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Acrylic acid–methyl methacrylate (2.5:7.5/2:8) enteric copolymer for colon targeted drug delivery

Saurabh Vijay · O. P. Sati · Dipak K. Majumdar

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Abstract Enteric copolymers of acrylic acid and methyl methacrylate (2.5:7.5 and 2:8) were prepared using tetrahydrofuran as solvent and AIBN as free radical initiator for colon targeting. FTIR and ^{1}H NMR spectra of the copolymers showed absence of vinyl bond/protons present in the monomers suggesting successful polymerization. Flurbiprofen sodium microspheres (M1 and M2) made with the copolymers, by oil/oil solvent evaporation, were spherical, anionic (zeta potential –57.8 and –53.7 mV) and contained 5.47 and 5.89% drug. FTIR spectrum of microspheres showed peaks for aromatic C = C stretching and substituted benzene ring, indicating entrapment of flurbiprofen. PXRD revealed crystalline structure of flurbiprofen while copolymer and microspheres were amorphous. DSC thermograms showed a sharp melting endotherm of flurbiprofen sodium at 129.26°C against broad endotherms of copolymers and microspheres. The microspheres released 43 and 36% drug at pH 6.8 in 2 h and 99 and 96% at pH 7.4 in next 3–4 h.The microspheres did not adhere on gastric-mucosa at pH 1.2 but showed mucoadhesion time of 18 min and 9 min on intestinal mucosa at pH 6.8. Thus, the microspheres on oral administration, would release the drug in colon, suggesting the potential of the hemocompatible copolymers for pH dependent colon targeted drug delivery system.

Keywords AA · MMA · Microspheres · Enteric · Release · Colon

1 Introduction

Oral colon-targeted drug-delivery systems have recently gained importance for delivering a variety of therapeutic agents for the treatment of colon diseases. The major obstacles to colonic drug delivery are the absorption and degradation pathways in the upper gastrointestinal tract (GIT). The interest of research in the area of colonic delivery is due to the need to better treat the diseases of the colon. These diseases range in seriousness from constipation and diarrhea to the debilitating inflammatory bowel diseases (ulcerative colitis and Crohn's disease) through to colon carcinoma, the third most prevalent form of cancer in both men and women [1]. Colon targeted drug delivery would therefore, ensure direct treatment at the disease site and, therefore, lower dosing and reducing systemic side effects. A variety of approaches and systems have been developed for the purpose of achieving colon targeting. In general four primary approaches have been proposed for delivery of drugs to the colon via oral route namely pH dependent system, prodrugs, time dependent systems and the utilization of carriers that are degraded exclusively by the colonic micro-flora [2]. Among the various systems for

S. Vijay
Department of Pharmaceutical Chemistry, Dolphin (PG) Institute of Biomedical and Natural Sciences, Manduwala, Dehradun, Uttarakhand 248007, India

O. P. Sati
Department of Chemistry, H.N.B. Garhwal University, Srinagar, Garhwal, Uttarakhand, India

D. K. Majumdar (✉)
Department of Pharmaceutics, Delhi Institute of Pharmaceutical Sciences and Research, (Formerly College of Pharmacy), University of Delhi, Pushp Vihar, Sector III, New Delhi 110017, India
e-mail: dkmajumdaar@yahoo.com; dkmajumdar@gmail.com

targeted drug delivery to colon, common are prodrug approach [3] and multi-unit systems like microsponges [4] and microspheres [5] as drug delivery vehicles.

The single-unit colon targeted drug delivery systems may suffer from the disadvantage of unintentional disintegration of the formulation due to manufacturing deficiency or unusual gastric physiology that may lead to drastically compromised systemic drug bioavailability or loss of local therapeutic action in the colon. Recently much emphasis is being laid on the development of multi-unit dosage forms in comparison to single unit systems because of their potential benefits like increased bioavailability; reduce risk of local irritation and predictable gastric emptying [6]. Multiparticulate approaches aimed for colonic delivery consist of formulations in the form of pellets, granules, microparticles and nanoparticles. The preference of multiparticulate system over single unit dosage forms for colon targeting purposes dates back to 1985 when Hardy et al. [7] showed that multiparticulate systems enabled the drug to reach the colon quickly and were retained in the ascending colon for a relatively long period of time. Because of their smaller particle size as compared to single unit dosage forms these systems are capable of passing through the GI tract easily, leading to less inter- and intra subject variability. Moreover, multiparticulate systems tend to be more uniformly dispersed in the GI tract and also ensure more uniform drug absorption [8–10].

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used to treat the chronic disorders caused by inflammation [11, 12]. Flurbiprofen, a phenyl propionic acid derivative NSAID, possesses analgesic and anti-inflammatory actions and is used in rheumatoid arthritis, ankylosing spondylitis, osteoarthritis and gout [13]. In addition, the drug showed a promising activity for prevention and treatment of colon cancers [14–18]. However, when administered orally, flurbiprofen is absorbed from the upper GIT and causes gastric irritation, a side effect shared by most of the NSAIDs [13]. Therefore, it is preferable to deliver the drug site-specifically to the colon [19]. The various approaches used for the colon targeted delivery of flurbiprofen are prodrugs [20], multi-unit systems like microsponges [21] and microspheres [22] as drug delivery vehicles.

In our previous study copolymers of AA and MMA having molar feed ratios of 3:7, 4:6, 5:5, 6:4 and 7:3 were synthesized and evaluated for swelling/dissolution. The release profile of flurbiprofen entrapped in AA and MMA (3:7) microspheres were also evaluated [23]. The present study is aimed at synthesis of acrylic acid–methyl methacrylate copolymers with 2.5:7.5 and 2:8 molar ratios for microencapsulating flurbiprofen for colon targeting.

