Polym. Bull. (2011) 66:583–598
DOI 10.1007/s00289-010-0294-x

ORIGINAL PAPER

Controlled release of 5-aminosalicylic acid from a new pH responsive polymer derived from tamarind seed polysaccharide, acrylic acid, and polyamidoamine

R. K. Dey · G. S. Tiwary · Tanushree Patnaik · Usha Jha

Received: 10 October 2009 / Revised: 30 March 2010 / Accepted: 14 May 2010 /
Published online: 25 May 2010

Abstract A new pH-responsive polymer (TKP–AA–PAA) was synthesized from the combination of tamarind kernel powder (TKP), acrylic acid (AA), and polyamidoamine (PAA) which was utilized for controlled release of 5-aminosalicylic acid (5-ASA) in buffer medium. The network structure of TKP–AA–PAA was obtained by irradiating the mixture of TKP, AA, and PAA in different proportion in presence of 2,2-dimethoxy-2-phenyl acetophenone as a photoinitiator. The dynamic and equilibrium swelling properties of the polymeric materials were studied as a function of pH and time in different buffer solutions similar to that of gastric and intestinal fluids. The controlled release kinetics of 5-ASA in simulated body fluid showed a Fickian diffusion behavior.

Keywords Polymer · pH-sensible · Poly(amido amine) · Polysaccharide · 5-Aminosalicylic acid

Introduction

Biologically sensitive materials mimic biological activities and thus interact with living tissue both in vitro and in vivo [1, 2]. Such kind of materials often find promising applications in the emerging fields of tissue engineering and controlled drug release applications [1–5]. The use of hydrogel materials in controlled release applications is preferred because of various advantages such as high water retention capacity and soft consistency similar to that of natural tissue system in addition to good biocompatibility in comparison to other class of synthetic biomaterials.

R. K. Dey (✉) · T. Patnaik
Post Graduate Department of Chemistry, Ravenshaw University, Cuttack 753 003, Orissa, India
e-mail: rkdey@rediffmail.com

G. S. Tiwary · U. Jha
Department of Applied Chemistry, Birla Institute of Technology, Mesra, Ranchi 835 215, India

The crosslinking in hydrogel material results in formation of an insoluble network structure which facilitates swelling and hydration of polymeric material in simulated body fluid without complete dissolution of the polymer [6]. The high permeable nature of hydrogel material facilitate exchange of oxygen, nutrient, and other water-soluble metabolites, thus finding immense utility as targetable carriers of bioactive agents with desirable biological properties [7]. The advantages as well as limitations of commonly employed stimuli sensitive polymers prepared from poly(acrylic acid) and poly(*N*-isopropylacrylamide) have been recently discussed by Sun et al. [8]. Studies regarding the development of new responsive systems, which would more closely resemble the normal physiological process and the amount of drug released can be monitored according to physiological needs, have been conducted by a number of research groups [3, 9].

Natural polysaccharides, as well as their derivatives, represent a group of polymers that have been widely used in pharmaceutical formulations and controlled drug release studies [10]. For example, the critical role of saccharide moieties in cell signaling schemes for immune recognition has contributed to the growing recognition of polysaccharide-based biomaterials [11]. Tamarind seed polysaccharide (obtained from seed kernel of *Tamarindus indica* also known as tamarind kernel powder—TKP) is non-carcinogenic, mucoadhesive, biocompatible, and found to exhibit properties like high viscosity, broad pH tolerance, and good adhesion behavior [12]. Formation of hydrogel material involving tamarind polysaccharide is a suitable option from biomaterial point of view because the structure involves the backbone as that of cellulose which is also water soluble.

Polyamidoamines (PAAs) are a family of polymers characterized by the presence of amido and tertiary amino groups regularly arranged along the macromolecular chain. Linear PAAs are obtained by the polyaddition reaction of primary monoamines or *bis*(secondary amine)s to *bis*-(acrylamide)s [13]. PAAs find potential applications in biomedical field of research. For example, PAA-grafted biomaterials and PAA-based crosslinked enzymatic biodegradable hydrogels are being considered as soluble carriers for anticancer drugs, intracellular delivery of proteins, and genes [14–18].

This investigation reports the preparation of new polymeric materials using combination of PAA, TKP, and acrylic acid (AA). In this combination, PAA could act as a suitable carrier material for drug molecule and simultaneously as a crosslinker, whereas AA is being used to form semi-interpenetrating (semi-IPN) network structure and to incorporate the pH responsive behavior in the materials. Selective delivery of contents at desirable sites may also possible where appropriate biological enzymes are present.

Experimental

Materials

TKP was a gift sample from Hindustan Gum and Chemicals, Bhiwani, Haryana, India. AA (Fluka A.G.) was vacuum distilled at 63 °C/12 mmHg prior to use in order to remove the inhibitor. Piperazine (Pip) and *N*,*N*′-methylenebisacrylamide

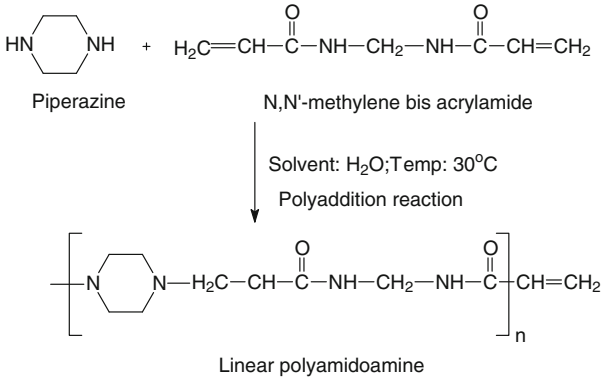
(MBA) (Fluka A.G.) were used without further purification. 2,2-dimethoxy-2-phenylacetophenone was purchased from Acros Organics. 5-aminosalicylic acid (5-ASA) was used as drug in the release processes and phosphate buffers with different pH (2.4, 6.8, and 7.4) were used as physiological mediums. The buffer solutions were prepared from a mixture of phosphoric acid (54.0 mmol), boric acid (40.0 mmol), and acetic acid (42.0 mmol) then adjusting the pH to the required value by dropwise addition of 0.2 N NaOH solutions.

