide)/O-carboxymethyl chitosan interpenetrating polymer networks, *Biomaterials*, **28**, 1258-1266, 2007.
19. Sutar PB, Mishra RK, Pal K, Banthia AK, Development of pH sensitive polyacrylamide grafted pectin hydrogel for controlled drug delivery system, *J Mater Sci*, **19**, 2247-2253, 2008.
20. Ranjha NM, Mudassir J, Swelling and aspirin release study: crosslinked pH-sensitive vinyl acetate-*co*-acrylic acid (VAC/AA) hydrogels, *Drug Dev Ind Pharm*, **34**, 512-521, 2008.
21. Mudassir J, Ranjha NM, Dynamic and equilibrium swelling studies: crosslinked ph sensitive methyl methacrylate-*co*-itaconic acid (MMA-*co*-IA) hydrogels, *J Polym Res*, **15**, 195-203, 2008.
22. Katime I, Valderruten N, Quintana JR, Controlled release of aminophylline from poly (*N*-isopropylacrylamide-*co*-itaconic acid) hydrogels, *Polym Int*, **50**, 869-874, 2001.
23. Berens AR, Hopfenberg HB, Diffusion and relaxation in glassy polymer powders. II separation of diffusion and relaxation parameters, *Polymer*, **19**, 489-496, 1978.
24. Wang J, Wu W, Swelling behaviors, tensile properties and thermodynamic studies of water sorption of 2-hydroxyethyl methacrylate/epoxy methacrylate copolymeric hydrogels, *Eur Polym J*, **41**, 1143-1151, 2005.
25. Chang HI, Perrie Y, Coombes, Delivery of the antibiotic gentamicin sulphate from precipitation cast matrices of polycaprolactone, *J Control Release*, **110**, 414-421, 2006.