2 Materials and methods

2.1 Materials

Acrylic acid (AA) and methyl methacrylate (MMA) were purchased from Merck, India and distilled under vacuum prior to use. αα'-azobis-isobutyronitrile (AIBN) of analytical grade was recrystallised before use. Tetrahydrofuran (THF), dichloromethane (DCM), petroleum ether (40–60°), methanol, acetone and all other chemicals were of analytical grade. Flurbiprofen was received as gift from Laborate Pharmaceuticals India Ltd. Paonta Sahib, (HP), India.

2.2 Methods

2.2.1 Synthesis of copolymers of AA with MMA

The copolymers of AA and MMA with 2.5:7.5 and 2:8 molar ratios were prepared by the same method described earlier [23] using THF as solvent and AIBN as free radical initiator. Appropriate quantities of the monomer, comonomer, THF and AIBN were taken in a polymerization tube and mixed vigorously. Then the reaction mixture was flushed with nitrogen gas for 10 min, sealed under nitrogen atmosphere and was immersed in a thermostatic water bath at 65 ± 2°C. After 16 h the excess solvent was removed from the polymer under vacuum. The polymer was then dissolved in minimum amount of methanol and DCM mixture (5:2 ratio) and precipitated in ice cold petroleum ether. The precipitated polymers were dried under vacuum. Subsequently, the polymers were boiled in distilled water for 5–10 min to get rid of unreacted monomers/materials. Then the polymers were collected by filtration and dried to constant weight before use.

2.2.2 Copolymer characterization

Both the copolymers AA:MMA (2.5:7.5 and 2:8) were subjected to Fourier-transform–infrared-spectroscopy (Thermo Nicolet NEXUS-FTIR) in the range of 4000–400 cm^{-1} as KBr pellets. The FTIR spectroscopy of the monomers was taken as such i.e. as neat film. The chemical structure of the copolymers was characterized with ^{1}H NMR (Bruker Avance II 400 MHz NMR Spectrometer). Samples were dissolved in deuterated DMSO and $CDCl_3$ (1:1 ratio). Powder XRD of copolymers was carried out employing Bruker D8 Advance X-ray diffractometer in the range of 3–50° 2θ. Differential scanning calorimetry (DSC) of both the copolymers was carried out using Q 10 differential scanning calorimeter (TA System, USA) at a heating rate of 10°C/min in the temperature range of 38–200°C in N_2 atmosphere. Molecular weights were determined by gel permeation chromatography (GPC) (Hitachi, [Elite La EZChrom, L2130]) relative to polystyrene

standards (Mw = 42,900) with tetrahydrofuran as mobile phase at a flow rate of 0.3 ml/min.

2.2.3 Estimation of apparent monomer composition in copolymer

The AA content of the copolymers was analyzed by slight modification of the method described for methacrylic acid polymers in USP27/NF22 as follows. The dried copolymer (500 mg) was dissolved in 50 ml acetone–methanol mixture (1:1 ratio) and titrated with 0.1 N NaOH using phenolphthalein as an indicator. The AA composition was estimated from the NaOH titer that of MMA as the difference between weight of analysis sample and weight of AA.

2.2.4 Preparation of polymeric membrane

To demonstrate swelling, membrane of above copolymers was prepared as follows: About 500 mg (10% w/v) of each copolymer was dissolved in 5 ml methanol the resulting solution was spread uniformly on to horizontally supported teflon coated Petri dish of 2 cm diameter and kept at room temperature for few hours and then dried over night. The membrane was removed from the petri dish, dried for 24 h at room temperature and stored in a desiccator under vacuum until analysis.

2.2.5 Swelling behavior

Membrane swelling was measured in four different buffers, 0.1 M HCl, i.e. enzyme-free simulated gastric fluid (SGF), (pH 1.2), 0.1 M mixed phosphate buffer (pH 5.5), and 0.2 M phosphate buffer (pH 6.8 and 7.4) prepared as per Indian Pharmacopoeia 1996 Vol.-II. The dry polymer membrane of fixed weight was used and placed in 10 ml of buffer. The buffer was changed to higher pH (starting with pH 1.2 and then 5.5, 6.8 and up to 7.4) after every 2 h. After fixed intervals the membrane was removed from buffer and wiped dry with tissue paper and reweighed. The percentage weight gain (swelling) was determined using Eq. (1).

$$\text{Percentage weight gain} = (W_w - W_d)/W_d \times 100 \quad (1)$$

Where W_w is the weight of wet membrane and W_d is the weight of dry membrane.

2.2.6 Dissolution behavior

The membrane dissolution was measured in the same buffers used in swelling study. The membrane dried to constant weight at 100°C was weighed and placed in 10 ml of buffer. The buffer was changed to higher pH (starting with pH 1.2 and then 5.5, 6.8 and up to 7.4) after every 2 h. After fixed intervals the membrane was removed from buffer washed with water and dried to constant weight at 100°C. The same dried membrane was then put in to higher pH buffer. The percentage dissolution was determined using Eq. (2).

$$\text{Percentage dissolution} = (W_2 - W_1)/W_1 \times 100 \quad (2)$$

Where W_2 is the weight of membrane following buffer treatment, washing and drying and W_1 is the initial weight of membrane.

2.2.7 Hemolysis assay

The hemolytic assay was conducted according to previously reported method [24]. Acid citrate dextrose (ACD) sheep blood was used for this purpose. ACD blood was prepared by adding 1 ml of ACD solution to 9 ml of fresh blood. ACD solution was prepared by mixing 0.544 g of anhydrous citric acid, 1.65 g of trisodium citrate dihydrate and 1.84 g of dextrose monohydrate, to 75 ml of distilled water. Blood testing solution was prepared by diluting 4 ml fresh ACD blood with 5 ml saline. The membrane of both the copolymers was cut into small pieces (approximately 1×1 cm^2) and equilibrated in 4 ml saline for 30 min at 37 ± 1°C. Diluted blood (0.2 ml) was added to each sample and incubated for 60 min at 37 ± 1°C. Positive or negative controls, which did not contain polymer membrane, prepared by adding 0.2 ml of blood to 4 ml of distilled water or normal saline, were similarly incubated for 60 min at 37 ± 1°C. All solutions were centrifuged for 5 min. The optical density (OD) of the supernatant was measured at 545 nm. The degree of hemolysis was calculated as follows:

$$\%\ \text{Hemolysis} = \left(\frac{\text{OD of test sample} - \text{OD of } (-)\text{ control}}{\text{OD of } (+)\text{ control} - \text{OD of } (-)\text{ control}}\right) \times 100 \quad (3)$$