Preparation of poly(amido amine)

The preparation of poly(amido amine) from piperazine and methylene-*bis*-acrylamide was carried out using procedure similar to Tanzi and Levi [19]. PAA was prepared by dissolving 7.7 g of MBA and 4.19 g of Pip (1:1.5 M ratio) in 50 mL double-distilled water. The reaction mixture was stirred under nitrogen atmosphere for 48 h at 30 °C. The viscous solution was poured into 50 mL of acetone. The PAA, consisting of Pip-MBA, was separated out as a white crystalline product, which was filtered, washed with acetone, and recrystallized from diethyl ether. The product was stored in air-sealed bottle. The schematic reaction is shown in Scheme 1.

Preparation of semi-IPN polymeric gel

Semi-IPN polymeric gel was prepared by variation of ratios of TKP:PAA:AA (Table 1). The samples were prepared using the appropriate amount of PAA dissolved in 20 mL of methanol, added to a mixture of solution of TKP in AA. To this mixture, a solution of 2,2-dimethoxy-2-phenyl acetophenone (2 wt% based on the PAA) was added to 5-mL methanol with agitation. The reaction mixture was poured into a glass Petri dish and was maintained at room temperature. The polymerization was initiated by irradiation with an incandescent broad-spectrum lamp (Philips Comptalux, 150 W), positioned 25 cm above the Petri dish. Irradiation was continued for 3 h until gelation occurred. The reaction leading to the formation of crosslinked semi-IPN structure is shown in Scheme 2. The polymer



Scheme 1 Synthesis of poly(amido amine)

Table 1 Composition of semi-IPN prepared

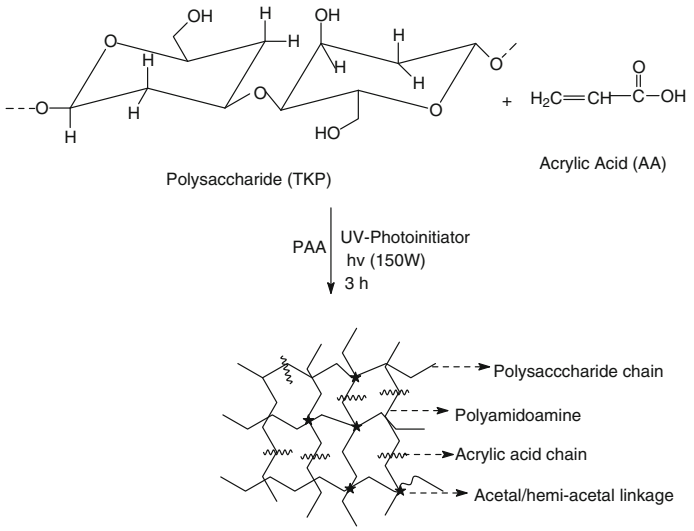
Sample	PAA (g)	TKP (g)	AA (g)
TKP–AA–$PAA_{0.1}$	0.1	1.0	0.5
TKP–AA–$PAA_{0.4}$	0.4	1.0	0.5
TKP–AA–$PAA_{0.6}$	0.6	1.0	0.5
TKP–AA–$PAA_{0.8}$	0.8	1.0	0.5

material was extensively washed with methanol to remove any residual monomer, then freeze-dried, and stored until further use. The resultant product was cut in films, dry in air for 3 days, and place in a vacuum oven at 25 °C until constant weight. The dry disks (gels) were polished to achieve a smooth and uniform surface of 1.00 ± 0.05 mm thickness.

Measurements

PAA was characterized by FTIR (Perkin Elmer Paragon 1000 FTIR spectrometer), and ^{1}H NMR (JEOL-GX 300 FT NMR spectrometer, deuterium oxide solvent) spectroscopy. Scanning electron microscope (SEM) of the sample material was taken using JSM-6390 LV (Jeol, Japan). ^{13}C NMR spectra were recorded at 300 MHz with a Bruker 300P spectrometer.

The quantity of drug entrapped in the polymer was determined using the difference between the amount of drug initially added to the gel and that estimated in the washings [20]. Thus, after the preparation of polymer the washings were collected, filtered with a 0.45-mm Millipore filter, and amount of 5-ASA present estimated from the absorption at 214 nm.



Scheme 2 Reaction scheme showing proposed network (Semi-IPN) structure

The swelling behavior of the polymeric materials was measured at 37 °C temperature in buffer solutions similar to that of gastric and intestinal fluids. The buffer solutions were prepared from a mixture of phosphoric acid (54.0 mmol), boric acid (40.0 mmol), and acetic acid (42.0 mmol) then adjusting the pH to the required value by the dropwise addition of 0.2 N NaOH solutions. The pH values were precisely checked by a pH-meter [Systronic digital pH meter, model 335 equipped with calomel glass electrode (accuracy ±0.1)]. The swollen weights of the gels were determined at intervals, after removal of the surface liquid using tissue paper, until equilibrium swelling was attained. The percent swelling was calculated by as follows

$$\%\text{Swelling} = 100\,[W_t - W_0]/W_0$$

where W_0 is the initial weight and W_t the final weight of the gel at time t. Data points are means of three determinations. Less than 5% variation from the mean was observed in all cases.

