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Synthesis of Poly[3-(methacryloylamino) propyl trimethylammonium chloride-*co*-methacrylic acid] Copolymer Hydrogels for Controlled Indomethacin Delivery

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ABSTRACT: Stimulus responsive hydrogels are being considered as one of the most exciting biomaterials of current generation. A novel hydrogel based on poly 3-[(methacryoylamino) propyl trimethylammonium chloride-*co*-methacrylic acid] (PMAPTACMAAc) copolymer was synthesized by free radical aqueous copolymerization. The water uptake of the hydrogels was investigated as a function of temperature and pH. The *in vitro* release properties of the PMAPTACMAAc hydrogels were analyzed under simulated body fluid (pH 7.4) by loading indomethacin (IND) as a model drug. The XRD study of the hydrogel revealed the amorphous nature of the co-polymer and provided evidence that crystalline IND was entrapped in the hydrogel matrix. The DSC study showed the presence of single glass transition temperature in the thermogram which indicated the formation of random copolymers. The prepared hydrogels showed typical polyampholyte behavior and swelling was affected by the monomer feed ratios in the hydrogels. The morphological study on the hydrogels showed the three dimensional (3D) porous nature of the PMAPTACMAAc-5 gel with pore size ranging from 10 to 40 μm. The kinetics of the cumulative IND release shows that the gel follows a non-Fickian release mechanism. *In vitro* cytotoxicity of the hydrogel (PMAPTACMAAc-5 gel) on RAW 264.7 murine macrophages showed that the hydrogel was biocompatible and not toxic as the viability was maintained. The hydrogel with a 90 : 10 feed ratio of MAAc and MAPTAC was better than the other developed formulations. The results suggest that the hydrogels could be employed as a sustained release formulation for targeted delivery of IND, for example to the colon. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2012

KEYWORDS: amorphous; biocompatibility; biomedical applications; biomaterials; drug delivery systems

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INTRODUCTION

Recent progress in biotechnology and conventional chemical synthesis has made it possible to prepare sufficient amount of proteins and polypeptides for the treatment of diseases. Nevertheless, a remaining issue is how to deliver them to specific targets in the body. Oral drug delivery is one of the safest ways of drug administration because of its convenience and patient compliance.[1] It provides an administrative route that gives preferable treatments for chronic diseases. However, the oral route poses many challenges such as low absorption, negative effect on the stomach and low bioavailability can be encountered.[2–4] Stimuli-responsive hydrogels are excellent class of functional materials due to their inherent structural and compositional features as well as peculiar physicochemical properties, such as tunable swelling, degradability, mechanics, and permeability.[5] These smart hydrogels can be classified according to their response towards various stimuli such as pH, temperature, ionic strength, light and electric and magnetic fields.[6–9] Interest in stimuli-responsive materials has persisted over the years and a tremendous amount of work has been dedicated to devising examples of environmentally sensitive macromolecules that can be designed into new smart materials.[10] Polymethacrylic acid (PMAAc) is an ionizable hydrophilic polymer and has received considerable recognition as a pH sensitive hydrogel.[11] It is already established that PMAAc can exhibit pH-induced conformational phase transition behavior.[12,13] 3-[(methacryloylamino) propyl trimethylammonium chloride (MAPTAC) is a cationic monomer used for the preparation of superabsorbent hydrogels.[14] Prausnitz et al. have carried out experimental and theoretical studies on the swelling of cationic copolymer

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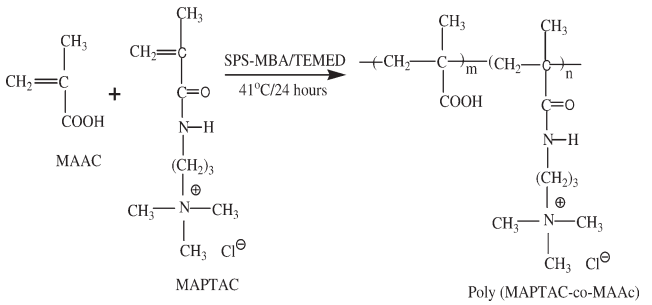

Scheme 1. Synthesis of poly MAPTACMAAc copolymer hydrogel.

hydrogels based on MAPTAC.[15–17] Indomethacin (IND) is a non-steroidal anti-inflammatory drug (NSAID), which is of low molecular weight and moderately hydrophobic in nature. IND has been commonly used for treatment of rheumatoid arthritis, ankylosing spondylitis, osteoarthritis, acute pain shoulder, and acute gouty arthritis.[18] Recent reports suggest that IND can be used as an anticancer agent against various *in vitro* and *in vivo* colon cancer models.[19] The oral administration of IND causes gastrointestinal side effects.[20] A formulation of IND is sought which causes limited exposure to the upper gastrointestinal (GI) tract and sustained release of the drug in colonic region with maximum therapeutic concentration of the drug in the colon.[21] There are various approaches used for successfully targeting the drugs to the colon includes pro drug approach (including azo polymers and hydrogels), time-dependent delivery system (including osmotic pumps, swelling and coating controlled), pH-sensitive polymer-based coating systems, and microbial enzyme controlled systems.[22,23] Some researchers has reported various formulations for colon targeted delivery of IND, which include pH sensitive polymer coated pellets,[24] compression coating using guar gum,[25] pectin and chitosan mixtures,[26] and drug embedding in HPMC/pectin/calcium chloride matrix bases.[27] It was reported earlier that pH sensitive hydrogels could be a promising system for targeted drug delivery of various antibiotics in effective concentration to the colon.[28–30] Herein we report the synthesis of poly [3-(methacryloylamino) propyl] trimethylammonium chloride-*co*-methacrylic acid] (PMAPTACMAAc) for controlled colon specific delivery of IND. The equilibrium swelling and thermal sensitive behavior of the hydrogels were investigated in simulated body fluid (SBF). The morphological and thermal properties of the stimuli-responsive hydrogels were investigated with field scanning electron microscopy, wide angle X-ray diffraction, differential scanning calorimetric and thermogravimetric analysis. The cytocompatibility of the copolymer hydrogels was also assessed under *in vitro* conditions with a RAW 264.7 murine macrophages cell lines.

