J Mater Sci: Mater Med (2008) 19:2247–2253
DOI 10.1007/s10856-007-3162-y

Development of pH sensitive polyacrylamide grafted pectin hydrogel for controlled drug delivery system

Prashant B. Sutar · Rakesh K. Mishra · Kunal Pal · Ajit K. Banthia

Received: 22 May 2006 / Accepted: 21 August 2006 / Published online: 10 July 2007

Abstract In the present study an attempt was made to graft polyacrylamide on pectin. The grafted polymer was characterized by FTIR spectroscopy, differential scanning calorimetry and X-ray diffraction. Rheological property of pectin solution was compared with the product solution. The grafted polymer was cross-linked with varying amount of glutaraldehyde. The swelling properties of the cross-linked product were also studied. The salicylic acid, an antipyretic drug, was incorporated in the cross-linked gel as a model drug and the drug release studies were done in a modified Franz's diffusion cell. The effect of cross-linking density on the release property of salicylic acid was studied through the cross-linked product. The product showed better film forming property and gelling property than pectin. The comparative rheological properties of pectin and grafted copolymer indicated change in the property of the product. FTIR studies indicated incorporation of amide group. Differential scanning calorimetry and XRD suggested formation of a new polymer. Swelling study indicated pH dependent swelling of the cross-linked hydrogel. Salicylic acid release indicated pH dependent release from the hydrogel.

Introduction

Hydrogels are three-dimensional cross-linked hydrophilic polymer networks capable of holding large amounts of water [1–3]. Hydrogels have long been used for various biomedical applications, such as soft contact lenses, wound dressing, super-absorbents, drug delivery systems etc [4–9]. Natural polysaccharides are now extensively used for the development of solid dosage forms for delivery of drug. Polysaccharides are, in general, non-toxic, biocompatible, biodegradable, and abundant [10–12]. But due to easy solubility of polysaccharides in water, they cannot form stable hydrogel. An effective method is to chemically modify them to form a stable polymer network. Pectin is a heterogeneous polysaccharide containing linear chains of α-(1-4)-linked-D-galacturonic acid residues. Pectin is found in fruit and vegetables and is mainly prepared from citrus peel and apple pomace. Pectin is mainly used in the food industry and research is currently going on the development of pectin based drug delivery system. Pectin is totally degraded in the proximal colon [13–14].

Colon is liable to numerous pathological conditions, such as constipation, Crohn's disease, ulcerative colitis, carcinomas and infections. Recommended treatments include the administration of anti-inflammatory drugs, chemotherapy drugs and/or antibiotics, which must be released in the colon [14]. From a biomedical point of view, it is also useful to have dosage forms that are able to specifically release drugs, such as peptides, proteins [15], vermifunges and diagnostic agents, in the colon due to the capacity of this part of the gastrointestinal tract to absorb these drugs. To achieve an optimum pharmacological action of these drugs, it is necessary to release the drug after reaching the upper part of the colon. Currently research is being carried out for the development of colon specific drug delivery systems. Pectin gels have poor gelling and film forming properties as well as less stability and it is not pH sensitive [16]. Since pectin is easily degraded in colon, in the current study an attempt has been made to modify

P. B. Sutar · R. K. Mishra · K. Pal · A. K. Banthia (✉)
Materials Science Centre, Indian Institute of Technology, Kharagpur 721302, India
e-mail: ajitbanthia2000@yahoo.co.in

the properties of the pectin gels by graft copolymerization with polyacrylamide and use the same as a drug delivery system.

Materials and methods

Materials

Pectin (MW ~30000–100000) and salicylic acid (SA) was obtained from Loba-chemie indoaustranal Co. Mumbai, India. Acrylamide was obtained from Spectrochem pvt. Ltd., Mumbai. Hydrochloric acid 35% and Acetone pure was obtained from Merk Limited, Mumbai, India. Glutaraldehyde (GA) 20% solution and Ammonium ceric sulphate (ACS) was obtained from Loba-chemie Pvt. Ltd. Mumbai, India. Oleic acid was obtained from S-d fine chem. Ltd., Mumbai, India. Double distilled water used throughout the experiments.