2.2.8 Microencapsulation

Flurbiprofen was encapsulated in the copolymer AA:MMA (2.5:7.5 and 2:8) by oil/oil solvent evaporation technique [10]. In a typical experiment 400 mg of the copolymer and 100 mg of drug were dissolved in a mixture of methanol and DCM (2:3 v/v, 10 ml). The resulting solution was poured drop wise with the help of a burette into 200 ml paraffin oil, containing span 80(0.5 % v/v) for emulsification under constant stirring with a mechanical stirrer at 1200 rpm (Remi Motors Ltd., India—RQ/24A). The system was stirred for 4 h at room temperature to allow the evaporation of the solvent. The microspheres obtained were separated by filtration and washed with petroleum

ether (40–60°) and dried under vacuum. The microspheres prepared with copolymer AA: MMA (2.5:7.5) and AA: MMA (2:8) were designated as M1 and M2 respectively.

2.2.9 Microspheres characterization

Microspheres were characterized for shape by scanning electron microscopy (SEM) (S-3400 N, HITACHI, Japan) and for Zeta potential (Zeta sizer-ZEN 3600—Malvern Instruments, UK).

Surface carboxylic acid concentration of microspheres was determined by potentiometric titration as follows: The microspheres were dispersed in 0.1 N NaOH by stirring for 10 min and the NaOH were titrated with 0.1 N HCl potentiometrically. The titration was conducted with 794 Basic Titrino Autotitrator (Metrohm) using pH glass electrode (capable of working in the range 0–14 pH and temperature up to 80°C) and setting the following parameters.

Titration parameters: Mass point density 4, minimum increment 10 µl, equilibration time 26 s, temperature 25°C; *Stop condition*: stop end point, end point criteria 30 mV.

FTIR spectroscopy, X-ray diffraction and differential scanning calorimetry of the microspheres were done using the same instruments and methods described under polymer characterization.

2.2.10 Determination of drug loading

Hundred milligram microspheres were crushed and dissolved in 100 ml mixture of phosphate buffer (pH 8.0) and methanol (1:1 ratio) and further dilution was made in phosphate buffer (pH 7.4). The drug was estimated by measuring absorbance at 247 nm in a UV spectrophotometer (Thermo Electron Corporation, UK–UV-1).

$$\text{Actual drug loading } (\%) = \text{mg of entrapped drug}/100 \text{ mg microspheres} \quad (4)$$

$$\text{Drug loading efficiency } (\%) = \text{Actual drug loading}/\text{Theoretical drug loading} \times 100 \quad (5)$$

2.2.11 Muco-adhesion testing

The mucoadhesive property of the flurbiprofen loaded microspheres (M1 and M2) prepared using both the copolymers was evaluated by an in vitro adhesion testing method known as the wash-off method [25]. Freshly excised piece of stomach or intestine of sheep obtained from a local butcher shop was cut to expose the mucosa and washed with normal saline. The serosal side of the excised stomach or intestine ($\sim$1.5 $\times$ 1 inch) was mounted on a glass slide (3 $\times$ 1 inch) with cyanoacrylate glue. The microspheres were fixed on the mucosa of the wet tissue specimen by gentle pressure and kept for few seconds, there after the glass slide was hung on to the arm of a tablet disintegration test apparatus with the help of a clamp and thread. When the disintegration apparatus was operated, the tissue specimen was given a slow, regular up and down movement in the test fluid (0.1 N HCl for stomach, phosphate buffer pH 6.8, for intestine) at 37 $\pm$ 2°C contained in a 1 L vessel of the apparatus. The time needed for all the microspheres to get detached from the mucosal surface was considered as mucoadhesion time.

2.2.12 In vitro drug release

The in vitro release of flurbiprofen from microspheres (equivalent to 50 mg of drug) was evaluated in a USP paddle apparatus (Lab India Instrument Pvt. Ltd., India–disso 2000) using 500 ml release medium at 50 rpm and 37 $\pm$ 1°C. To simulate gastro-intestinal pH conditions, the drug release from microspheres was evaluated in buffer of pH 1.2 (i.e. 0.1 N HCl), pH 5.5, 6.8 and 7.4 by changing buffer every 2 h. After every 2 h the release medium was filtered and the microspheres were replaced in dissolution flask containing buffer of higher pH. The buffers used were same as described under swelling study. Samples were withdrawn at predetermined intervals, filtered and assayed spectrophotometrically at 247 nm.

3 Results and discussion

3.1 Swelling behavior

The swelling behavior of the dried membranes of synthesized copolymers is shown in Table 1. The copolymer AA: MMA (2.5:7.5) showed 18.28% weight gain after 2 h at pH 1.2 and the weight gain of the copolymer increased with increase in pH. The observed weight gain was 27.35% at pH 5.5 (after 4 h), 38.81% at pH 6.8 (after 6 h) and 44.08% at pH 7.4 (after 8 h). The same trend was observed for the copolymer AA:MMA (2:8), i.e. weight gain increased with increase in pH. The weight gain for the copolymer AA: MMA (2:8) was minimum (16.83%) at pH 1.2 (after 2 h) and maximum was 38.13% at pH 7.4 after 8 h. The reduced swelling/weight gain of AA:MMA (2:8) copolymer appears to due to its lower AA content.