EXPERIMENTAL

Materials

The [3-(methacryloylamino) propyl] trimethylammonium chloride (MAPTAC), methacrylic acid (MAAc), sodium persulfate (SPS), *N*,*N*,*N*,*N*-tetramethyleneethylenediamine (TEMED), *N*,*N*-methylenebisacrylamide (MBA) and indomethacin (IND) a model drug was purchased from Sigma (St. Louis, USA). Deionized water was used for all copolymerization reactions and in the preparation of buffer solutions.

Synthesis of Copolymer Hydrogels

The copolymer hydrogels were synthesized by free radical aqueous copolymerization of MAPTAC and MAAc (Scheme 1). SPS, TEMED and MBA were used as initiator, accelerator, and cross-linker respectively.[28]

Before the reactions, five different monomer mixtures were prepared by controlled addition of MAPTAC and MAAc with simultaneous stirring over a hot plate. Predetermined amount of water was then added to each of the reaction mixtures. The first step of the reaction was carried out at 20°C in 100-mL three necked flask equipped with stirring system and nitrogen line. The nitrogen gas was continuously purged for 15 min into the monomer mixtures, followed by the addition of SPS (0.5 mole %), MBA (1 mole %) and TEMED (3 mole %). To accomplish the second phase of the reaction the individual reaction mixtures were transferred to rectangular molds. The molds 60 cm/70 cm/0.06 cm in dimension were prepared using Teflon spacers along the three edges of a pair of glass plates. The molds were then placed on thermostated water bath at 41°C ± 1°C and dipped up to the height of reaction mixtures. The nitrogen gas was purged into the reaction mixtures through one of the orifices of the mold. After 10 min the nitrogen purging was stopped and orifices were closed using paraffin grease. After 24 h of reaction the hydrogels were removed from the molds, cut into pieces, repeatedly washed up to 3 days to remove the unreacted monomers and dried under vacuum. The feed compositions of five PMAPTACMAAc hydrogels and corresponding synthetic parameters were enlisted in Table I.

The PMAPTAC homopolymer was synthesized according to method described elsewhere.[31] In a typical reaction 0.011 mol of MAPTAC and 0.7 mmol of the initiator ammonium persulfate (APS) were dissolved in 25 mL water in a 100 mL flask. The reaction was carried out at 70°C temperature for 24 h. After the completion of the reaction the solution was precipitated in 1,4-dioxane. The precipitate was separated by centrifugation at 4000 rpm for 15 min and washed three times with 1,4-dioxane. The white product was dried to a constant weight in a vacuum oven. The PMAAc homopolymer was synthesized by the similar method used for the copolymer hydrogels.

Characterization

FTIR-ATR Spectroscopy. The infrared spectra of homopolymer and copolymer hydrogels (PMAPTACMAAc) were recorded

Table I. Composition of Poly(PMAPTACMAAc) Hydrogels[a]

Sample	MAAc (mole %)	MAPTAC (mole %)	Water (mole %)[b]
1	50	50	200
2	60	40	150
3	70	30	100
4	80	20	50
5	90	10	25

[a]The concentrations of MBA, SPS, and TEMED in the feed were 2, 0.5, and 3 mole %, respectively.
[b]Molar percentage to total monomer content.

within the range of 400–4000 cm^{-1} as KBr pellet on a Varian 640-IR FT-IR spectrophotometer. Finely ground powder of the freeze dried samples was used to prepare the KBr pellets.

Wide Angle X-ray Diffraction (WAXD). Dry PMAPTACMAAc copolymers and homopolymers were ground to fine powder. X-ray powder diffraction data were collected on a XPERT PRO X-ray diffractometer (PAN Analytical). The scanning 2θ angle was from 5° to 65°.

Differential Scanning Calorimetry (DSC). DSC of the polymeric gels and IND were recorded with NETZSCH differential scanning calorimeter (DSC 200 F3) at a 10°C min^{-1} heating rate and N_2 flow speed of 50 mL min^{-1}.

Thermogravimetric Analysis (TGA). Thermal stability of the copolymer networks were analyzed with NETZSCH TG 209 F3 at a heating rate of 20°C min^{-1} with nitrogen flushed at 100 mL min^{-1}.

Field Emission Scanning Electron Microscopy (FESEM). A JEOL JSM-670 IF FESEM was used to investigate the surface morphology of the hydrogels. All the hydrogels (PMAPTACMAAc) were allowed to swell in simulated body fluid and stored in a deep freezer at -80°C for 2 days. The samples were then freeze dried at -50°C using LABCONCO (USA) freeze drying system for 3 days. Samples were kept under vacuum before platinum sputtering treatment.

