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Acrylic acid–methyl methacrylate copolymer for oral prolonged drug release

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Abstract Acrylic acid (AA)–methyl methacrylate (MMA) based copolymers, in different molar ratios (3:7, 4:6, 5:5, 6:4, and 7:3) were synthesized using tetrahydrofuran as solvent and AIBN as free radical initiator. Increase in acrylic acid concentration promoted pH-dependent swelling of copolymer and copolymer AA:MMA (3:7) was selected due to minimum swelling. ATR/FTIR and ^{1}H NMR spectra of the copolymer showed absence of vinyl bond/protons present in the monomers suggesting successful polymerization. The copolymer was hemocompatible. Flurbiprofen sodium microspheres made with the copolymer, by oil/oil solvent evaporation, were spherical, anionic (zeta potential −59.0 mV) and contained 4.53% drug. ATR spectrum of microspheres showed peaks for aromatic C=C stretching and substituted benzene ring, indicating entrapment of flurbiprofen. XRD analysis 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 copolymer and microspheres having peaks at 82.24 and 86.59°C, respectively. The thermogram of microspheres did not show the melting peak of flurbiprofen. The microspheres exhibited no drug release at pH <6.8 and released 83.4 and 99% drug at pH 6.8 and 7.4 in 3 h. The microspheres did not adhere on gastric mucosa at pH 1.2 but showed mucoadhesion time of 28 min on intestinal mucosa at pH 6.8. Thus, the microspheres on oral administration, would release the drug in distal ileum, suggesting the potential of the hemocompatible copolymer for enteric coating for prolonged drug release.

Keywords AA · MMA · Microspheres · Enteric · Release

1 Introduction

In last decades chemists have been vigorously involved in designing new polymeric materials using acrylic monomers for the pharmaceutical and biomedical applications due to the need of advanced drug delivery system to improve drug efficacy. Acrylic and methacrylic polymers are very attractive candidates for drug delivery systems as they can sense a stimulus as a signal and induce structural changes by themselves and release the drug in desired manner. The stimulus against which, the polymers responded are such as pH [1], temperature [2] and others [3–5]. These polymers have been reported as biocompatible [6]. Several studies have been done for the synthesis of acrylic copolymers as carrier for drug delivery. Xiaoliang and Richard [7] developed poly (acrylic acid-*co*-methyl methacrylate) microparticles for tumor chemotherapy where carboxylate containing monomers were included to complex therapeutic agents like cisplatin. The authors found low in vivo acute toxicity (LD 50 > 170 mg/kg) of

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the microparticles. Katime et al. [8] evaluated hydrogels from poly (acrylic acid-*co*-methyl methacrylate) for nafcillin release, in three different compositions: (90/10), (80/20) and (60/40) and observed that the molecular diffusion of nafcillin through hydrogels is controlled by the swelling. Müller et al. [9] also prepared acrylic acid copolymer nanoparticles for drug delivery.

These studies revealed that the acrylic copolymers can be used as good drug delivery vehicle. Literature review revealed no evidence for the use of acrylic acid–methylmethcarylate copolymer for pH dependent drug delivery system. Taking the aforesaid information in view attempts have been made to synthesize copolymers of acrylic acid and methyl methacrylate in different molar ratios and study the swelling of the copolymers in simulated gastro-intestinal pH condition followed by characterization of the selected copolymer by FTIR and ^{1}H NMR spectroscopy. Attempts have also been made to prepare microspheres of flurbiprofen, a model drug, with the selected copolymer and characterize the microspheres by FTIR spectroscopy, X Ray Diffraction and Differential Scanning Calorimetry and evaluate the drug release profile.

Flurbiprofen, a nonsteroidal anti-inflammatory drug (NSAID) was chosen as model drug for encapsulation which possesses anti-inflammatory, analgesic and antipyretic actions and is used in rheumatoid arthritis, ankylosing spondylitis, osteoarthritis and gout. Like other NSAIDs, flurbiprofen administered orally, causes gastro-intestinal irritation [10]. Considering activity of NSAIDs for prevention and treatment of colitis and colon cancer, colon targeted delivery of flurbiprofen has been studied [11]. Various approaches used for the colon targeted delivery of flurbiprofen are prodrugs [12] and multi-unit systems like microsponges [13].