3.2 Dissolution behavior

The study was conducted to ascertain if the swelling of membrane was accompanied with dissolution. The results

Table 1 Percent weight gain and swelling of polymeric membranes in buffers of increasing pH

pH	1.2		5.5		6.8		7.4	
Time	1 h	2 h	3 h	4 h	5 h	6 h	7 h	8 h
AA:MMA (2.5:7.5)	17.53 ± 1.04	18.28 ± 1.23	22.63 ± 1.52	27.35 ± 1.80	33.16 ± 2.13	38.81 ± 2.54	41.99 ± 2.28	44.08 ± 1.75
AA:MMA (2:8)	16.08 ± 1.46	16.83 ± 1.21	20.86 ± 1.36	24.31 ± 1.16	28.92 ± 1.32	31.78 ± 1.48	36.42 ± 2.06	38.13 ± 1.94

Values are mean ± SD of three observations

Table 2 Percent dissolution of polymeric membranes in buffers of increasing pH

pH Time	1.2 2 h	5.5 4 h	6.8 6 h	7.4 8 h
AA:MMA (2.5:7.5)	–0.51 ± 0.02	–1.33 ± 0.44	–14.5 ± 0.71	–24.46 ± 0.92
AA:MMA (2:8)	–0.27 ± 0.09	–1.10 ± 0.03	–8.89 ± 0.72	–17.52 ± 1.13

Values are mean ± SD of three observations, (–) sign indicate weight loss

of the dissolution study of the membrane revealed dissolution of both the copolymers with increase in pH being higher at pH 7.4 (Table 2). Successive exposure of copolymers at pH 1.2 for 2 h and pH 5.5 for 2 h showed around 1% dissolution for copolymer AA:MMA (2.5:7.5 and 2:8) and dissolution increased to 14.5 and 8.9% following subsequent exposure to pH 6.8 for 2 h. Therefore, the polymers, if used for encapsulation of drug, may protect the drug from exposure in stomach and small intestine. The dissolution reached up to 24.46% for copolymer AA: MMA (2.5:7.5) at pH 7.4 suggesting that the copolymer, as encapsulating agent, may release the drug in colon. Comparatively, the dissolution for the copolymer AA: MMA (2:8) was less at pH 7.4 (~17%) which implies that the polymer would provide slow release of drug in colon compared with the copolymer AA: MMA (2.5:7.5). The reduced dissolution of AA: MMA (2:8) copolymer appears to due to its lower AA content.

3.3 Characterization of copolymer by FTIR

The FTIR spectra of both the copolymers AA: MMA (2.5:7.5), (2:8) and the monomers (AA and MMA) are shown in Fig. 1. The spectrum of acrylic acid shows a broad absorption band at 3350–2800 cm^{-1} indicating the presence of hydroxyl group in acid, which is also present in both the polymers. The broad absorption band in copolymers between 1734 and 1727 cm^{-1} is due to C = O stretching of the ester group and weaker band at ~1445 cm^{-1} in the copolymer is due to the symmetric absorption of carboxylate anion. The C = C stretching at 1634 cm^{-1} and out of plane =C–H bending at 921 cm^{-1} and 934 cm^{-1} observed in the spectra of acrylic acid and

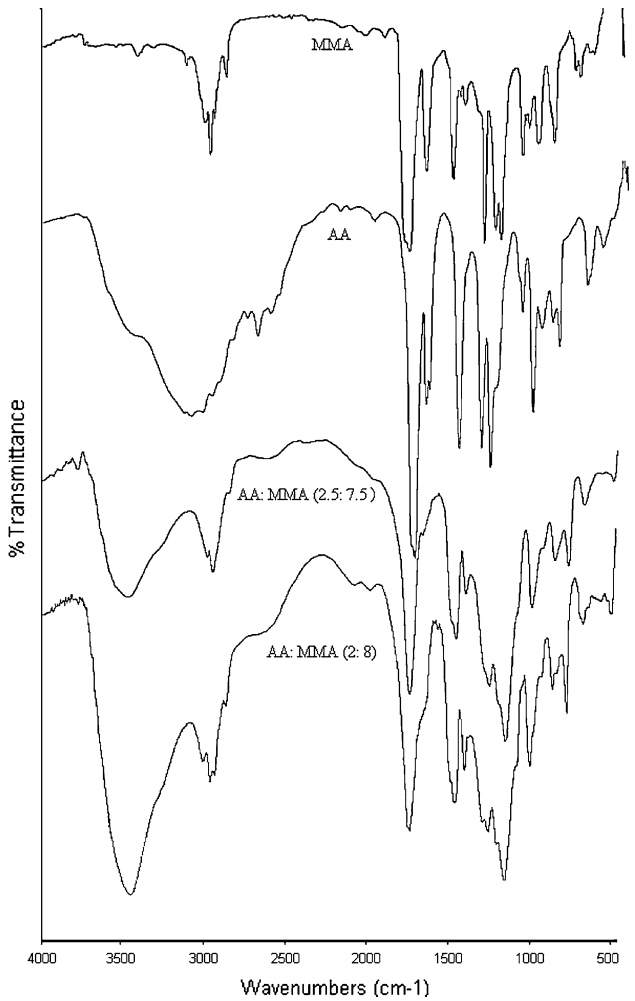

Fig. 1 FTIR spectra of monomers AA and MMA and copolymers AA: MMA (2.5:7.5) and (2:8)

methyl methacrylate, are absent in the FTIR spectrum of the copolymer suggesting successful polymerization.

3.4 Characterization of copolymer by ^{1}H-NMR

The ^{1}H NMR spectra of both the copolymers AA: MMA (2.5:7.5 and 2:8) and monomers (AA and MMA) are shown in Fig. 2. The spectra of monomers indicate the presence of vinyl protons, at 5.5 and 6.1 ppm in methyl methacrylate and at 5.9, 6.1 and 6.5 ppm in acrylic acid and the absence of these peaks in NMR spectrum of the copolymers reveals that all the double bond present in the monomers has been converted to the aliphatic back bone indicating successful polymerization. The peak at 7.26 ppm in the spectra is due to $CDCl_3$ and at 2.58 ppm is due to DMSO solvent. The peak assignments for the monomers and copolymer are shown in Table 3.