In vitro cumulative release studies by ultraviolet spectroscopy

The release of entrapped 5-ASA in vitro was determined by placing the pre-weighed gel loaded with drug in a buffer solution (pH 2.4, simulating gastric fluid, or pH 6.8 and 7.4, simulating intestinal fluid at 37 °C. At definite time intervals, an aliquot was withdrawn and its absorbance at 214 nm measured. The withdrawn sample was replaced with an equal volume of fresh buffer, to keep the volume of release media constant. Data points were means of three determinations. The amount of drug at any selected time was calculated from the 5-ASA calibration curve. This study was carried out for 24 h. The goodness of the kinetic results was verified by using the program Origin 6.0 for Windows XP Professional. Less than 5% variation from the mean was observed in all cases.

Results and discussion

Characterizations

The FTIR spectrum (Fig. 1) of PAA displayed a peak at 1530 cm^{-1}, assigned to C=C stretching. Peaks at 1630 and 1498 cm^{-1} were assigned to amide linkage, whereas signals due to N–H stretching appeared at 3314 cm^{-1} [21]. The ^1H NMR spectrum for PAA shows characteristic vinylic proton signals at 4.5 ppm. Peaks observed in the range 2.43–2.92 ppm were assigned to the $–CH_2–$ bonded to carbonyl group and nitrogen. Presence of broad peak at 4.8 ppm attributed to the DOH used to solubilize PAA.

In the FTIR spectrum of TKP, a broad peak at 3556 cm^{-1} was assigned to –OH stretching vibrations. The bands at 1120 and 2926 cm^{-1} are assigned to C–O stretching and C–H stretching, respectively. One strong band at 1039 cm^{-1} is attributed to $CH_2–O–CH_2$ stretching vibrations. The ^{13}C NMR spectrum of TKP, in Fig. 2, shows three distinct peaks. The absorption peak at δ 105 ppm is assigned to anomeric carbon atom and the peak at δ 78 ppm is assigned to the carbon atoms

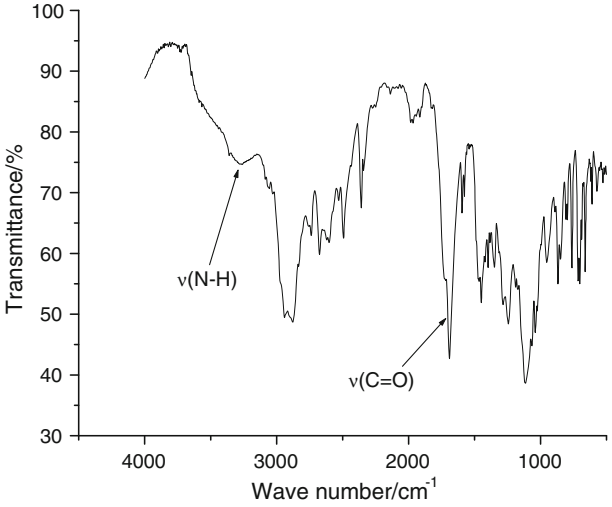


Fig. 1 FTIR Pip-MBA

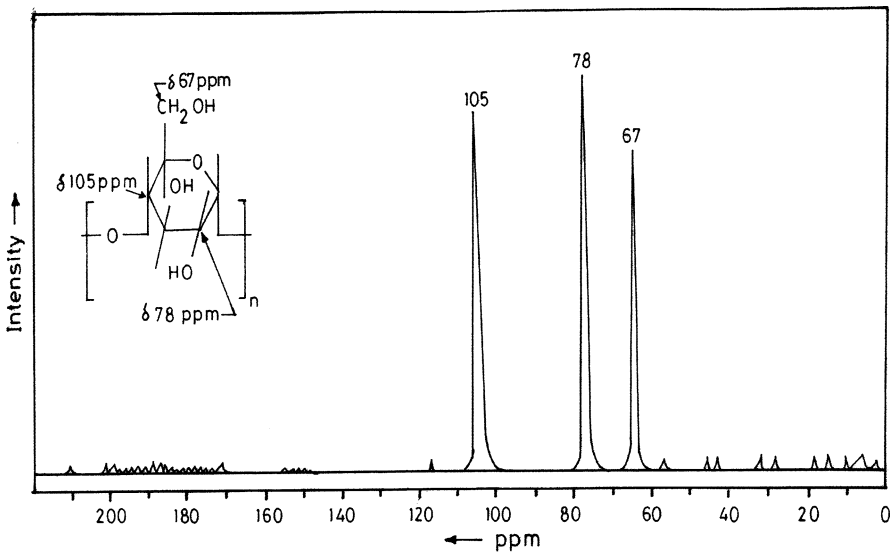


Fig. 2 ^{13}C NMR of TKP

connected by –OH groups (i.e., the carbon atoms in the six-membered ring except anomeric carbon atom). The presence of peak at δ 67 ppm is attributed to the carbon atom of CH_2OH group.