2 Materials and methods

2.1 Materials

Acrylic acid (AA) and methylmethacrylate (MMA) were purchased from Merck, India and distilled under vacuum prior to use. $\alpha\alpha'$-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 sodium was received as gift from Laborate Pharmaceuticals India Ltd. Paonta Sahib, (HP), India.

2.2 Synthesis of copolymers of acrylic acid with methylmethacrylate

Copolymers of acrylic acid with methyl methacrylate having different molar feed ratios (3:7, 4:6, 5:5, 6:4 & 7:3) were synthesized in glass tube sealed with Teflon film, using THF as solvent and AIBN (0.25% w/v) 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. The polymers were boiled in distilled water for 5–10 min to remove unreacted monomers. Then the polymers were dried to constant weight and used.

2.3 Copolymer characterization

The copolymer was characterized with following techniques.

2.3.1 Fourier-transform-infrared spectroscopy (FTIR)/ attenuated total reflectance (ATR) spectroscopy

The selected copolymer AA:MMA (3:7) was subjected to Attenuated total reflectance (ATR) spectroscopy (Brooker, Model: alpha T) in the range of 4000–500 cm^{-1}. Fourier-transform-infrared spectrum (Shimadzu 8400 S) of the monomers was taken as such, i.e. as neat film in the range of 4000–400 cm^{-1}.

2.3.2 ^{1}H nuclear magnetic resonance (^{1}H NMR)

The chemical structure of the selected copolymer AA:MMA (3:7) 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).

2.3.3 Differential scanning calorimetry (DSC)

Differential Scanning Calorimetry (DSC) of selected copolymer AA:MMA (3:7) 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.

2.3.4 Powder X ray diffraction (PXRD)

Powder XRD of selected copolymer AA:MMA (3:7) was carried out employing Bruker D8 Advance X-ray diffractometer in the range of 3–50° 2θ.

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 polymer was dissolved in 5 ml of methanol and the resulting solution was spread uniformly onto 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.

2.4.1 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), 0.2 M phosphate buffer (pH 6.8) and 0.2 M phosphate buffer (pH 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, W_d is the weight of dry membrane

2.4.2 Dissolution behavior

The membrane dissolution was measured in the same buffers used in swelling study with membranes of all synthesized copolymers. 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 into 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; W_1 is the initial weight of membrane

2.4.3 Muco-adhesion testing

The mucoadhesive property of the prepared microspheres of the copolymer AA:MMA (3:7) containing flurbiprofen was evaluated by an in vitro adhesion testing method known as the wash-off method [14]. 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 onto 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 or phosphate buffer of 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.

2.4.4 Hemolysis assay

Hemolytic assay was done as previously reported [15]. ACD sheep blood was used for this purpose. ACD blood was prepared by adding 1 ml of acid citrate dextrose (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. Film of the copolymer AA:MMA (3:7) was cut into small pieces (approximately 1 $\times$ 1 cm^2) and equilibrated in 4 ml saline for 30 min at 37 $\pm$ 1°C. Diluted blood (0.2 ml) was added to each sample and incubated for 60 min at 37 $\pm$ 1°C. Positive or negative controls, which did not contain polymer film, prepared by adding 0.2 ml of blood to 4 ml of distilled water or saline solution, were similarly incubated for 60 min at 37 $\pm$ 1°C. All solutions were centrifuged for 5 min. The optical density (OD) of the supernatant was measured at 545 nm. Hemolysis was calculated as follows:

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

2.5 Microencapsulation

Flurbiprofen was encapsulated in the copolymer AA:MMA (3:7) by oil/oil solvent evaporation technique [16]. In a typical experiment 400 mg of the copolymer and 100 mg of drug were dissolved in a mixture of methanol & 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 maintained under constant stirring 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.

2.6 Microspheres characterization

2.6.1 Scanning electron microscopy (SEM)/Zeta potential

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

ATR Spectroscopy, X-ray Diffraction and Differential Scanning Calorimetry of the microspheres were done using the same instruments and methods described under polymer characterization.