Swelling Experiment. To determine the equilibrium swelling *in vitro* the dried PMAPTACMAAc gel discs were incubated in simulated body fluid (SBF) at 37°C. At equilibrium the swollen discs were removed from the solution and reweighed after careful removal of excess surface water. The temperature-sensitive swelling of the hydrogels was measured at 20–70°C in a pH 7.4 buffer (SBF) solution. The swelling ratio (Q) was then calculated from the following equation:

$$Q(W_f W_i)/W_i 100 \quad (1)$$

where W_f and W_i are the final weight of the swollen disc and initial weight of swollen disc respectively. Each experiment was performed in triplicate.

Indomethacin Release Experiment. The dry PMAPTACMAAc hydrogels were equilibrated in 30 mg drug/10 mL of alcohol solution (alcohol: water = 8 : 2 volume ratio) for loading IND at 25°C for 2 days. Then the IND loaded hydrogels were dried at 25°C for 1 day and further vacuum dried to get IND loaded gel. Indomethacin loaded dried hydrogel discs (GEL-1) were placed into a conical flask containing 50 mL physiological fluid (SBF). The flask was kept at constant temperature-shaking incubator at 37°C at 100 rpm. At predetermined time points, 2 mL of solution was taken out and replaced with the same amount of buffer solution to maintain the same volume of solution. The IND concentration in the removed solution was measured by UV–vis spectrophotometer at 320 nm.[32]

Cytotoxicity Tests. The RAW 2664.7 a murine macrophage cell line was obtained from American Type Culture Collection (Manassas, VA) and was maintained in Dulbecco's modified Eagles medium (DMEM, PAA Laboratories, UK) supplemented with 10% heat activated fetal bovine serum (PAA Laboratories, UK) and 1% penicillin/streptomycin (PAA Laboratories, UK). The cell line was maintained in a humidified incubator at 37°C in an atmosphere of 5% CO_2. The hydrogel was immersed in phosphate buffer saline (pH 7.4), stored for 4 h and autoclaved at 121°C, 20 min. The samples were then incubated for 0 h, 24 h, and 5 days of incubation and immediately frozen at -80°C. The cytotoxicity effect of the hydrogel was carried out using the 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay as described by Mossman (1983).[33] Briefly, 180 μL cells were seeded in 96-well plates with a density of 20,000 cells per well for all cell lines. All the cells were incubated for overnight at 5% CO_2 and 95% humidity. After 24 h of incubation, 20 μL of sterile hydrogels were added to each well at various concentrations (0.0001–1 mg mL^{-1}) followed by incubation of the plates for 72 h in 5% CO_2 incubator at 37°C. At the end of the treatment 50 μL of MTT solution (2 mg mL^{-1}) (Sigma Chemical, Louis, MO) was added to each well and incubated for an additional 4 h. The purple blue MTT formazen precipitate was dissolved in 100 μL DMSO (Sigma Chemical, Louis, MO). The optical density of the wells was measured with an Elisa plate reader (Magellan Tecan, USA) at 520 nm. The experiment was performed in triplicate.

Statistical Analysis

All experiments were done at least thrice and figures have been expressed along with respective error bars and standard deviations, respectively.

RESULTS AND DISCUSSION

Synthesis of Hydrogels

The methacrylic acid and MAPTAC based copolymer hydrogels were synthesized by free radical copolymerization method. We observed that the prepared hydrogels were rod shaped and the physical appearance of the copolymer hydrogels changed from transparent to opaque during the course of polymerization reaction. The FTIR spectrum of respective homopolymers (PMAPTAC and MAAc) is shown in Figure 1(a,b). The IR spectrum of PMAPTAC showed important peaks at 3356, 2860, 1650, and 1542 cm^{-1} which belonged to CON—H, C—H stretch, C=ONH, and CON—H groups in the homopolymer. The IR spectrum of PMAAc homopolymer showed characteristic intense peaks at 1700, 3352, 2929 due to C=O, CON—H, C—H stretching vibration group in the homopolymer.[34] The FTIR spectrum of PMAPTACMAAc-1 hydrogels showed peaks at 3459, 2994 cm^{-1} which is associated with —O—H and C—H stretching vibration peaks [Figure 1(c)]. Other important peaks were observed at 1642 (C=O), 1550, 1482 cm^{-1} shows the incorporation of MAPTAC moieties into the copolymer gel. The IR spectrum of PMAPTACMAAc-5 showed an additional peak at 1706 cm^{-1} which is attributed to the acid group (C=O) of methacrylic acid [Figure 1(d)]. The weak peak at 1706 cm^{-1} and strong absorption band at 1551 cm^{-1} indicated that MAAc and MAPTAC units predominantly remain as electrolytic complex in the copolymer hydrogels. The incorporation of ester group and MAPTAC moieties confirms the formation of the copolymer hydrogel.