The FTIR spectrum of TKP–AA and TKP–AA–$PAA_{0.8}$ is illustrated in Fig. 3. The spectrum of TKP–AA shows a peak in the range 2900–3000 cm^{-1}, assigned to –OH stretching vibrations. The presence of peak 1720 cm^{-1} is assigned to the

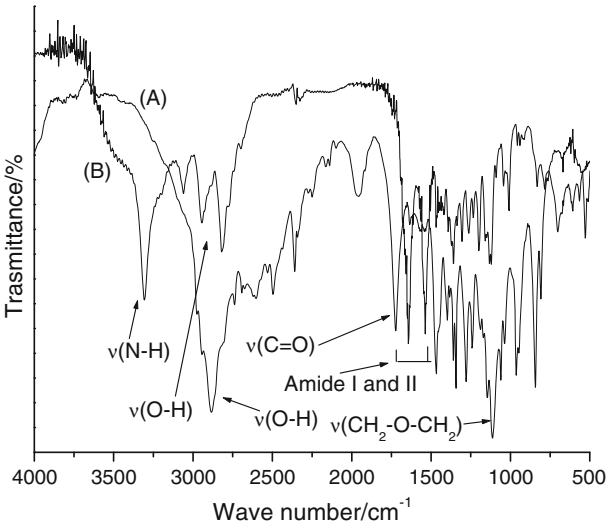


Fig. 3 FTIR spectra of *a* TKP–AA, *b* TKP–AA–PAA

carbonyl group vibrational frequency resulted from free carboxylic group in the material. In addition, presence of one strong band at 1090 cm^{-1} is attributed to CH_2–O–CH_2 stretching vibrations of polysaccharide moiety. The FTIR spectrum of TKP–AA–PAA shows the presence of carbonyl carbon at 1680 cm^{-1} due to the presence of carboxylic acid group as well as the amide-I (CONH) group. The characteristic amide-II linkage found at 1500 cm^{-1}. The appearance of small shoulder peaks on the carbonyl peak indicates the formation of intermolecular bonding [3]. Absorption bands found at 1500 and 514 cm^{-1} are characteristics of AA [22]. The presence of NH vibrational frequency is observed at 3300 cm^{-1}. With the formation of semi-IPN structure, the spectrum shows more complexity in finger print region. Further, the ^{13}C NMR spectrum of TKP–AA–$PAA_{0.8}$, illustrated in Fig. 4, shows the presence of various carbon atoms as indicated in the inserted structures. The presence of carbon atom of CH_2OH is observed at 75 ppm. Other carbon atoms pertaining to the presence of ethylenic linkage are observed in the range 10–30 ppm. The presence of peaks in the range 30–50 ppm shows the presence of –C–O–C– which is the exclusive characteristics of polysaccharide moiety in the structure. Thus, all the above characterizations confirm the formation of polymer where PAA has been incorporated in the structure containing TKP and AA.

The morphological features of PAA and TKP–AA–PAA were observed in SEM using the technique of Xue et al. [23]. The micrograph (Fig. 5) shows a unique feature of PAA where a number of long chain PAA got agglomerated. On the other hand, the SEM of TKP–AA–PAA, as shown in Fig. 6, was observed to be more open and porous structure.

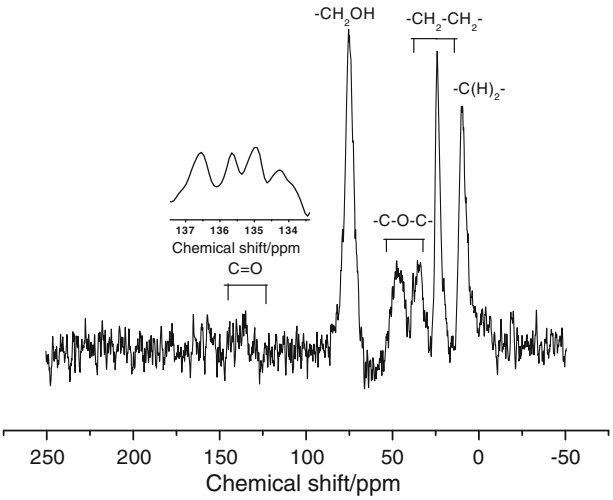


Fig. 4 ^{13}C NMR of TKP–AA–$PAA_{0.8}$

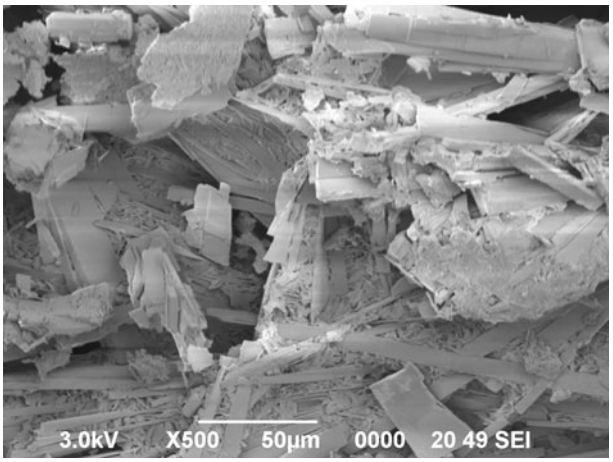


Fig. 5 SEM of poly(amido amine) of Pip-MBA

Equilibrium swelling studies

The % equilibrium swelling values of the prepared polymers TKP–AA–PAA_x and control TKP–AA are measured at different time interval at 37 °C temperature. Figure 7 shows the equilibrium swelling of TKP–AA–PAA_x (x = ratio of PAA in the matrix) at constant pH of 7.2 and at different time intervals. On comparison of amount of swelling at same pH, it was found that with increase in PAA content, the % swelling decreases, indicating an increase in extent of crosslinking in the structure. For TKP–AA–$PAA_{0.1}$, a higher swelling value of 1402% was noted within a time period of 8 h. Whereas the % swelling for corresponding control