2.7 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).

$$\text{Drug loading}(\%) = \text{mg of entrapped drug}/100\ \text{mg microspheres} \times 100$$

Microencapsulation efficiency (%) = Actual drug content/theoretical drug content $\times 100$

2.8 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 ± 1°C. For enteric coated article United States Pharmacopoeia (USP) recommends to test the drug release from the product in 0.1 N HCL for 2 h and then in phosphate buffer of pH 6.8 by replacing acid with phosphate buffer. 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

Swelling behavior of the dried membranes of synthesized copolymers is shown in Table 1. The copolymers AA:MMA (7:3 &6:4) showed weight gain at pH 1.2 for 2 h and at pH 5.5 up to 1 h and then started dissolving at pH 5.5 and completely dissolved at pH 7.4. Similarly, the copolymers AA:MMA (5:5 & 4:6) showed weight gain at pH 1.2 for 2 h, at pH 5.5 for 2 h and at pH 6.8 up to 1 h, but these copolymers showed less weight gain at pH 1.2 and 5.5 as compared to AA:MMA (7:3). Subsequently the copolymers showed enormous swelling and copolymer AA:MMA (5:5) dissolved at pH 7.4 while copolymer AA:MMA (4:6) broke into pieces. The copolymer AA:MMA (3:7) showed least weight gain at pH 1.2 (19% after 2 h); the weight gain of copolymer increased with increase in pH being maximum at pH 7.4 (48.62% in 8th h). The results suggest increased swelling or weight gain on increase in acrylic acid (AA) content of the copolymer.

3.1.1 Dissolution behavior

The study was conducted to ascertain if the swelling of membrane was accompanied with dissolution. The results of the dissolution study of the membrane revealed dissolution of all copolymers with increase in pH being higher at pH 7.4 (Table 2). The membrane dissolution was increased with increase in AA content. The dissolution was 2.29% for the copolymer AA:MMA (7:3) and 0.60% for copolymer AA:MMA (3:7) at pH 1.2 in 2 h. The first four copolymers, i.e. AA:MMA (7:3, 6:4, 5:5 & 4:6) swelled at pH 1.2 (Table 1) and showed 2.29, 1.6, 1.08 and 0.98% dissolution at the said pH in 2 h (Table 2), therefore, it appears that all these copolymers will start releasing the drug in the stomach itself and hence, may not be good candidates for enteric coating purpose. The copolymer AA:MMA (3:7) showed least dissolution at pH 1.2 (0.60% in 2 h), and therefore it may protect the drug from exposure in stomach and release the drug at pH 6.8 in distal small intestine, as the membrane dissolution reaches to ~19%, which is desirable. The copolymer AA:MMA (3:7) was selected for further studies based on the results.

3.2 Characterization of copolymer by FTIR

The ATR spectrum of the copolymer AA:MMA (3:7) and the FTIR spectra of monomers (AA and MMA) are shown

Table 1 Percent weight gain and swelling of the polymeric films in different buffers

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 (7:3)	67.41 ± 4.61	74.10 ± 3.94	105.68 ± 5.79	73.19 ± 6.57	Start dissolving	Around 60% dissolved	Dissolved	–
AA:MMA (6:4)	39.30 ± 1.54	45.00 ± 3.27	72.48 ± 2.47	64.38 ± 3.85	Start dissolving	Around 40% dissolved	Dissolved	–
AA:MMA (5:5)	29.52 ± 2.22	34.37 ± 1.47	51.53 ± 2.91	63.64 ± 3.43	106.04 ± 7.02	Swelled enormously	Almost dissolved	Dissolved
AA:MMA (4:6)	21.32 ± 1.87	23.78 ± 2.02	41.35 ± 5.12	47.98 ± 2.58	78.80 ± 4.46	Swelled enormously	Swelled unable to weigh	Break in pieces
AA:MMA (3:7)	18.22 ± 1.54	19.22 ± 0.94	26.04 ± 0.61	33.03 ± 2.28	39.28 ± 1.85	42.02 ± 1.45	46.31 ± 3.14	48.62 ± 3.47

Values are Mean ± SD of 3 observations

in Fig. 1. The spectrum of acrylic acid shows a broad absorption band at 3350–2500 cm^{-1} indicating the presence of hydroxyl group in acid, which is also present in the spectrum of polymer. The broad absorption band in copolymer at 1721 cm^{-1} is due to C=O stretching of the ester group and weaker band at 1389 cm^{-1} in the copolymer is due to the symmetric absorption of carboxylate anion. The C=C stretching at 1640 cm^{-1} and out of plane =C–H bending at 930 cm^{-1} and 940 cm^{-1} observed in the spectra of acrylic acid and methyl methacrylate, are absent in the ATR spectrum of the copolymer suggesting successful polymerization.