Synthesis of pectin-polyacrylamide graft co-polymer and cross-linked hydrogels

The reaction was carried out at room temperature. Pectin (0.5 g) was dissolved in 40 mL of water with continuous stirring. Nitrogen was purged through the resulting solution for 30 min to remove dissolved oxygen. Acrylamide (2.5 g) and 2 mL of freshly prepared solution of ceric ammonium sulphate in water (0.04 g/mL) were added subsequently to the reaction mixture with constant stirring. The flask was then kept under nitrogen atmosphere to avoid the interference of the oxygen for 24 h with continuous stirring. After the completion of the reaction, the graft copolymer (GP) was first precipitated in an excess of acetone. Figure 1 shows the probable mechanism of the reaction.

The product was purified by extracting the homopolymer of polyacrylamide (that might be produced during the polymerization) from the crude product by washing with acetone-water mixture (30:70). The procedure was repeated for 10 times. The pure graft copolymer (GP) so obtained was finally washed with pure acetone and was allowed to dry in laminar flow air drier for 72 h at room temperature [18].

Then GP was cross-linked with different amount of GA and were converted into membrane by conventional solution casting technique. A stock solution of GP in water (10% w/v) was prepared with continuous stirring for 7 h at room temperature. To the 5 mL of 10% (w/v) GP solution, 0.2 mL or 0.4 mL or 0.6 mL or 0.8 mL of glutaraldehyde (25% v/v) was added and each solution was acidified with a 0.05 mL of HCl. The resulting solutions were heated at 70 °C for 20 min to complete the cross-linking reaction. The thick cross-linked gels so obtained was further sonicated to remove the trapped air bubbles and used for study. The hydrogels so obtained were washed with water to remove HCl from the product. The products so formed were termed as CGP1 (0.2 mL of GA) or CGP2 (0.4 mL of GA) or CGP3 (0.6 mL of GA) or CGP4 (0.8 mL of GA).

Characterization

The FTIR spectrum of Pectin and GP were taken in the range of 4000–400 cm^{-1} as KBr pellet and Attenuated total reflectance (ATR) technique with the help of FTIR spectrophotometer (NEXUS–870,Thermo Nicolet Corporation).

For the X-ray diffraction study the raw materials were subjected to X-ray diffraction (XRD-PW 1700, Philips, USA) using Cu Kα radiation generated at 40 kV and 40 mA; the range of diffraction angle was 10°–100° 2θ.

In differential scanning calorimetry was done in the DSC unit (Netzsch DSC-200 PC Phox, Germany). The samples were heated in an aluminium pan at a rate of 10 °C/min from –20 to 200 °C. Nitrogen was used as a purge gas with a flow rate of 50 mL/min.

The rheological measurements of aqueous solutions (5% w/v) of pectin and the graft copolymer GP were carried out in controlled stress AR-1000 Advanced RheometerThe temperature of the system was maintained at 30.8 °C throughout the experiment.

Swelling study

The swelling characteristics of the prepared hydrogels were determined by immersing the xerogels of known weight in

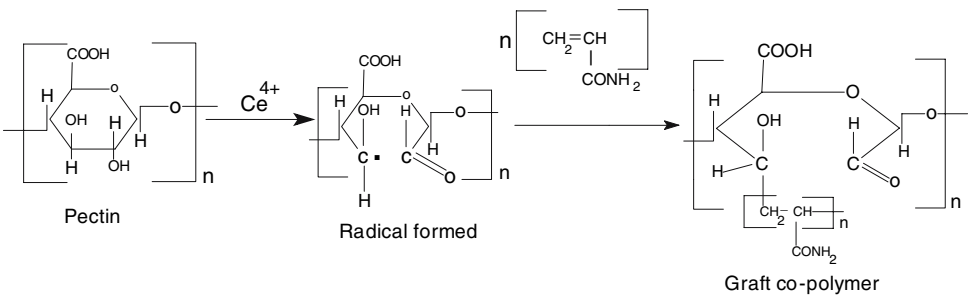

Fig. 1 Mechanism of graft polymerization

20 mL of buffer solutions of pH 1.4, 5.4, 7.4, and 9.4 for 24 h. At specific time intervals, the samples were removed from the buffer solutions and were blotted with a piece of tissue paper to absorb excess water on the surfaces. The % swelling (% Sw) of test samples were calculated from the following expression

$$\% \text{ Sw} = (W_s - W_d)/W_d \times 100$$

where 'W_s' is the weight of the swollen test sample and 'W_d' is the weight of the dried test sample.