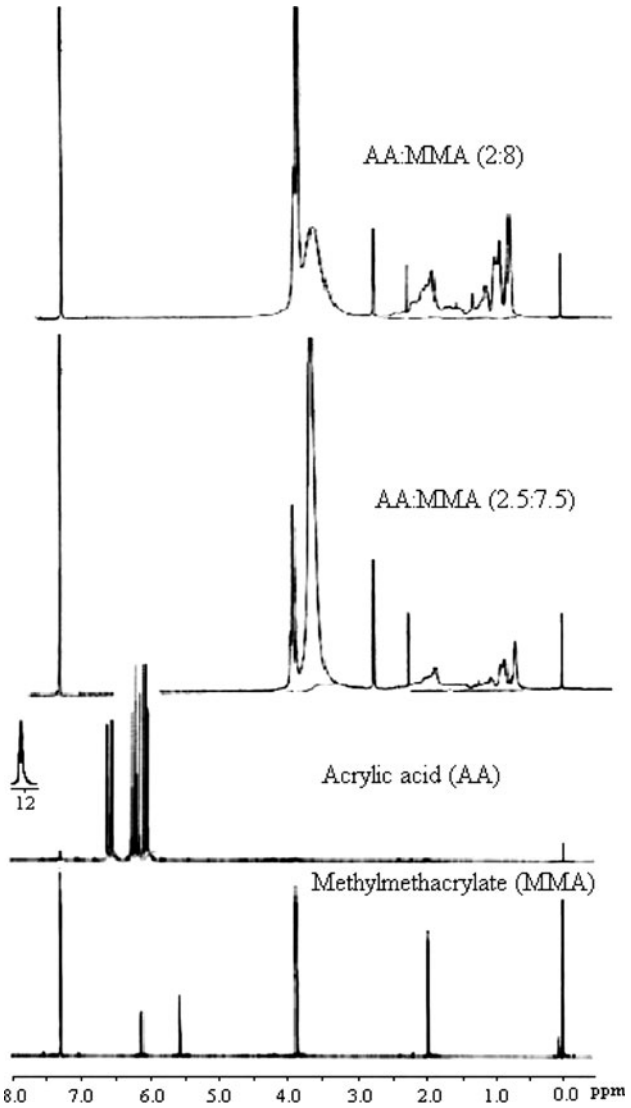

Fig. 2 ^{1}H NMR of monomers and copolymers AA:MMA (2.5:7.5) and (2:8)

Table 3 Chemical shift values for the monomers and the copolymers AA:MMA (2.5:7.5 and 2:8)

S. No.	Chemical shift values (ppm)			
	AA	MMA	AA:MMA (2.5:7.5)	AA:MMA (2:8)
1	5.94 (1H)	1.949 (3H)	0.80	0.80
2	6.17 (1H)	3.69 (3H)	0.85	0.85
3	6.53 (1H)	5.54 (1H)	0.87	0.92
4	12.0 (1H)	6.10 (1H)	0.92	0.98
5			0.98	1.10
6			1.10	1.25
7			1.25	1.80
8			1.49	1.93
9			1.79	2.14
10			2.14	3.34
11			3.39	3.59
12			3.59	–

3.5 Molecular weight determination by GPC

The number-, weight- and viscosity- average molecular weights of both the copolymers AA:MMA (2.5:7.5) and (2:8) determined by gel permeation chromatography are given in Table 4.

3.6 Estimation of apparent monomer composition in copolymer

The mole contents of AA: MMA (2.5:7:5) were AA = 2.07 and MMA = 8.50 and the corresponding data for AA: MMA (2:8) were AA = 1.62 and MMA = 8.82 moles. Thus, the copolymer becomes richer in MMA with its increase in the monomer feed. Kerber and Glamann [26] studied the copolymerization of methyl methacrylate–acrylic acid and reported the reactivity ratios of methyl methacrylate (r_1) and acrylic acid (r_2) to be $r_1 = 2.46$,

Table 4 Weight, number and viscosity average molecular weights of the copolymers AA:MMA (2.5:7.5) and (2:8)

	AA:MMA (2.5:7.5)	AA:MMA (2:8)
Weight average molecular weight (Mw)	164,500	211,160
Number average molecular weight (Mn)	110,828	156,914
Viscosity average molecular weight	155,835	202,248

$r_2 = 0.25$ in tetrahydrofuran. Formation of hydrogen bond between acrylic acid and tetrahydrofuran has been implicated for the low reactivity ratio of acrylic acid and same could result in a polymer of low acrylic acid content. Thus our results are in accordance with published results.

3.7 Hemolysis assay

According to the ASTM standard for biomaterials the copolymers were found to be highly hemocompatible (% hemolysis was less than 5%) [27] which suggests their use as biomaterial for specific purposes (Table 5).

3.8 Microspheres characterization

The microspheres were spherical (Fig. 3a and b), anionic particles having zeta potential of –57.8 mV for M1 and –53.7 mV for M2 formulations. The surface carboxylic acid concentrations of the formulations were 1.44 mmol/g and 1.45 mmol/g respectively. The M1 formulation had flurbiprofen loading and loading efficiency of 5.47 and 27.34 % respectively whereas M2 formulation showed flurbiprofen loading and loading efficiency of 5.89 and 29.46%. The mucoadhesion time for M1 formulation was 20 s on stomach mucosa (pH 1.2) and 18 min on intestinal mucosa (pH 6.8) while the mucoadhesion time for M2 formulation was 8 s on stomach mucosa (pH 1.2) and 9 min on intestinal mucosa (pH 6.8) (Table 6).