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

The ^{1}H NMR spectra of the copolymer AA:MMA (3:7) 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 methylmethacrylate and at 5.9, 6.1 and 6.5 ppm in acrylic acid and the absence of these peaks in NMR spectrum of the copolymer reveals that all the double bond present in the monomers has been converted to the aliphatic back bone indicating successful polymerization. The NMR spectra of the monomers and copolymer, however, contain peaks associated with the solvents used. The peaks at 7.26 and 2.58 ppm are due to $CDCl_3$ and deuterated DMSO, respectively. The NMR peak assignments for the monomers and copolymer are shown in Table 3.

3.4 Microspheres characterization

The microspheres were spherical (Fig. 3), anionic particles (Zeta potential = −59.0 mV) having flurbiprofen loading and loading efficiency of 4.53 and 22.65% respectively. The mucoadhesion time for microspheres was 48.46 s on stomach mucosa (pH 1.2) and 28 min on intestinal mucosa (pH 6.8) (Table 4).

3.5 Hemolysis assay

According to the ASTM standard for biomaterials the copolymer AA:MMA (3:7) was found to be highly hemocompatible (% hemolysis was less than 5%) (Table 5) and it can be used as biomaterial for specific purposes [17].

3.6 Characterization of microspheres by ATR spectroscopy

ATR spectrum of flurbiprofen sodium (Fig. 4) showed asymmetric carboxylate anion and aromatic C=C ring stretching at 1546 cm^{-1}, symmetric carboxylate anion stretching at 1403 cm^{-1} and aromatic C=C ring stretching

Table 2 Percent dissolution of the polymeric films in different buffers

pH Time	1.2 2 h	5.5 4 h	6.8 6 h	7.4 8 h
AA:MMA (7:3)	−2.29 ± 0.49	−14.36 ± 1.09	Unable to weigh	Dissolved
AA:MMA (6:4)	−1.60 ± 0.26	−7.24 ± 0.22	Unable to weigh	Dissolved
AA:MMA (5:5)	−1.08 ± 0.12	−4.54 ± 0.36	Unable to weigh	Dissolved
AA:MMA (4:6)	−0.98 ± 0.02	−3.60 ± 0.27	−38.70 ± 2.55	−53.87 ± 2.64
AA:MMA (3:7)	−0.60 ± 0.01	−1.65 ± 0.20	−19.17 ± 1 .10	−30.50 ± 2.04

Values are Mean ± SD of 3 observations. (−) sign indicates weight loss

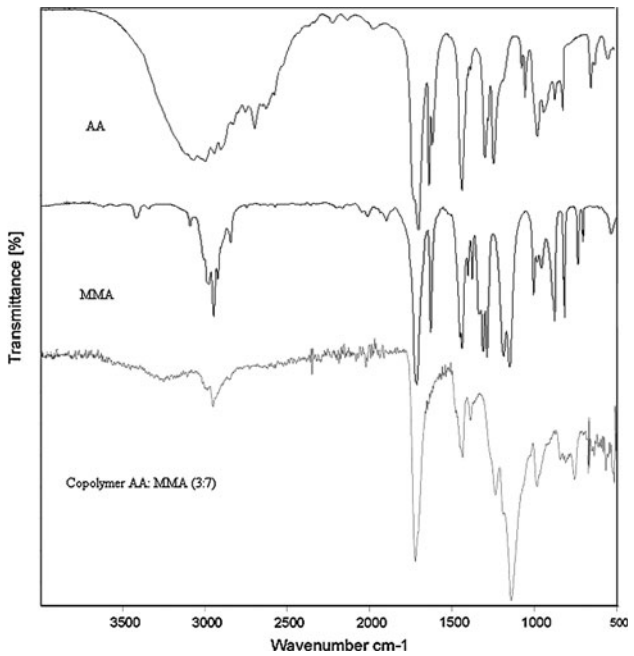

Fig. 1 FTIR spectra of monomers AA & MMA and ATR spectra of copolymer AA:MMA (3:7)