Drug release study of Salicylic Acid from GP hydrogel

The rat skin was prepared using reported article [19, 20]. The skin of albino mice was obtained from Biochemistry laboratory, Department of Biotechnology, I.I.T. Kharagpur. The cross-linked GP (CGP) were used for the drug release study. For preparing drug loaded cross-linked hydrogel [21], the CGPs were kept in an ethanolic solution of SA (5% w/v) for 5 h. Finally the drug loaded hydrogels were washed with distilled water to remove the drug adhered to the surface of hydrogel. The release study was carried out at 37 °C.

The prepared rat skin was mounted on the Franz's diffusion cell (Fig. 2). The diffusion surface area and volume of the acceptor compartment of the diffusion cell was 2.503 cm^2 and 40 mL respectively. For diffusion study through the hydrogel, 200 mg of the hydrogel was kept in the donor and acceptor contained distilled water (pH adjusted to 1.4 with HCl, or 7.4 with Na_2CO_3). At regular interval of time 1 mL of acceptor fluid was taken out using a 1 mL pipette and was replaced by equal volume of fresh acceptor medium. Samples were analyzed spectrophotometrically at 294 nm.

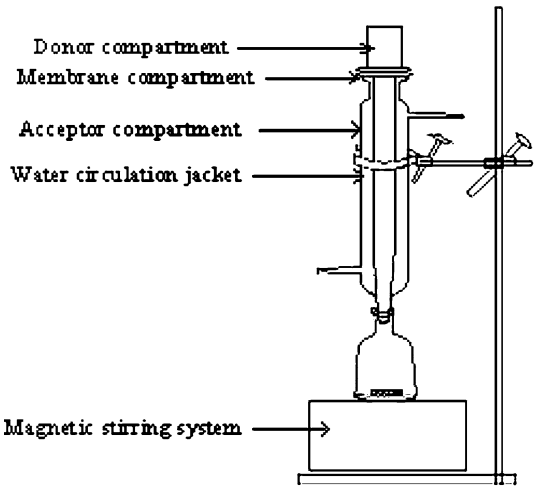

Fig. 2 Diagrammatic representation of Franz's diffusion cell

Hemocompatibility test

In the present work the hemocompatibility tests were carried out broadly on the basis of ASTM standard [22]. The test is mainly aimed at finding the extent of hemolysis caused in the presence of the sample prepared. The hemolysis percentage was calculated from given formula

$$\% \text{ Hemolysis} = \{[OD_{test} - OD_{negetive}] \div [OD_{Positive} - OD_{negetive}]\} \times 100$$

Cytotoxicity test

In cytotoxicity test B16 melanoma cells were used. Polymer cytotoxicity was assessed by a MTT assay [23]. MTT (3-[4,5-dimethylthiazole-2-yl]-2,5 diphenyltetrazolium bromide) is oxidized by mitochondrial dehydrogenase in living cells and gives a dark purple formazan product. Damaged or dead cells show little or no dehydrogenases activity [24]. Cells were seeded into 96-well microplates at 3000 cells per well. The medium was removed 24 h after plating and fresh media containing different concentrations of polymers (1–100 μg/mL) were added. After incubation for 1 h the medium was discarded, the cells were washed twice with phosphate-buffered saline (PBS: 150 mM NaCl; 1.9 mM NaH_2PO_4; 8.1 mM NaH_2PO_4; pH 7.4) and 50 mL of 5 mg/mL MTT solution in PBS were added to each well. The plates were incubated under cell culture conditions for 4 h and the formazan crystals were dissolved by adding 100 mL dimethyl sulfoxide (DMSO) to each well. The absorptions were measured in triplicate at 570 nm, with a background correction at 630 nm, using a microplate ELISA reader. Results were recorded as percentage absorbance relative to untreated control cells. The cytotoxicity assay results were used to calculate cell viability after incubation with polymers as follows:

$$\text{Cell Viability}\% = \frac{X}{X_c} \times 100\%$$

where X is the absorbance in a well containing a particular polymer concentration and X_c is the absorbance for untreated control cells.