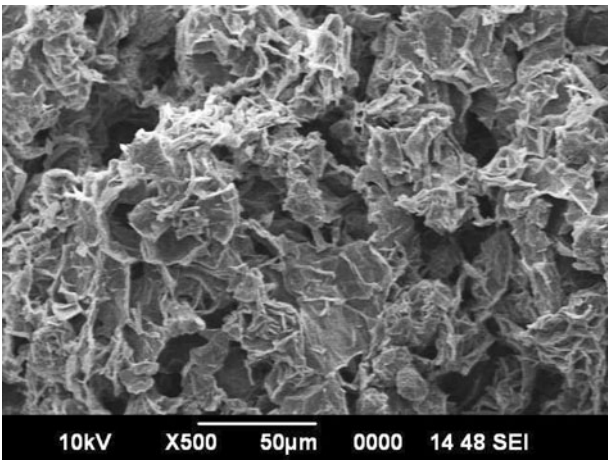


Fig. 6 SEM of TKP–AA–PAA

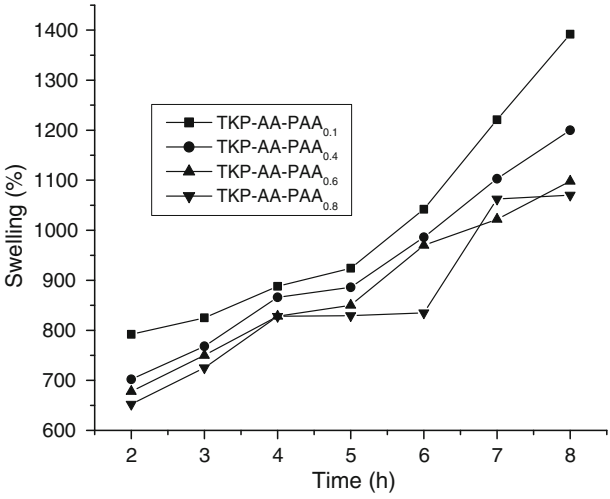


Fig. 7 Equilibrium swelling of TKP–AA–PAA_x (x = ratio of PAA), at pH 7.2 and different time intervals

TKP–AA polymer at various pH and time interval demonstrated a lesser value. Equilibrium swelling of TKP–AA–$PAA_{0.8}$ at different time intervals and with variation of pH of the medium was illustrated separately in Fig. 8. The result indicated that with lowering of pH of the medium the percentage of equilibrium swelling decreases. TKP–AA–$PAA_{0.8}$ as a matrix for controlled release application is judiciously chosen for maximizing incorporation of drug molecule to the matrix. The combination of hydrophilic nature of polysaccharide unit and PAA could better facilitate the swelling of gel by attracting water toward the core of the gel. In this

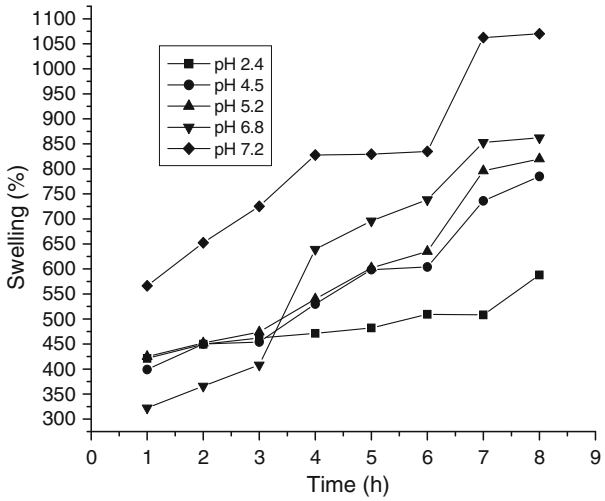
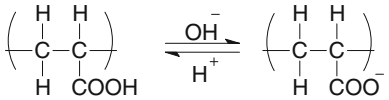


Fig. 8 Equilibrium swelling of TKP–AA–$PAA_{0.8}$ at various pH and different time intervals

Scheme 3 Ionization of carboxylic acid with variation of pH of the medium



investigation it was found that the % equilibrium swelling values were found to be higher at pH 7.4 than corresponding values at pH 6.8 and 2.4 for all polymeric materials under consideration. The observation can be attributed to the fact that anionic hydrogels are basically three-dimensional polymer networks capable of swelling by absorbing large amounts of water or aqueous solvents. Therefore, the swelling behavior is directly dependent on changes in external environmental conditions, such as the pH, ionic strength, solvent composition, and temperature [24, 25]. In particular, anionic hydrogels exhibit a drastic change in swelling that depends on the environmental pH change which makes them comparatively more suitable candidates for designing carrier systems for oral drug delivery [26]. A pictorial demonstration of pH-responsive swelling behavior of the prepared polymer is shown in Scheme 3, where the swelling of the polymeric material could be attributed mainly to the ionization of functional groups in the matrix. Ionization process is known to affect significantly the penetrant transport mechanism of the polymer networks. In case of anionic polymeric gel material, an increase in the degree of ionization contributes to electrostatic repulsion between charged groups present in the polymer matrix which swells the gel material to a high degree. A highly swollen gel contains large amounts of unbound water that allows greater solute release.

Polymeric materials made of polyacrylic acid or polymethacrylic acid found immense usefulness in developing formulations that release drugs in a neutral environment [27]. Polymers made of poly-ions crosslinked with azoaromatic

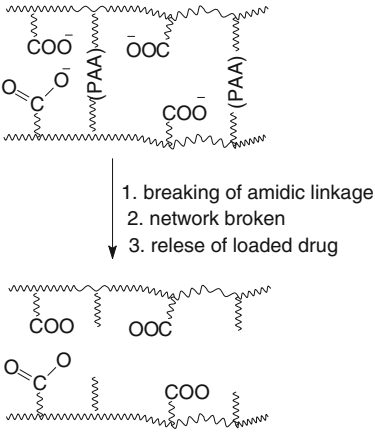


Fig. 9 Semi-IPN structure showing the possible way of breaking of amidic bond in PAA in simulated environment

crosslinkers were developed for colon-specific drug [24]. Swelling of such polymers in the stomach is minimal and thus the drug release is also minimal. The extent of swelling increases as the polymers passes down the intestinal tract due to increase in pH which leads to ionization of the carboxylic groups. For example, the study conducted by Ghandehari et al. [28] shows that at low pH, in the simulated environment of the colon, azoaromatic crosslinks of polymeric materials can be degraded by azoreductase by the microbial flora of the colon. In this context, Fig. 9 shows the schematic process of release of drug molecule from the prepared polymeric material mainly due to the degradation of the polymeric chain (semi-IPN structure) following the breaking of amidic linkage in simulated body's physiological environment.