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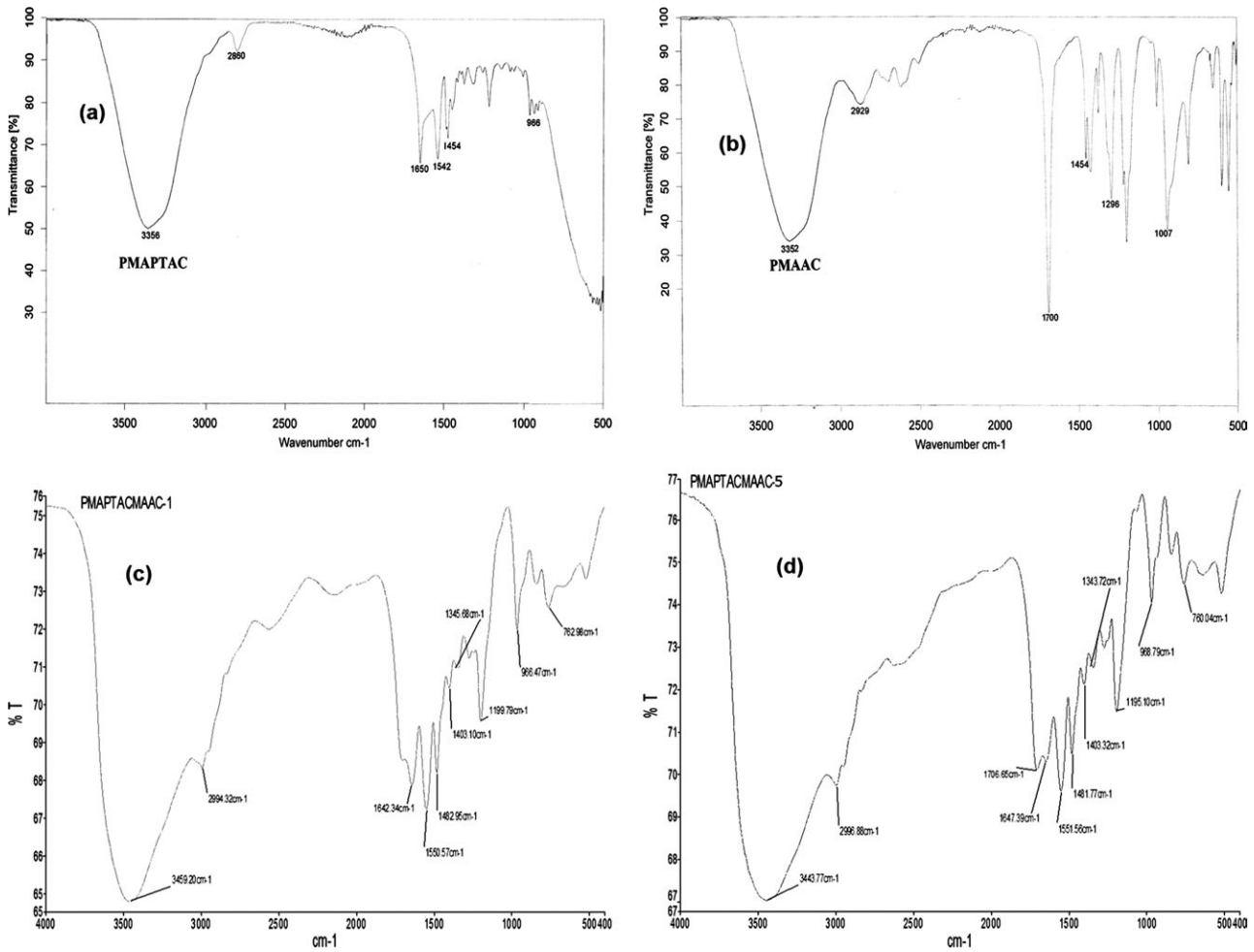

Figure 1. (a–d) FTIR spectrum of homopolymers (PMAPTAC and PMAAc) and PMAPTACMAAc hydrogels.

X-Ray Diffraction Analysis

The powder X-ray diffractogram of the homopolymers (PMAPTAC and PMAAc) and copolymer hydrogels (PMAPTACMAAc) are presented in Figure 2(a). The XRD of PMAPTAC shows an intense peak at 2θ equals to 19.47° which shows the semicrystalline nature of the homopolymer. The XRD pattern of PMAAc shows two peaks at $2\theta = 15.91°, 31.11°$. The X-ray diffractogram of PMAPTACMAAc hydrogels shows broad peaks which indicate the amorphous nature of the copolymer hydrogels. Incorporation of more PMAAc units in the hydrogels significantly reduces the crystallinity of the polymer hydrogels.[35,36] We hypothesize that PMAAc molecules are relatively small; they cannot be incorporated into the regular structure of the crystallite regions. They are forced out of the crystal packing and aggregate in the amorphous matrix surrounding the crystals. Figure 2(b) shows the XRD pattern of indomethacin (IND) and IND loaded hydrogel (PMAPTACMAAc-5). The X-ray diffractogram of IND showed the completely crystalline nature of the drug. It showed crystalline peaks at 2θ equals to 11.63°, 19.63°, 21.82°, 26.63°, and 29.35°, but these peaks have disappeared in the IND loaded gel. The XRD peak usually depends on the crystal size, but for the IND loaded gel formulation, characteristic peaks of IND have overlapped with those of the polymer. Thus IND loaded gel exhibited amorphous nature, making it hard to measure at the detection limit of the crystal size, confirming that IND was dispersed at a molecular level in the polymer matrix, as no crystals were found in IND-loaded formulations.