Results and discussion

FTIR characterization

Figure 3 a shows the FTIR spectra of the pure pectin. The spectrum showed peak at 3415 cm^{-1} due to the stretching of –OH groups. The peaks at 2913 cm^{-1} indicated –C–H

stretching vibration. The peak at 1756 cm^{-1} indicated >C = O stretching vibrations due to the presence of –CO-OCH_3 group. The peaks at 1441 cm^{-1} and 1342 cm^{-1} could be assigned to $–CH_2$ scissoring and –OH bending vibration, respectively. The peak at 1023 cm^{-1} suggested –CH–O–CH– stretching. The peak at 1150 cm^{-1} suggested the presence of –CH–OH in aliphatic cyclic secondary alcohol.

Figure 3b shows FTIR spectra of graft copolymer (GP). The peaks at 3200 –3260 cm^{-1} indicated –OH stretching and/or –NH stretching. The characteristic peak at 1648 cm^{-1} was due to the amide-I band of the amide group of polyacrylamide (>C = O stretching vibration frequency) and the another band at 1571 cm^{-1} was due to –N–H bending frequency (amide-II). The presence of a band at 1752 cm^{-1} was due to free carboxylic groups. The peaks at 2937 cm^{-1} and 2910 cm^{-1} indicated C–H stretching of $–CH_2$ groups. The peak at 1419 cm^{-1} suggested –CH bending of $–CH_2$ group. The peak at 1085 cm^{-1}suggested –OH bending, 1041 cm^{-1} indicated the presence of secondary alcohol (characteristic peak of –CH–OH in cyclic alcohol C–O stretch). Presence of amide groups in the GP suggested the formation of a grafted polymer.

X-ray diffraction

The XRD patterns of GP (Fig. 4a) revealed that the GP peak was at ~20.10° 2θ while that of pectin (Fig. 4b) showed peak at ~13.58° 2θ. The XRD patterns of the GP and pectin indicated that there was decrease in crystallinity of pectin when it was modified with polyacrylamide. Also, the XRD pattern of the GP was totally changed from that of pectin indicating the formation of a new product. From the XRD plots the % crystallinity of pectin and graft copolymer was found to be 0.81 and 0.69 respectively. This decrease in crystallinity with copolymerization is might be due to incorporation of bulkier group within the polymeric network, which in turn decreases intermolecular hydrogen bonding.

Differential scanning calorimetry characterizations

The DSC of pectin (Fig. 5a) indicated that the Tg is at 95 °C while the Tg of GP (Fig. 5b) was found to be at 53 °C. This decrease in Tg may be due to the disruption of crystalline structure (as mentioned above) when grafted with polyacrylamide indicating the formation of a new polymer.

Rheological study

Viscosity versus shear rate (Fig. 6a and b) of the 5% polymer solution were plotted. The viscosity of the polymer solutions decreases with increase in shear rate. Similar results are observed by, Cheng, Takai and Ekong [25]; Gómez-Díaz and Navaza [26] for carboxy methyl cellulose, Rheological viscosity measurement study. Both the aqueous 5% solutions of GP and Pectin show strong pseudo plastic behavior. At low and high shear rate, the viscosity of the solutions of graft copolymer was found to be higher than that of pectin. The changes in viscosity are due to the presence of the grafted chains in the graft copolymer. This marked shear thinning behavior of polysaccharide and the graft copolymer solution may be explained by the conformational states of polymer molecules. Domains of associated polysaccharide and the graft copolymer chains exist at rest or low shear, which are stabilized by hydrogen bonds. When shearing takes place, the extent of aggregation was reduced resulting in a lower solution viscosity.