In vitro cumulative release studies

The cumulative release profiles of 5-ASA from the polymeric network structure at 37 °C, for pH 6.8 and 7.4, are shown in Figs. 10 and 11, respectively. The release profiles indicated that more amount of drug molecule could be released from the matrix with increase in pH of the medium. A comparison was made regarding the amounts of drug released from TKP–AA–$PAA_{0.1}$ and TKP–AA–$PAA_{0.8}$ at the same pH which shows that the extent of release at equilibrium is relatively higher in TKP–AA–$PAA_{0.1}$ than that of TKP–AA–$PAA_{0.8}$. This could be attributed to the swelling of polymers with variation in PAA content where the breaking of PAA chain in a simulated environment facilitated the controlled release of drug molecules to the environment. Present investigation shows that the cumulative release of drug molecules attained equilibrium after 10 h in a simulated environment which is just sufficient for its applications in various stomach-related diseases. A control polymer of TKP–AA was also prepared to compare the cumulative release profile of 5-ASA (Fig. 12) which indicated maximum release of drug molecules within 2 h in simulated body fluid. Therefore, by optimizing the amount of drug loading vis-à-vis PAA content, it could be possible to achieve the desired objective of efficient delivery in colon specific drug molecules. In this case,

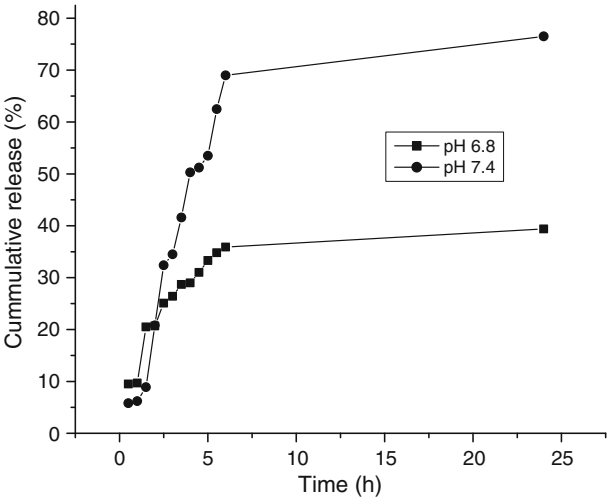


Fig. 10 Cumulative release profile of 5-ASA from TKP–AA–$PAA_{0.1}$ various pH and different time intervals

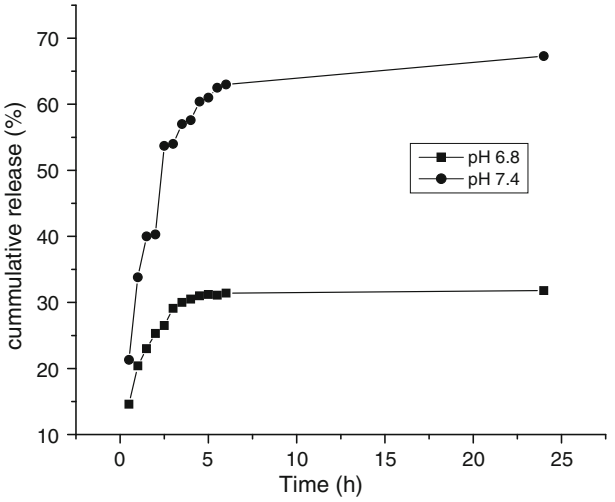


Fig. 11 Cumulative release profile of 5-ASA from TKP–AA–$PAA_{0.8}$ in different pH of medium

the preliminary study was carried out using a single-model drug (small and highly hydrophilic in nature) and therefore, application of matrix for entrapment of other suitable drug molecules worth investigation. The controlled release of quantitatively less amount of drug from the gel could be attributed to the fact that some drug molecules might be deeply buried in the network structure and a slower releasing pattern into the surrounding media is expected. In other words, the quantitative release of 100% of drug tends to be unlikely unless the carrier matrix have been completely dissolved.

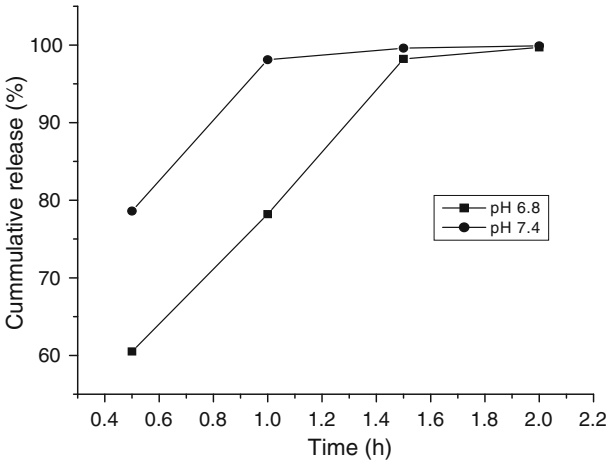


Fig. 12 Cumulative release profile of 5-ASA from TKP–AA in different pH of medium