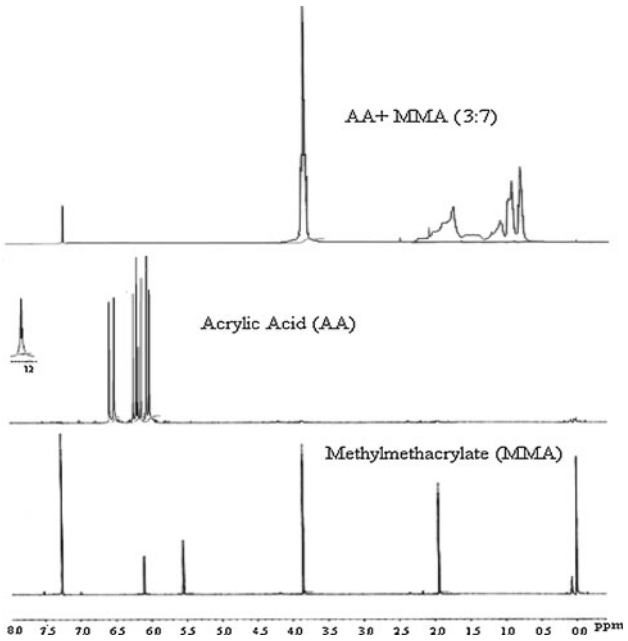

Fig. 2 ^{1}H NMR of the monomers and copolymer AA:MMA (3:7)

at 1481 cm^{-1}. Besides, it also showed peaks at 830, 765, 730 and 700 cm^{-1} for substituted benzene ring. The ATR spectrum of microspheres showed peaks at 1566, 840 and 757 cm^{-1} indicating entrapment of flurbiprofen.

3.7 Characterization of copolymer and microspheres by P-XRD

The powder XRD pattern of flurbiprofen sodium, copolymer and microspheres are shown in Fig. 5. The XRD indicated an amorphous structure of copolymer while flurbiprofen was crystalline. The diffractogram of microspheres also indicated an amorphous structure which was devoid of any crystalline peak of flurbiprofen. Considering the drug content of 4.5% in the microspheres, the peak heights of crystalline flurbiprofen would be relatively small compared with the amorphous copolymer.

3.8 Characterization of copolymer and microspheres by DSC

The DSC thermograms of flurbiprofen sodium, copolymer and microspheres are shown in Fig. 6. The thermograms showed a sharp melting endotherm of flurbiprofen sodium at 129.26°C while the polymer showed a broad endotherm with peak at 82.24°C which is typical of amorphous material. The thermogram of microspheres showed a broad endotherm having peak at 86.59°C and no melting peak of flurbiprofen was observed.

Maghsoodi [18] prepared naproxen-loaded microparticles with Eudragit L 100. 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

Table 3 Chemical shift values for the monomers and the copolymer AA:MMA (3: 7)

S.No.	Chemical shift values (ppm)		
	AA	MMA	Copolymer AA:MMA (3: 7)
1	5.94 (1H)	1.949 (3H)	0.82
2	6.17 (1H)	3.69 (3H)	0.98
3	6.53 (1H)	5.54 (1H)	1.12
4		6.10 (1H)	1.25
5			1.43
6			1.53
7			1.80
8			1.88
9			1.93
10			2.05
11			2.16
12			3.59
13			3.63

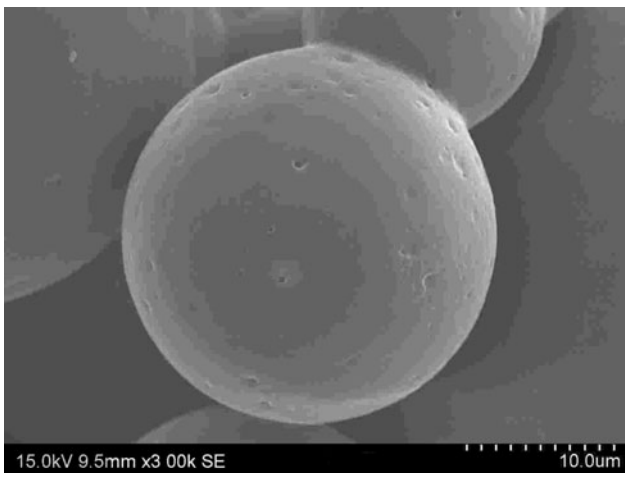

Fig. 3 SEM photograph of microspheres prepared using copolymer AA:MMA (3:7)