Hemocompatibility study

Hemocompatibility study indicated that Hemolysis due to test sample was 3.636%; suggesting that GP was hemocompatible [22]. Table 1.

Cytotoxicity study of GP (MTT assay Test)

The MTT assay was used to investigate changes in cell viability after the addition of the polymers. Reduction of

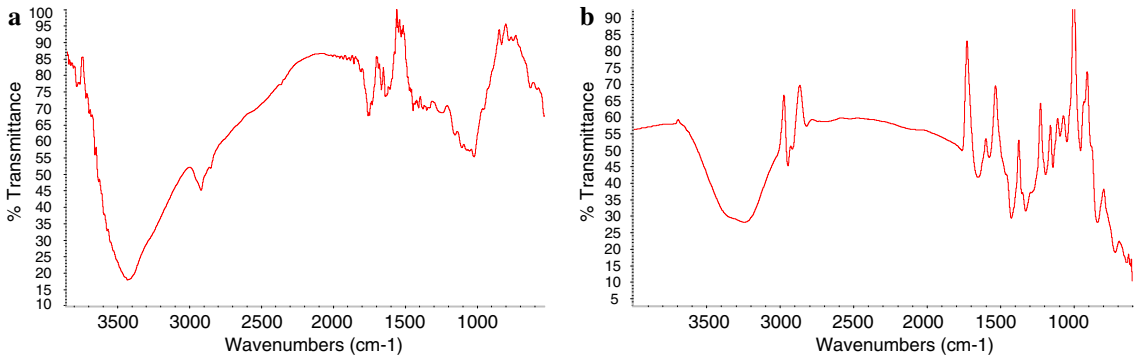

Fig. 3 (**a**) FTIR spectra of pectin, (**b**) FTIR spectra of GP

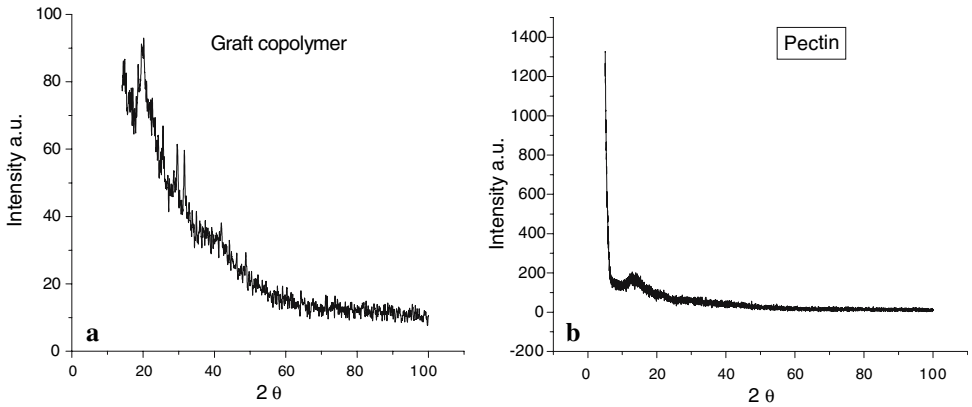

Fig. 4 (**a**) XRD of GP, (**b**) XRD of pectin

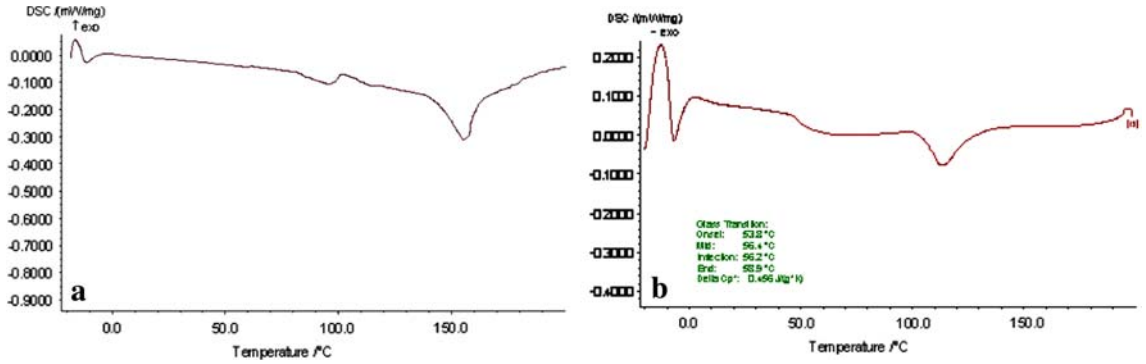

Fig. 5 (**a**) DSC of pectin, (**b**) XRD of GP

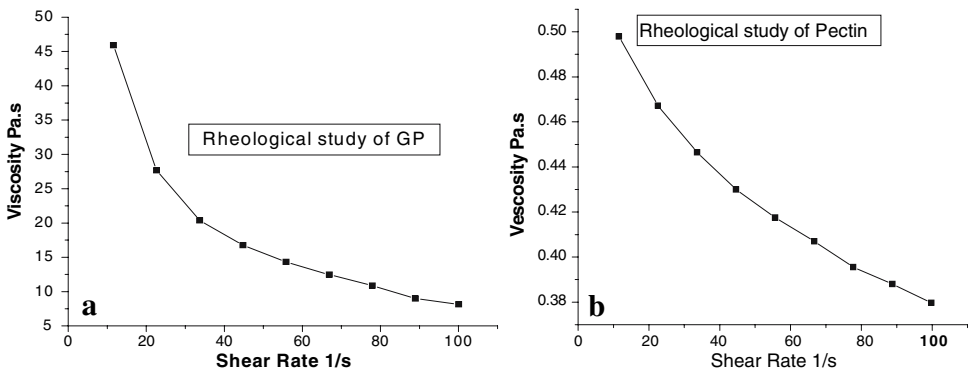

Fig. 6 (**a**) Viscosity versus shear rate curves of 5% GP solution, (**b**) Viscosity versus shear rate curves of 5% pectin solution

Table 1 Hemocompatibility observations and results

$OD_{Negative}$	$OD_{Positive}$	OD_{Test}	%Hemolysis
0.04	0.37	0.052	3.6

MTT by cells indicates mitochondrial activity, which may be interpreted as proof of cell viability. The Graft copolymers didn't induced significant cytotoxic effects even at the higher concentrations used (Fig. 7), indicating biocompatibility of the hydrogels.

Swelling behavior of the GP hydrogel

The cross-linked products were allowed to swell in 25 mL of buffer solutions of pH 1.4, 5.4, 7.4, and 9.4. The result indicated that cross-linked hydrogels swelled more significantly (swelling 295%) at pH 7.4 which can accounted due to large swelling forces created by the electrostatic repulsion between the ionized acid group as well as amide groups. Figure 8 shows the effect of amount of cross-linking agent on the swelling behavior of GP.