Diffusion process at different pH

To know the type of diffusion of drug molecules in different buffered solutions, Eq. 1 was used. In this investigation, the polymeric material TKP–AA–$PAA_{0.8}$ was used at the early stages of the swelling process (until the first 30 min of the experiment) where the thickness of the sample is almost constant. For polymer films with constant thickness, Fick's equation can be written as:

$$M_t/M_e = kt^n \tag{1}$$

where M_t and M_e are the amount of buffer solution (drug) absorbed by the polymeric material at time 't' and in the equilibrium, respectively. k is a characteristic constant of the system and n is an exponent related to the kind of transport of the buffer solutions. The value of $n = 0{:}5$ indicates a Fickian diffusion process, but $0.5 < n < 1$ indicates non-Fickian or anomalous diffusion. In the special case in which $n = 1$, the transport mechanism is named Type II diffusion. In the representation of ln (M_t/M_e) versus ln t, a linearity could be observed until values of the swelling fraction $M_t/M_e \leq 0.60$ (first 60% of the dynamics water uptake data), and n and k are obtainable from the slope and the intercept, respectively. In our case, value of n close to 0.50 was obtained; therefore, it can be considered a Fickian behavior for which the diffusion coefficients can be obtained from the slope (k) of the plot of M_t/M_e versus $t^{1/2}$ (Fig. 13) in buffer solutions of pH 6.8 and the expression can be written as (Eq. 2) [29]:

$$k = 4\left(D_i/\pi l^2\right)^{1/2} \tag{2}$$

where D_i can be interpreted as diffusion coefficient in the glass region and l is the thickness of the sample. The kinetic experiment shows a straightline with correlation coefficient of 0.9618. The corresponding value of D_i calculated to be 0.859×10^{-5} cm^2 seg^{-1}.

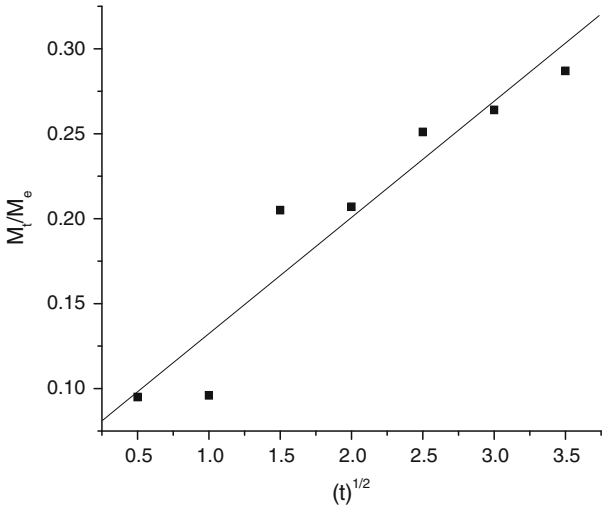


Fig. 13 Plot of M_t/M_e versus $t^{1/2}$ at pH 6.8 for TKP–AA–$PAA_{0.8}$

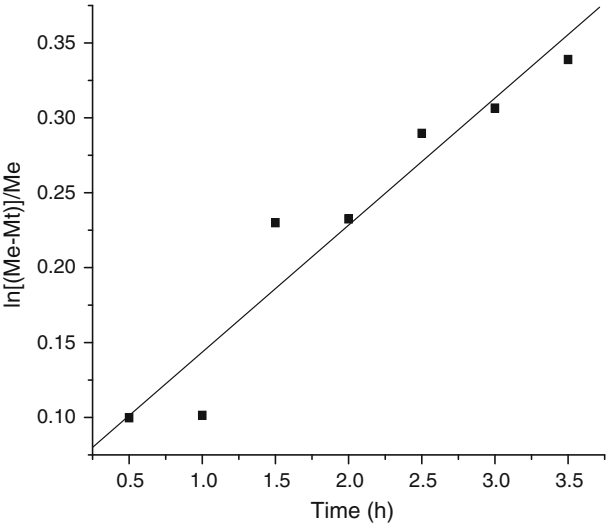


Fig. 14 Plot ln $[(M_e - M_t)/M_e]$ versus t at pH 6.8 for TKP–AA–$PAA_{0.8}$

However, for longer duration of swelling process, a modified Eq. 3 can be represented as:

$$\ln[(M_e - M_t)/M_e] = \ln 8/\pi^2 - \pi^2 Dt/l^2 \quad (3)$$

where M_t and M_e are the amount of buffered or drug solution in the polymeric material at time t and in the equilibrium, respectively, D the diffusion coefficient, t the time, and l the thickness of the sample material. The plot of ln $[(M_e - M_t)/M_e]$

versus t (Fig. 14) shows a straightline with correlation coefficient of 0.9688 from which the value of D for longer time of swelling process was found out to be 0.916×10^{-1} cm^2 seg^{-1}.

Conclusions

A new pH responsive drug delivery system constituting polysaccharide, AA, and poly(amido amine) was prepared using environment-friendly photo polymerization process at room temperature. The equilibrium swelling measurements of prepared polymers in simulated body fluid shows pH responsive characteristics. The preliminary in vitro release profiles of 5-ASA shows sustained release behavior over a period of time, indicating that the polymeric material could find potential applications for controlled oral delivery of various therapeutic agents. By variation of molar proportion of PAA in the network structure, the amount of loading and release can be monitored which worth for further investigations.

Acknowledgment R.K.D. is thankful to Professor A. R. Ray for useful discussions. Kind help of CIF, BIT, Mesra, is thankfully acknowledged.