4:1 polymer/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 [18]. In view of the aforesaid discussion, it appears quite likely that the DSC thermogram of flurbiprofen-loaded microspheres which contained 4.53% drug, (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 slightly 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-

Table 5 Hemocompatibility assay

	OD at 545 nm	% Hemolysis	Remarks
Positive	1.179	100	–
Negative	0.029	0	–
AA:MMA (3:7)	0.076	4.09	Highly hemocompatible

Values are Mean ± SD of 3 observations

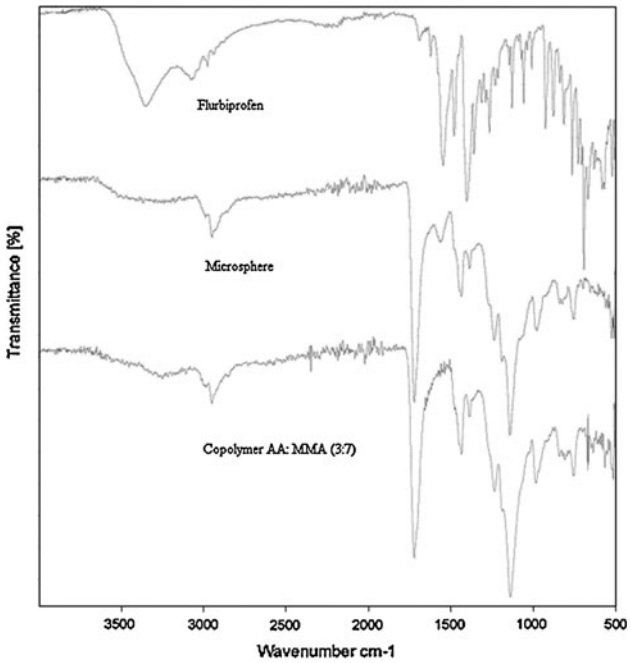

Fig. 4 ATR spectra of flurbiprofen sodium, microspheres and copolymer AA:MMA (3:7)

Table 4 Microspheres characterization

S.No.	Characterization of microspheres	
1	Flurbiprofen (%) loading	4.53 ± 0.248%
2	Loading efficiency (%)	22.65 ± 1.24%
3	Zeta potential	−59.0 mV
4	Mucoadhesion Time (Stomach mucosa)	48.46 s
5	Mucoadhesion time (Intestine mucosa at pH 6.8)	28 min

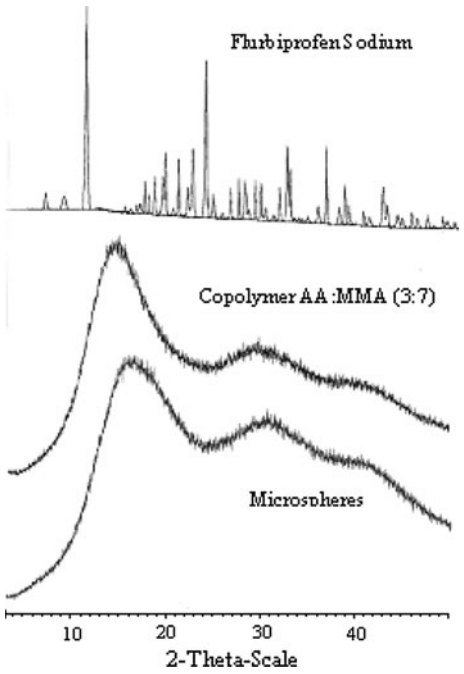

Fig. 5 P-XRD of flurbiprofen sodium, copolymer AA:MMA (3:7) and microspheres

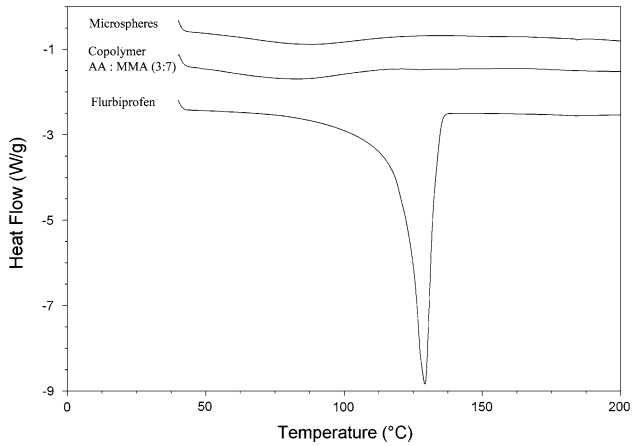

Fig. 6 DSC thermogram of microspheres, copolymer AA:MMA (3:7) and flurbiprofen sodium

loaded microspheres also did not show any crystalline peak of flurbiprofen. Thus the results of the present study are in accordance with published results.