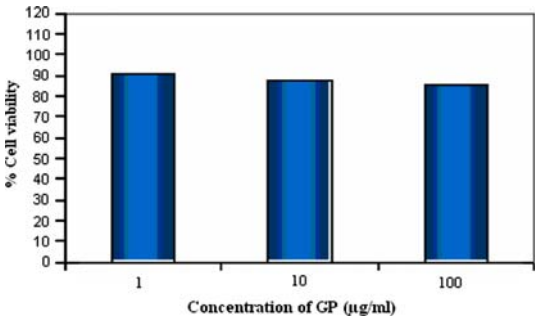

Fig. 7 MTT assay test on B16 melanoma cell line

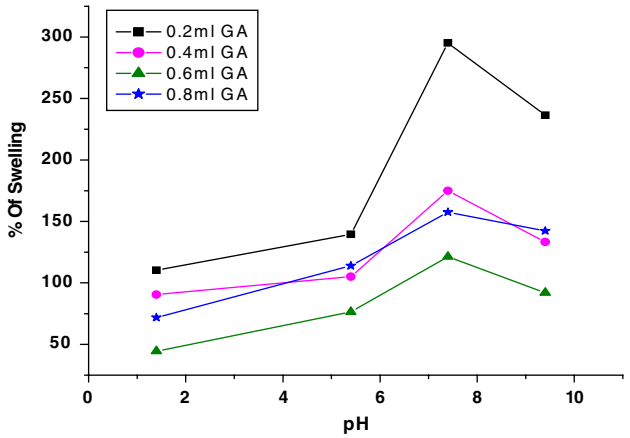

Fig. 8 Swelling behavior of cross-linked GP

The swelling behavior of the cross-linked GP indicated pH dependent swelling. The degree of swelling increased from pH 1.4 to 7.4 but at pH 9.4 swelling decreases again. The maximum swelling occurs at pH 7.4 which can be accounted due to the ionization of the carboxylic group at the said pH. The decrease in the solubility of the hydrogel at pH 9.4 can be accounted to the partial solubility of the cross-linked GP. The swelling behavior also depended upon the cross-linking density. As the amount of GA was increased, the % swelling decreased. This is due to the fact that as the amount of cross-linking agent was increased the stability of the polymeric structure increases. The increase in the stability of the polymeric structure is due to the increased number of cross-linking points within the polymeric network.

As the cross-linking density is increased the swelling of the cross-linked polymer decreases initially up to a certain optimum cross-link density. After this the swelling increases followed by a decrease. This might be the reason for the increase in the % swelling when 0.8 mL of GA was used for the preparation of the hydrogel [27].

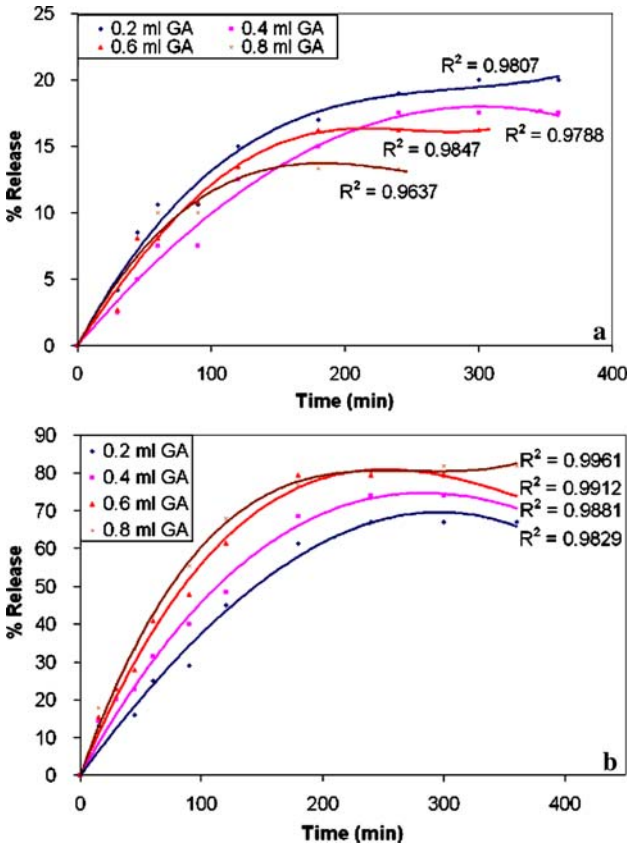

Fig. 9 (**a**) SA release profile from GA cross-linked GP hydrogel at pH ~1.4, (**b**) SA release profile from GA cross-linked GP hydrogel at pH ~7.4

Drug release study

Figure 9a and b shows the SA release profiles from cross-linked hydrogels at pH ~1.4 and ~7.4 respectively. The release study indicated pH dependent release. At pH 1.4 (gastric pH) the release was within 20% of the total drug loading while at pH 7.4 (simulated intestinal pH) it showed a greater release (~ 86%) than at pH 1.4. From this it can be inferred that the cross-linked hydrogels could be tried for controlled drug delivery.

Conclusions

A pH sensitive hydrogel was prepared by the grafting acrylamide on pectin followed by cross-linking with GA. The product developed showed pH dependent release and hence could be tried for controlled drug delivery system. Further the product was found to be biocompatible and can be tried for the development of the implantable devices.