References

1. Langer R, Peppas NA (2003) Advances in biomaterials, drug delivery, and bionanotechnology. AIChE J 49:2990–3006
2. Sokolsky-Papkov M, Agashi K, Olaye A, Shakesheff K, Domb AJ (2007) Polymer carriers for drug delivery in tissue engineering. Adv Drug Deliv Rev 59:187–206
3. Devine DM, Higginbotham CL (2005) Synthesis and characterisation of chemically crosslinked *N*-vinyl pyrrolidinone (NVP) based hydrogels. Eur Polym J 41:1272–1279
4. Choi KY, Lee S, Park K, Kim K, Park JH, Kwon IC, Jeong SY (2008) Preparation and characterization of hyaluronic acid-based hydrogel nanoparticles. J Phys Chem Solids 69:1591–1595
5. Dayananda K, He C, Park DK, Park TG, Lee DS (2008) pH- and temperature-sensitive multiblock copolymer hydrogels composed of poly(ethylene glycol) and poly(amino urethane). Polymer 49:4968–4973
6. García DM, Escobar JL, Noa Y, Bada N, Hernáez E, Katime I (2004) Timolol maleate release from pH-sensible poly(2-hydroxyethyl methacrylate-co-methacrylic acid) hydrogels. Eur Polym J 40:1683–1690
7. Tanaka Y, Gong JP, Osada Y (2005) Novel hydrogels with excellent mechanical performance. Prog Polym Sci 30:1–9
8. Sun JX, Luo YF, Peng H, Han ZW (2008) Design, synthesis and characterization of a novel pH-sensitive hydrogel. Chin Chem Lett 19:1475–1478
9. Kost J, Langer R (2001) Responsive polymeric delivery systems. Adv Drug Deliv Rev 46:125–148
10. Coviello T, Palleschi A, Grassi M, Matricardi P, Bocchinfuso G, Alhaique F (2005) Scleroglucan: a versatile polysaccharide for modified drug delivery. Molecules 10:6–33
11. Francis Suh J-K, Matthew HWT (2000) Application of chitosan-based polysaccharide biomaterials in cartilage tissue engineering: a review. Biomaterials 21:2589–2598
12. Xiao C, Li H, Gao Y (2009) Preparation of fast pH-responsive ferric carboxymethylcellulose/poly(vinyl alcohol) double-network microparticles. Polym Int 58:112–115
13. Ferruti P, Marchisio MA, Duncan R (2002) Poly(amido-amine)s: biomedical applications. Macromol Rapid Commun 23:332–355
14. Bignotti F, Sozzani P, Ranucci E, Ferruti P (1994) NMR studies, molecular characterization, and degradation behavior of poly(amido amine)s. 1. Poly(amido amine) deriving from the polyaddition of 2-methylpiperazine to 1,4-bis(acryloyl)piperazine. Macromolecules 27:7171–7178

15. Ferruti P, Manzoni S, Richardson SCW, Duncan R, Pattrick NG, Mendichi R, Casolaro M (2000) Amphoteric linear poly(amido-amine)s as endosomolytic polymers: correlation between physico-chemical and biological properties. Macromolecules 33:7793–7800
16. Kosmala JD, Henthorn DB, Brannon-Peppas L (2000) Preparation of interpenetrating networks of gelatin and dextran as degradable biomaterials. Biomaterials 21:2019–2023
17. Richardson SCW, Pattrick NG, Man YKS, Ferruti P, Duncan R (2001) Poly(amidoamine)s as potential nonviral vectors: ability to form interpolyelectrolyte complexes and to mediate transfection in vitro. Biomacromolecules 2:1023–1028
18. Pattrick NG, Richardson SCW, Casolaro M, Ferruti P, Duncan R (2001) Poly(amidoamine)-mediated intracytoplasmic delivery of ricin A-chain and gelonin. J Control Release 77:225–232
19. Tanzi MC, Levi M (1989) Heparinizable segmented polyurethanes containing poly-amidoamine blocks. J Biomed Mater Res 23:863–881
20. Wiwattanapatape R, Lomlim L, Saramunee K (2003) Dendrimers conjugates for colonic delivery of 5-aminosalicylic acid. J Control Release 88:1–9
21. Nakanishi K (1966) Infrared absorption spectroscopy practical, 4th edn. Nankodo Company Ltd., Tokyo, pp 142–143
22. Borzacchiello A, Ambrosio L, Netti PA, Nicolais L, Peniche C, Gallardo A, Roman SJ (2001) Chitosan-based hydrogels: synthesis and characterization. J Mater Sci Mater Med 12:861–864
23. Xue W, Champ S, Huglin MB, Jones TGJ (2004) Rapid swelling and deswelling in cryogels of crosslinked poly(*N*-isopropylacrylamide-*co*-acrylic acid. Eur Polym J 40:467–476
24. Qiu Y, Park K (2001) Environment-sensitive hydrogels for drug delivery. Adv Drug Deliv Rev 53:321–339
25. Firestone BA, Siegel RA (1991) Kinetics and mechanisms of water sorption in hydrophobic, ionizable copolymer gels. J Appl Polym Sci 43:901–914
26. Kim B, Flamme KL, Peppas NA (2003) Dynamic swelling behavior of pH-sensitive anionic hydrogels used for protein delivery. J Appl Polym Sci 89:1606–1613
27. Brannon-Peppas L, Peppas NA (1990) Dynamic and equilibrium swelling behaviour of pH-sensitive hydrogels containing 2-hydroxyethyl methacrylate. Biomaterials 11:635–644
28. Ghandehari H, Kopeckova P, Kopecek J (1997) In vitro degradation of pH-sensitive hydrogels containing aromatic azo bonds. Biomaterials 18:861–872
29. Bajpai SK, Tankhiwale R (2006) Investigation of dynamic release of vitamin B_2 from calcium alginate/chitosan multilayered beads: part II. React Funct Polym 66:1565–1574