Thermal Analysis

DSC is a useful tool for measuring the temperature and energy variation involved in phase transitions of copolymer hydrogels. DSC thermograms of the corresponding copolymer hydrogels are presented in Figure 3(a). The thermogram of PMAPTACMAAc-1 did not show any glass transition temperature peak; however it displays a sharp endothermic peak at 178.5°C which correspond to its melting temperature peak. The thermogram of PMAPTACMAAc-2 shows an inflexion at 121.1°C which indicates the glass transition temperature (T_g) of the hydrogel and the melting transition is seen at 171.7°C. The DSC trace of PMAPTACMAAc-3 shows an onset temperature at 125.5°C which is responsible for T_g of the copolymer and a melting temperature peak at 169.4°C. PMAPTACMAAc-4 shows a sharp melting transition at 174°C and the glass transition at 125.2°C. Figure 3(b) shows the thermogram of the model drug (IND), with a sharp melting peak at 163.5°C which confirms the crystalline nature of IND. However, the thermogram of IND loaded

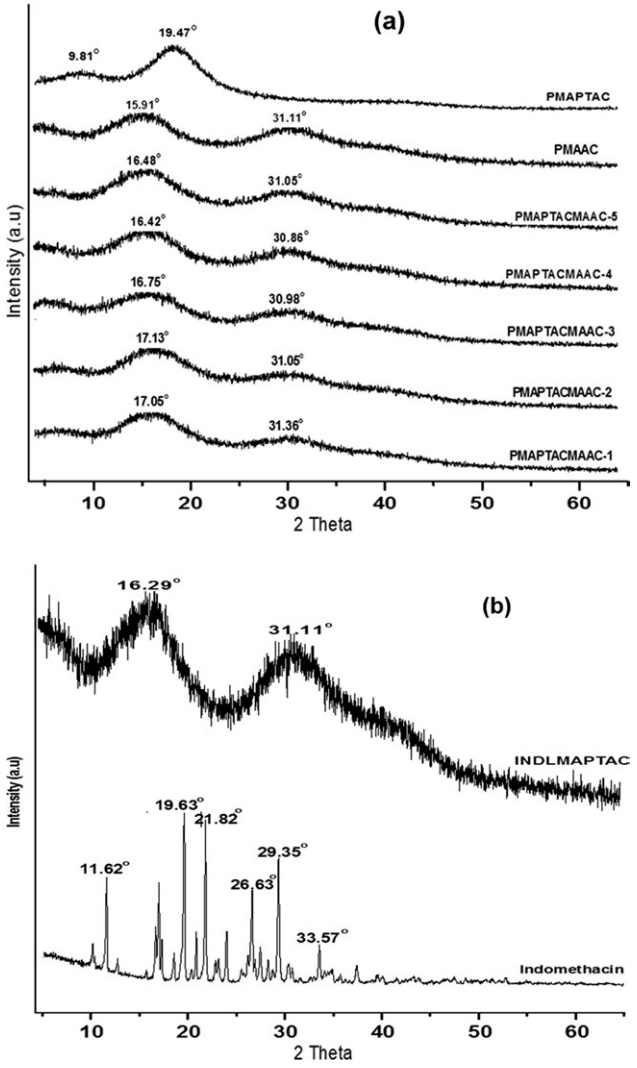

Figure 2. (a) Wide angle X-ray diffraction pattern of homopolymer and copolymer hydrogels. (b) Wide angle X-ray diffraction pattern of IND and IND loaded gel.

gel (PMAPTACMAAc-5) gives two endothermic inflexions at 90 and 126.7°C, which are due to elimination of bound crystallized water and T_g of the hydrogel. The thermogram displays a decrease in the melting transition peak (167.9°C). We observed that an increase in PMAAc units in the hydrogels lead to an increase in glass transition temperature. The presence of single T_g in the copolymers showed the formation of random copolymers.

TGA is a powerful technique to determine polymer state and evaluate the intermolecular association between polymers in hydrogels. The TGA thermogram of the copolymer hydrogels (PMAPTACMAAc-1 and 5) are shown in Figure 4(a). The first weight loss appeared to occur at 180–240°C, where the sample lost 5.68% of its weight. This step may be attributed to release of moisture from the copolymer sample. The second weight loss was evidenced at 280–380°C, and in this region the sample rapidly lost 16.54% of its weight. In the third degradation event the mass change was quite fast (53.60%) and final decomposition temperature (FDT) was observed at 460°C. The thermogram of PMAPTACMAAc-5 shows an almost similar thermal degradation pattern [Figure 4(b)]. It can be observed from the thermogram that the thermal degradation is quite fast having the final decomposition temperature at 470°C. The thermogram of PMAPTACMAAc-1 showed residual mass of 11.35% as compared to 8.05% residual mass in PMAPTACMAAc-5. This confirms that thermal stability of hydrogel decreases with increase in MAAc ratio in the gel network.

Equilibrium Swelling

The equilibrium swelling profile of the PMAPTACMAAc hydrogels in simulated body fluid (SBF) at 37°C is shown in Figure 5. A considerable variation in the degree of swelling was observed in all hydrogels. PMAPTACMAAc-1, having stoichiometric ratio of MAPTAC and MAAc, undergoes a minimum swelling ratio of 1355%. We observed that swelling degree of the hydrogels increased with increase in MAAc content, reaching maximum in PMAPTACMAAc-5 with a swelling ratio of 3128%. The increase in swelling ratio is due to dissociation of the available —COOH group in MAAc, thereby increasing the osmotic pressure inside the polyampholyte hydrogels. This is because of electrostatic repulsion among the carboxylic group which can accept or release proton in response to various pH aqueous media.[11] It is worthwhile to mention here all the hydrogels were dimensionally stable in the swollen state. We hypothesize that a lot of hydrogen bonds are formed in the