References

1. S. P. VYAS and R. P. KHAR, in "Targeted & Controlled Drug Delivery Novel Carrier Systems" (1st edn. CBS Publishers and distributors, New Delhi, India, 2002) p. 1–5
2. N. A. PEPPAS and A. G. MIKOS, in "Hydrogels in Medicine and Pharmacy—Vol. 1", Edited by: N. A. PEPPAS (CRC Press, Boca Raton, FL, 1986), pp. 1–27
3. L. BRANNON-PEPPAS, in "Absorbent Polymer Technology", Edited by: L. BRANNON-PEPPAS and R. S. HARLAND (Elsevier, Amsterdam, 1990), pp. 45–66
4. Y. KIMURA, in "Biomedical Applications of Polymeric Materials", Edited by: T. TSURUTA et al. (CRC Press, Boca Raton, FL, 1993) pp. 8–9
5. B. D. RATNER and A. S. HOFFMAN, in "Hydrogels for Medical and Related Applications, ACS Symposium Series, No. 31", Edited by: J. D. ANDRADE (American Chemical Society, Washington, DC, 1976), p. 1–36
6. N. A. PEPPAS, in "Hydrogels in Medicine" (CRS Press, Boca Raton, FL, 1986)
7. N. A. PEPPAS and R. LANGER, *Science* **263** (1994) 1715–1720
8. K. PARK, in "Controlled Release: Challenges and Strategies", (American Chemical Society, Washington, DC, 1997)
9. N. A. PEPPAS, *Curr. Opin. Coll. Int. Sci.* **2** (1997) 531–537
10. O. FELT, P. BURI and R. GURNY, *Drug Dev. Ind. Pharm.* **24** (1998) 979–993
11. L. HOVGAARD and H. BRONDSTED, *Crit. Rev. Ther. Drug Carrier Syst.* **13** (1996) 185–223
12. V. R. SINHA and R. KUMRIA, *Int. J. Pharm.* **224** (2001) 19–38
13. S. C. WERCH and A. C. IVY, *Am. J. Digest. Dis.* **8** (1941) 101–105
14. M. ASHFORD and J. T. FELL, *J. Drug Target.* **2**(3) (1994) 241–257
15. J. P. F. BAI and J. H. GUO, *Crit. Rev. Ther. Drug Carrier Syst.* **12**(4) (1995) 339–371
16. F. M. ROMBOUTS and J. F. Thibault, in "Chemistry and Function of Pectins", Edited by: M. L. FISHMAN and J. J. JEN (Washington, DC: American Chemical Society, ACS Series 310, 1986) p. 49–60
17. J. JOHN MEISTER, in "Principle, Techniques and Application" (Marcel Dekker, Inc., 2000) p. 331
18. G. F. FANTA, in "Synthesis of graft and block copolymers of starch, block and graft copolymerisation—Vol 1", Edited by: R. J. CERESA (Wiley, New york, 1973) p. 11
19. A. K. JAIN, N. S. THOMAS and R. PANCHAGNULA, *J. Control Release* **79** (2002) 93–101
20. R. PANCHAGNULA, K. STEMMER and W. A. RITSCHEL, *Exp. Clin. Pharmacol.* **19** (1997) 335–391
21. T. W. WONG, H. Y. LEE, L. W. CHAN and P. W. S. HENG, *Int J Pharm* **242** (2002) 233–237
22. EN 30993 Standard. Biological evaluation of medical devices * Part 4: selection of tests for interactions with blood, 1993
23. M. B. HANSEN, S. E. NIELSEN and K. BERG, *J Immunol Methods*, **119** (1989) 203e10
24. T. MOSMANN, *J Immunol Methods* **65** (1983) 55e63
25. H. N. CHENG, M. TAKAI and E. A. EKONG, *Macromol. Symp.* **140** (1999) 145–153
26. D. GÓMEZ-DÍAz and J. M. NAVAZA, *J. Food Eng.* **56** (2003) 387–392
27. A. ANIS, A. K. BANTHIA, S. MONDAL and A. K. THAKUR, *Chin. J. Polym. Sci.* **5** (2006) 449