Table 5 Hemocompatibility assay

	OD at 545 nm	% Hemolysis	Remarks
Positive	1.179	100	–
Negative	0.029	0	–
AA:MMA (2.5:7.5)	0.062 ± 0.005	2.87	Highly hemocompatible
AA:MMA (2:8)	0.055 ± 0.003	2.26	Highly hemocompatible

Values are mean ± SD of 3 observations

3.9 Characterization of microspheres by FTIR

FTIR spectrum of flurbiprofen sodium (Fig. 4) showed asymmetric carboxylate anion and aromatic C = C ring stretching at 1553 cm^{-1}, symmetric carboxylate anion stretching at 1356 cm^{-1} and aromatic C = C ring stretching at 1480 cm^{-1}. Besides, it also showed peaks at 817, 764, 728 and 697 cm^{-1} for substituted benzene ring. The FTIR spectrum of M1 and M2 microspheres showed peaks at 1570 and 1578 cm^{-1} and around 756 and ~700 cm^{-1} indicating entrapment of flurbiprofen.

3.10 Characterization of copolymers and microspheres by P-XRD

The powder XRD pattern of flurbiprofen sodium, copolymers and microspheres are shown in Fig. 5. The XRD

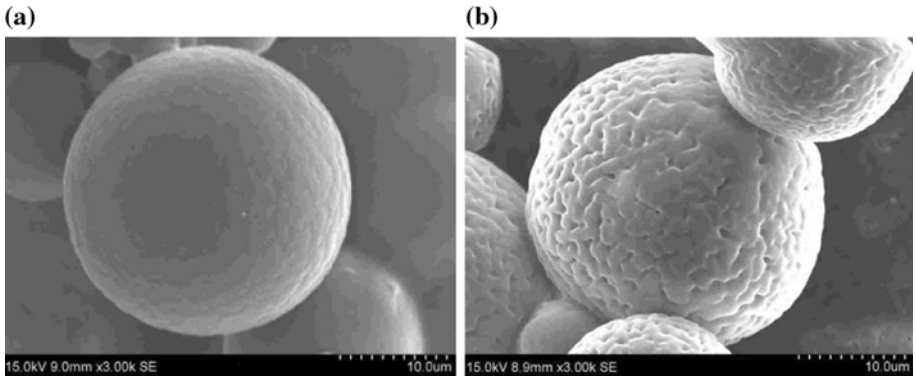

Fig. 3 SEM photograph of microspheres 3(**a**) formulation M1 and 3(**b**) formulation M2

Table 6 Microspheres characterization

S. No.	Characterization of microspheres	Formulation M1	Formulation M2
1	Flurbiprofen (%) loading	5.47 ± 0.83	5.89 ± 0.39
2	Loading Efficiency (%)	27.34 ± 1.79	29.46 ± 1.61
3	Zeta potential	–57.8 mV	–53.7 mV
4	Mucoadhesion time (stomach mucosa)	20 ± 5 s	8 ± 3 s
5	Mucoadhesion time (intestinal mucosa at pH 6.8)	17.82 ± 5.62 min	8.97 ± 3.41 min

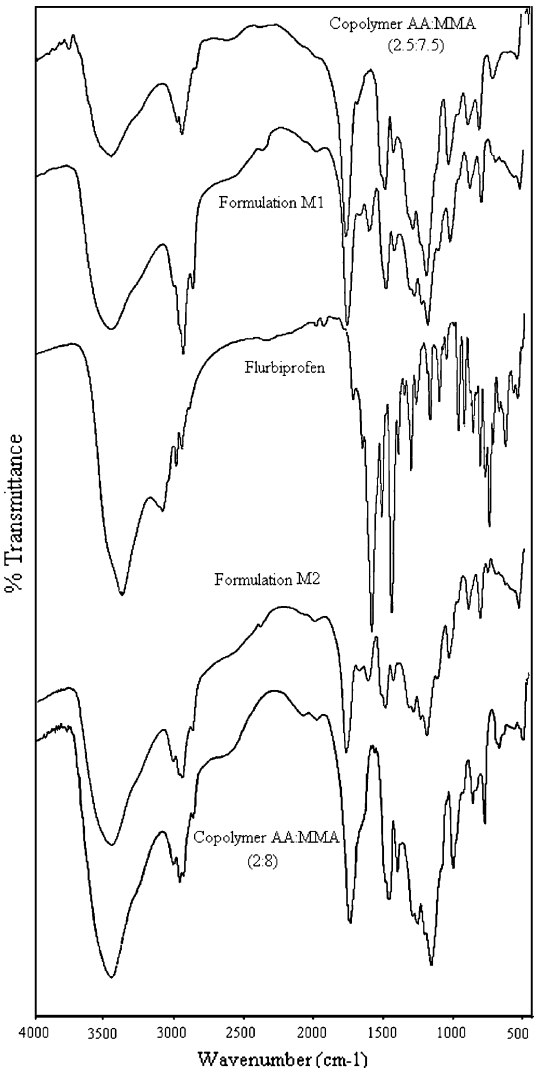

Fig. 4 FTIR spectra of the copolymer AA:MMA (2.5:7.5) and (2:8), flurbiprofen sodium and microspheres M1 and M2 formulations