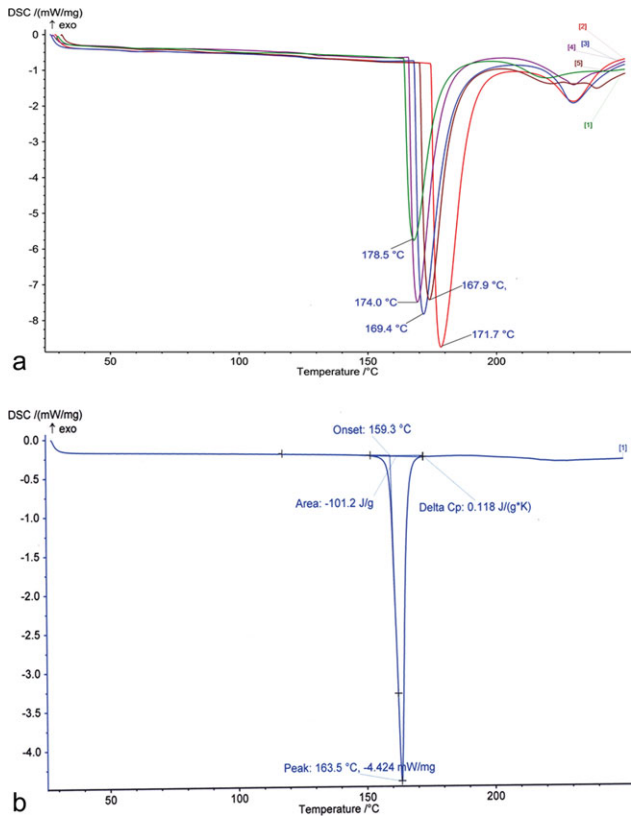

Figure 3. (a) DSC thermogram of the copolymer hydrogels. (b) DSC thermogram of indomethacin (IND). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

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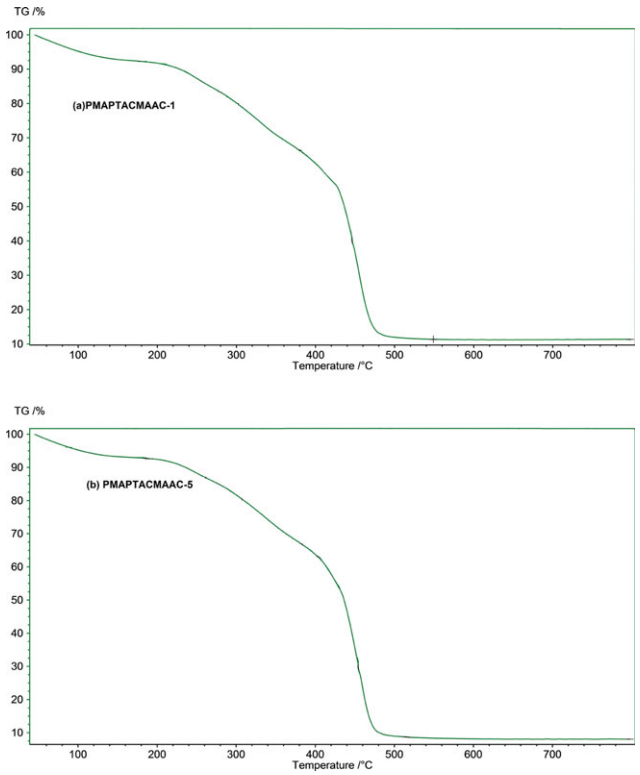

Figure 4. (a) TGA thermogram of PMAPTACMAAc-1 hydrogel (b) TGA thermogram of PMAPTACMAAc-5 hydrogel. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

simulated gastric fluid (SGF, pH 1.2) due to the presence of carboxylic group in the gel, which acts like a barrier that hinders the entrance of water molecules inside the hydrogel network (Supporting Information Figure S1). Because this pH is below the pK_a of methacrylic acid, which can explain the reduction in swelling ratio. For polyelectrolyte hydrogels a reduction of solution pH, modifies the degree of ionization. Hence it reduces the net ion concentration difference (osmotic swelling pressure) which subsequently leads to dehydration of the hydrogel. It was

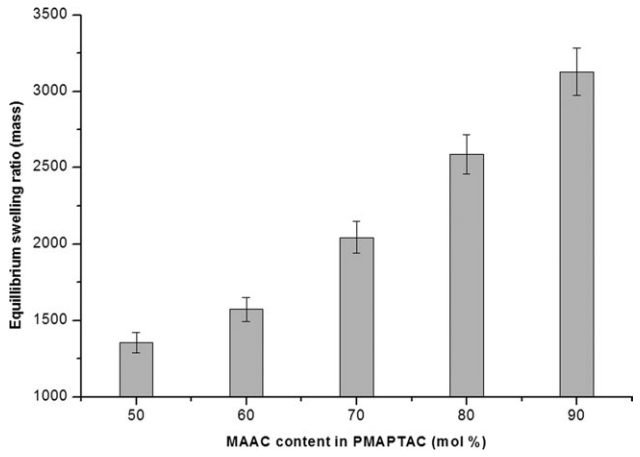

Figure 5. Equilibrium swelling ratio of the PMAPTMAAc hydrogels in SBF.