3.9 In vitro drug release

The prepared microspheres showed no drug release at pH 1.2 after 2 h. Similarly no drug release was observed on exposure of microspheres at pH 5.5 for 2 h (Table 6). The results suggest that the microspheres on oral administration will not release the drug either in the acidic environment of stomach which has pH between 1.0 and 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 39.8 and 83.4% drug was released in 1 h and 2 h, respectively and further increase in pH to 7.4 released 99% drug in next 1 h. The microspheres, thus, will release the drug at distal ileum (pH $\approx$ 7.0) and colon (pH between 7.0 and 8.0). The copolymer has been synthesized with AA:MMA molar ratio of 3:7, hence it contains small number of carboxylic groups in the polymer backbone. As a result the swelling of the polymer with increase in pH was less compared with rest of the polymers having higher acrylic acid content. However; being an anionic polymer, it showed pH dependent swelling on increase of pH from 1.2 to 7.4 (Table 1). 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 polymer also swells (about 40%) and dissolves (around 19%) 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. For enteric coated article United States Pharmacopoeia (USP) recommends to test the drug release from the product in 0.1 N HCL for 2 h and then in phosphate buffer of pH 6.8. As per USP, the drug should be released at pH 6.8 and not in 0.1 N HCL. Thus, copolymer AA:MMA (3:7) behaves like an enteric polymer and it could be used for oral delivery of drugs which cause gastro-intestinal irritation, e.g. NSAIDs or drugs which are unstable in acidic environment of the stomach, e.g. penicillin G. Flurbiprofen used in the present study is a NSAID which causes gastro-intestinal irritation. Considering gastro-intestinal transit time from mouth to

Table 6 Percent cumulative drug release from the microspheres

pH of buffer (time in hrs.)	% Cumulative drug release
1.2 (1 h)	0
1.2 (2 h)	0
5.5 (3 h)	0
5.5 (4 h)	0
6.8 (5 h)	39.84 $\pm$ 1.08
6.8 (6 h)	83.42 $\pm$ 0.93
7.4 (7 h)	99.14 $\pm$ 0.59

Values are Mean $\pm$ SD of three observations

cecum as 4–6 h [19] the drug release from microspheres was evaluated for 6 h in simulated gastro-intestinal pH conditions, by successively exposing the microspheres 2 h in 0.1 N HCL, 2 h at pH 5.5 and 2 h at pH 6.8 buffers. The microspheres 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 83.4% drug was released in 2 h. Since distal ileum has a pH around 6.8, where the 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 released about 99% drug at pH 6.8 and 7.4 over a period of 3 h. Assuming maximum gastro-intestinal transit time from mouth to cecum as 6 h [19], the complete release of drug from microspheres would take around 9 h. The mucoadhesive nature of the copolymer would also affect drug release/absorption. The microspheres exhibited a mucoadhesion time of 48.5 s on stomach mucosa at pH 1.2 indicating the formulation is not gastroretentive and 28 min on intestinal mucosa at pH 6.8 implying that the microspheres may be retained in distal ileum for a short period. However the exact drug release could only be ascertained by further studies in vivo. The hemocompatibility of the polymer adds safety to the formulation.

Thus, the copolymer AA:MMA (3:7) could be used as an enteric coating material for prolonged drug release or 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 systemic and local toxicity and maximize drug concentration at the target site. However further studies are required to comment more in this respect.

4 Conclusions

From the results of the study it can be concluded that the copolymer of AA and MMA with molar ratio of 3:7 could be used as an enteric coating material for prolonged drug release. Since the polymer releases the drug at pH 6.8 (i.e. distal ileum), it will eliminate/reduce the gastrointestinal irritation/toxicity caused by drugs like NSAIDs, systemic absorption from small intestine and target the drug to the colon. Thus the polymer could be useful for colon targeting of NSAIDs, glucocorticoids and anticancer drugs for inflammatory bowel disease and colon cancer. Direct targeting of drug to the colon will reduce the dose and systemic and local toxicity.

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