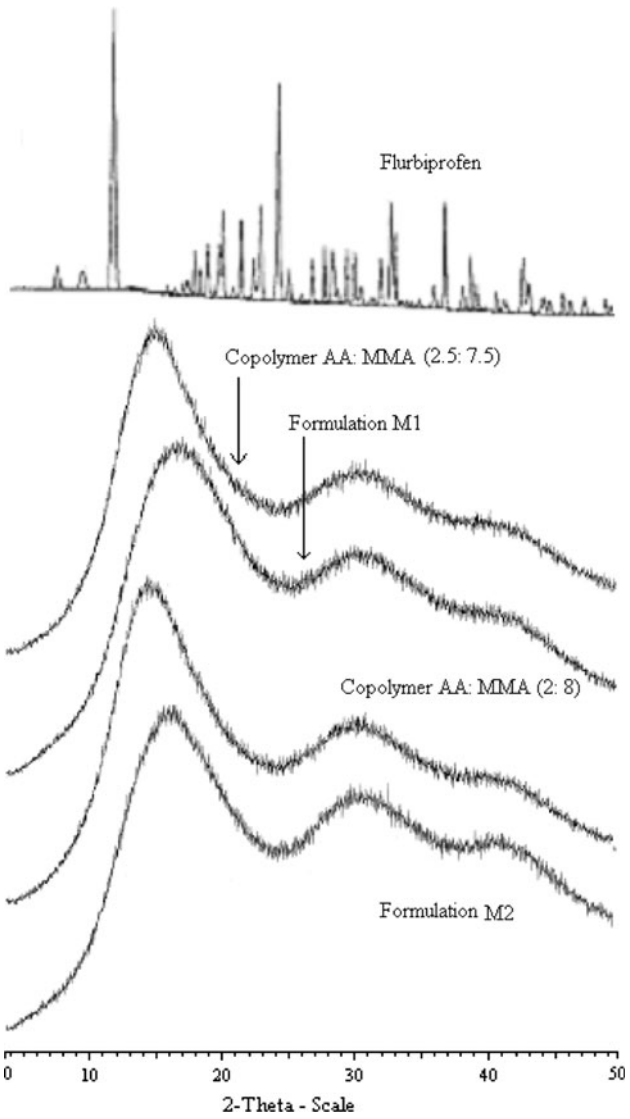

Fig. 5 P-XRD of flurbiprofen sodium, copolymers AA:MMA (2.5:7.5) and (2:8) and microspheres of formulations M1 and M2

indicated an amorphous structure of copolymer while flurbiprofen was crystalline. The diffractogram of both the microspheres formulations indicated an amorphous structure which was devoid of any crystalline peak of flurbiprofen. Considering the drug content of 5.47% in M1 formulation and 5.89% in M2 formulation, the peak heights of crystalline flurbiprofen would be relatively small compared with the amorphous copolymer.

3.11 Characterization of copolymer and microspheres by DSC

The DSC thermograms of flurbiprofen sodium, copolymers and microspheres are shown in Fig. 6. The thermograms showed a sharp melting endotherm of flurbiprofen sodium at 129.26°C while the copolymer AA: MMA (2.5:7.5) showed a broad endotherm with peak at 73.98°C and the copolymer AA: MMA (2:8) showed a broad endotherm with peak at 75.64°C. Thermograms of the copolymers suggested amorphous nature of the polymers. The thermogram of M1 and M2 microspheres showed a broad endotherm having peaks at 102° and 80.10°C respectively and no melting peak of flurbiprofen was observed in both the thermogram of microspheres.

Maghsoodi [28], prepared naproxen-loaded microparticles using Eudragit L 100 polymer. DSC thermograms of microparticles revealed that the melting peak of naproxen in the microparticles disappeared gradually with increasing the ratio of Eudragit L 100 to drug in the formulation. When the ratio of Eudragit L 100 to drug was 4:1, no melting peak of drug was observed. The results suggested that naproxen had been highly dispersed in the microparticles at 4:1 polymer/

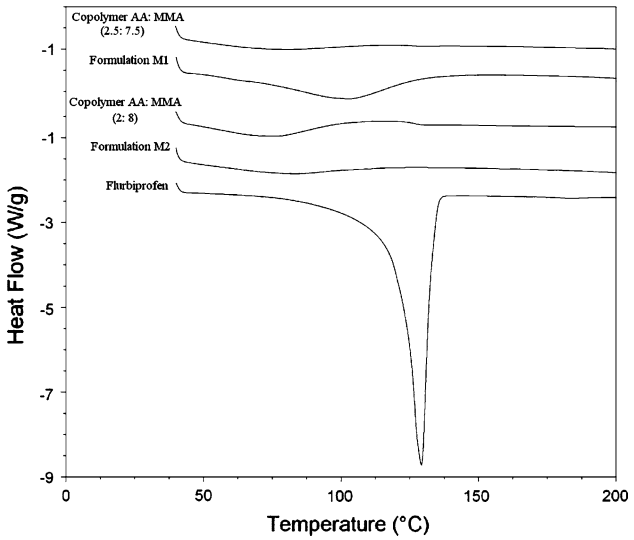

Fig. 6 DSC thermograms of flurbiprofen, copolymers AA:MMA (2.5:7.5) and (2:8) and microspheres formulations M1 and M2

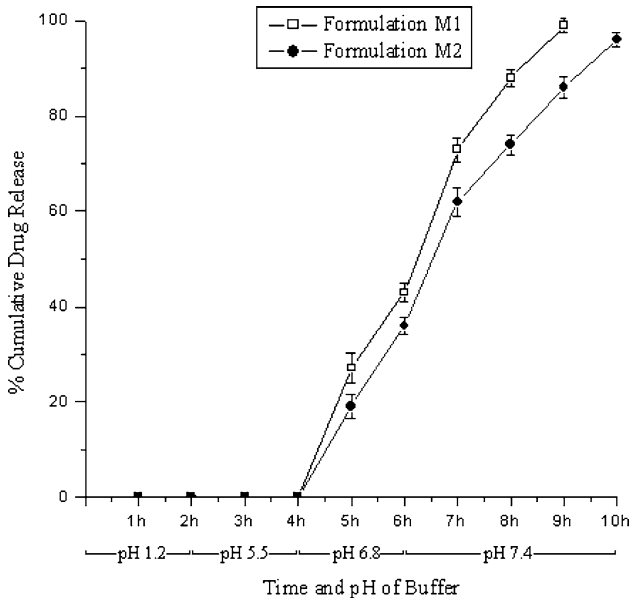

Fig. 7 In vitro drug release profile from microspheres formulations M1 and M2

drug ratio, similar to amorphous state. This was supported by X-ray analysis data where no crystalline peak of naproxen was found in the microparticles at 4:1 polymer/drug ratio [28]. In view of the aforesaid discussion, it appears quite likely that the DSC thermogram of flurbiprofen-loaded microspheres of formulation M1 and M2 which contained 5.47 and 5.89% drug respectively, (i.e. the drug is present as an impurity in the polymer) also would not show any melting peak of flurbiprofen, and the effect appears to be a dilution effect contributed by the amorphous polymer. Further, even in non-crystalline copolymers, there may be microphase separation, which can result in a 'mixing endotherm' in the DSC trace. The fact that this peak appeared to be at higher temperature in the microspheres may be due to the presence of the drug affecting mutual solubilities of the different monomer segments. The powder XRD of flurbiprofen-loaded microspheres also did not show any crystalline peak of flurbiprofen. Thus the results of the present study are in accordance with published results.