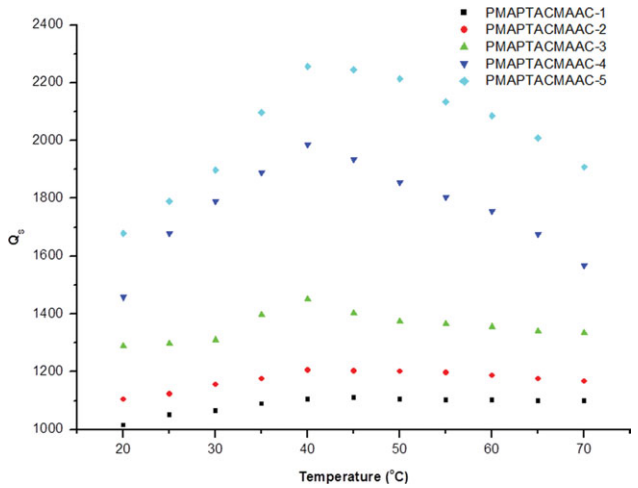

Figure 6. Thermal sensitive behaviors of the PMAPTACMAAc hydrogels. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

previously reported that hypercoiled structure of PMAAc in unionized state produces abrupt volume shrinkage at low pH.[37] Contrarily, in SBF the hydrogen bonds are broken and consequently electrostatic repulsive force is generated between the copolymer networks. This in turn will cause a greater expansion of polymer chains which leads to entrance of more water molecules through porous channels of the hydrogel.[38,39]

Thermal Sensitivity of the Hydrogels

The driving force for thermal sensitive volume phase transition is generally considered to be a subtle balance between the ability of the polymers to form H-bonds with water and the inter- and intramolecular hydrophobic forces. The thermal sensitive volume phase transition was strongly copolymer composition dependent (Figure 6). The same figure shows that PMAPTACMAAc gels (1–2) have almost similar volume phase transition behaviors. However, the temperature-induced swelling of PMAPTACMAAc-4 and 5 shows increase in Qs with temperature of up to 40°C but after that decrease in *Q*s was noticed. It is worthwhile to mention here that PMAPTACMAAc-4-5 has a higher ratio of MAAc in the hydrogel feed which affects the Qs of the hydrogel. This may be attributed to the more hydrophilic nature of MAAc in comparison to MAPTAC in the hydrogel. Nisha et al. has reported that PMAPTAC does not show temperature-induced volume phase transition behavior because of the complex nature of MAPTAC.[14] Hence MAAc plays a crucial role in improving the temperature sensitive behavior of the copolymer hydrogel.

Morphology Observation by FESEM

The surface morphology of the hydrogels was investigated by FESEM. The photomicrographs of the freeze dried PMAPTACMAAc hydrogels (a–h) are presented in Figure 7. The photomicrographs of PMAPTACMAAc-1 swelled in simulated body fluid exhibit- less porous nature. The micrograph shows that at low magnification (100×) few pores are visible on the surface of the gel network. When we increased the magnification, the morphology changed from porous to cauliflower type of

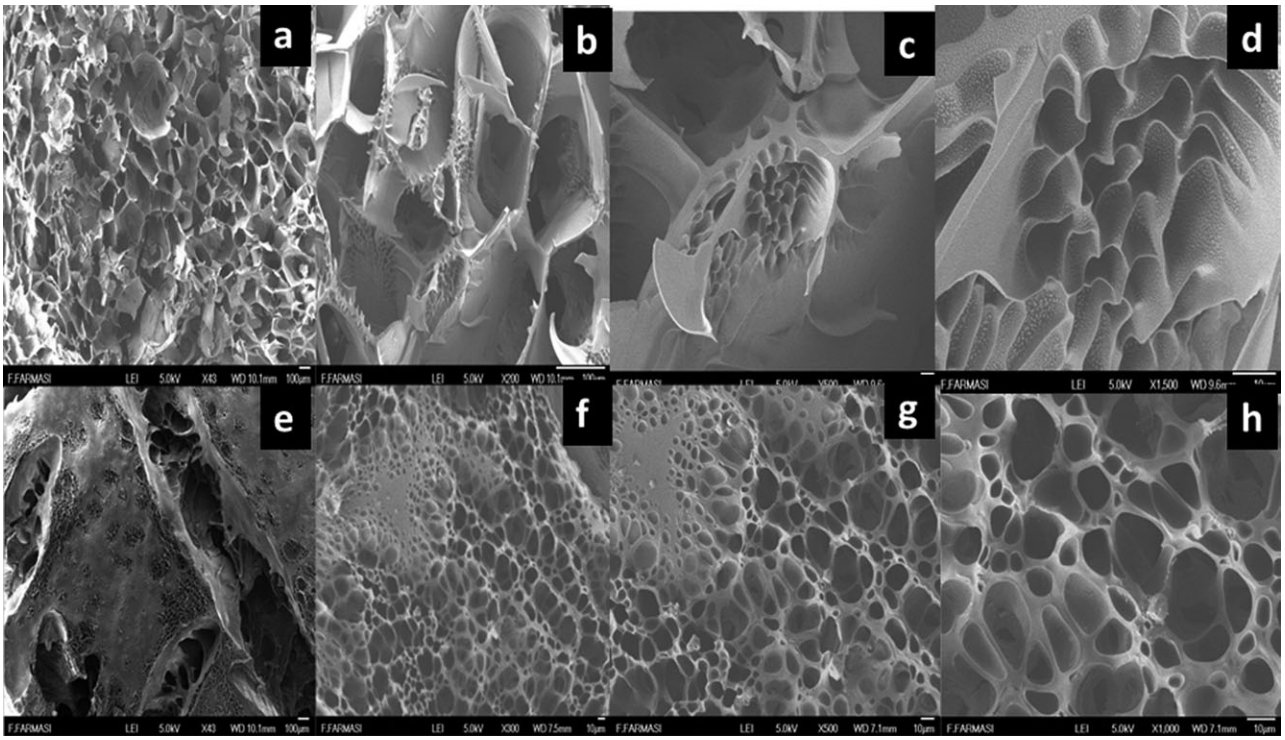

Figure 7. FESEM micrographs of the copolymer hydrogels (a–d) PMAPTACMAAc-1 (×43, ×200, ×500, and ×1500), (e–h) PMAPTACMAAc-5 (×43, ×200, ×500, and ×1000).

morphology. The photomicrographs of GEL-5 show more porous morphology as compared to GEL-1. It can be observed that gel swelled in SBF showed unique three-dimensional (3D) porous nature. This unique 3D porous network is responsible for the rapid increase in the swelling ratio of the hydrogels. The network structures formed during the polymerization may assume different size micropore structures depending on change of the swelling degree under different surroundings.[11] In SBF, a deprotonation that leads to electrostatic repulsion originating from the ionization of carboxylic groups caused more water to enter inside the hydrogel and hence polymer chains were stretched and presented larger porous structures.