3.12 In vitro drug release

The microspheres formulations M1 and M2 showed no drug release at pH 1.2 after 2 h and at pH 5.5 in next 2 h (Fig. 7). The results suggest that the formulations on oral administration will not release the drug either in the acidic environment of stomach (pH 1.0–3.0) or in the duodenum having pH around 5.0. As the pH of the dissolution medium was raised to 6.8, drug release started and 43.23 and 36.40% drug was released in next 2 h from M1 and M2 formulations and further increase in pH to 7.4, released 99.04 and 96.04% drug in next 3–4 h from the respective formulations, suggesting enteric nature of the polymers/microspheres. Following oral administration, the microspheres thus, will release the drug in distal ileum (pH $\approx$ 7.0) and colon having pH between 7.0 and 8.0. Earlier we have studied the swelling and dissolution behavior of copolymers of AA and MMA, synthesized in the molar ratio of 3:7, 4:6, 5:5, 6:4 and 7:3. It was observed that pH dependent swelling and dissolution of polymer was directly related to acrylic acid concentration of the feed [23]. The copolymers, described in the present study, have been synthesized with AA: MMA molar ratio of 2.5:7.5 and 2:8 and contain small number of carboxylic groups in the polymer backbone. As a result the swelling and dissolution of the copolymers with increase in pH was less compared with the copolymers having higher acrylic acid content described earlier [23]. However, being anionic, the polymers showed pH dependent swelling and dissolution on increase of pH from 1.2 to 7.4 (Tables 1 and 2). For drug release, the drug will have to diffuse through the polymer. For diffusion, drug will have to dissolve first to give a concentration, as diffusion occurs along a concentration gradient. Flurbiprofen is a weakly acidic (anionic) drug having pKa of 4.2. Hence at pH 1.2, since the pH is below the pKa of the drug, the drug will be in unionized form and will not dissolve and at pH 5.5, since pH exceeds pKa of the drug, some drug may dissolve due to ionization but the quantity dissolved could be too small to give a concentration for diffusion. Increase of pH to 6.8, promotes pH-induced ionization/dissolution of drug and copolymers also swell (more than 30%) and dissolve (around14% for AA: MMA [2.5:7.5], and 9% for AA: MMA [2:8]) and the dissolved drug diffuses through the swollen/dissolved

polymer. Subsequent increase in pH to 7.4 promotes drug dissolution and polymer swelling/dissolution which facilitate further drug release.

Considering gastro-intestinal transit time from mouth to cecum as 4–6 h [29] the drug release from microspheres was evaluated in simulated gastro-intestinal pH conditions, by successively exposing the microspheres 2 h in 0.1 N HCl, 2 h at pH 5.5, 2 h at pH 6.8 and then in pH 7.4 buffers until complete drug release was attained. The microsphere formulations did not release the drug in 0.1 N HCl or at pH 5.5 and the drug release started at pH 6.8 and 43 and 36% drug was released in 2 h for M1 and M2 formulations respectively. The complete drug release was observed at pH 7.4, 99% for M1 in next 3 h and 96% for M2 in next 4 h. In our earlier study conducted with flurbiprofen microspheres made with AA:MMA (3:7) copolymer, the drug release was 83.4% at pH 6.8 in 2 h and 99% at pH 7.4 in 1 h [23] while in the present study microspheres made of copolymer AA:MMA (2.5:7.5) and AA:MMA(2:8),which contained less AA in the polymer backbone, released 43 and 36% drug at pH 6.8 in 2 h. Thus reduced AA content of the polymers could retard the release of the drug due to reduced swelling and dissolution. Since distal ileum has a pH around 6.8 where 36–43% of drug is expected to be released from microspheres, on oral administration, the released drug will reach colon in no time. Accordingly the chances of absorption of drug from small intestinal tract would be diminished and most of the drug would be available to colon.

The microspheres formulations M1 and M2 showed a cumulative release of 99 and 96% drug at pH 6.8 and 7.4 over a period of 5–6 h. Assuming maximum gastro-intestinal transit time from mouth to cecum as 6 h [29] the complete release of drug from microspheres M1 and M2 formulations would take around 9–10 h . The mucoadhesive nature of the copolymer would also affect drug release/absorption. The microspheres formulation M1 exhibited a mucoadhesion time of 20 s on stomach mucosa at pH 1.2 indicating the formulation is not gastroretentive and 18 min on intestinal mucosa at pH 6.8 suggesting that the microspheres may be retained in distal ileum for a short period while the microspheres formulation M2 showed lesser mucoadhesion time of 9 min on intestinal mucosa at pH 6.8. However the exact drug release could only be ascertained by further studies in vivo. The hemocompatibility of both the copolymers adds safety to the formulations.

Thus, on the basis of results available, one might conclude that the enteric copolymers AA: MMA (2.5:7.5 and 2:8) could be used for colonic delivery of NSAIDs and glucocorticoids in inflammatory bowel disease or anticancer drugs like 5-fluorouracil in colon cancer. Direct targeting of the drug to colon will minimize the dose of the drug and reduce systemic and local toxicity and maximize drug concentration at the target site. However further studies are required to comment more in this respect.

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