Indomethacin Delivery

The cumulative releases of IND from PMAPTACMAAc hydrogels in SBF are shown in Figure 8. The percentage of IND loading was found to be 42.3%. The release of IND from the hydrogels was biphasic in nature characterized by initial rapid release (burst effect) phase followed by sustained release phase.[18] This biphasic nature mainly depends on the composition of MAAc and MAPTAC in the hydrogels. The initial burst release is evident in all the hydrogels and it decreases with increasing content of MAPTAC in the hydrogels. It can be observed that the amount of IND released after 10 min was reduced from 10.05% for PMAPTACMAAc-5 to only 5% for PMAPTACMAAc-1 hydrogels. This burst release is associated with rapid diffusion of water due to the formation of porous structure in the hydrogel when it comes in contact with water. The hydrogel composition plays a significant role in determining the sustained release performance of the hydrogel formulations. The sustained release from the PMAPTACMAAc-5 hydrogel was most efficient; it released 75% of entrapped IND in 8 h and at the end of 12 h 82% of IND had been released from the hydrogel. The other hydrogel formulations showed percentage cumulative IND release of 77.39, 71.23, 64.15, and 43.5%, respectively at the end of 12 h of study. The increase in MAAc ratio in the hydrogels leads to an increase in percentage of IND release of the hydrogels. The incorporation of more MAAc units leads to electrostatic repulsion of carboxylic group which leads to maximum expansion of the network. Similar results were suggested by

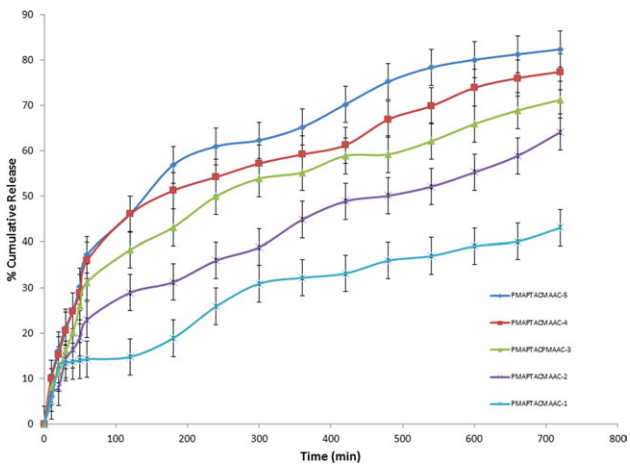

Figure 8. Percentage cumulative IND release profile of the copolymer hydrogels in SBF. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

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others who studied the effect of hydrophilic and hydrophobic matrices on drug delivery.[40–43] Huang et al. found that lidocaine release was faster from PEG/PLA copolymer than from PLA homopolymer. They suggested that PEG/PLA copolymer can imbibe more water as compared to PLA homopolymer which eventually results in faster drug release. The primary mechanism of transport of many drugs from hydrogels is the diffusion occurring through the space between the macromolecular chains of a polymer. The porous nature significantly affects the drug release from the hydrogel formulation. In case of the copolymer hydrogels (PMAPTACMAAc) the pore size significantly improves the indomethacin release. As evident by the photomicrographs of the gels (Figure 7) that the MAAc composition causes remarkable improvement in pore size that causes more efficient transport of IND through the hydrogel. The release study showed that these hydrogels could be tried as sustained release formulations for colon targeted drug delivery. It is an established fact that for an ideal colon targeted drug delivery system, the drug release should be prevented in stomach and small intestine. Hence one need to insure that drug must be released successfully within the residence time of oral dosage form in the colonic region. Because it was reported earlier that colonic residence time is highly variable (10 to 30–40 h), so we thought to design a probable drug delivery system in the intermediate range of 12 h that will insure maximum release of IND even in cases when colonic transit time is on the lower side during some pathological conditions of bowel disease.[21]

The drug release kinetics (F) from different matrices were determined by Ritger and Peppas[44]

$$FM_t/M_K t^n \tag{2}$$

where k is a constant representing the apparent release rate (%) that takes into account structural and geometrical characteristics of the release device and n is the diffusional exponent. The value of n is very important for investigating the drug release mechanism from different matrices. In case of nonswelling matrices, the drug release is generally expressed by Fickian diffusion, for which $n = 0.5$. In case of swelling matrices, the release mechanism is generally governed by combination of swelling and erosion. They follow non-Fickian release mechanism, for which n generally ranges from 0.5 to 1. For most erodible matrices the drug release follows zero order kinetics for which $n = 1$. Occasionally a value of $n > 1$ is observed in few reports, which have been regarded as super case-II kinetics.[45–47] The value of diffusional exponent (n) was calculated from the cumulative release of the hydrogels and it is listed in Table II. The calculated value of n (0.52–0.98) showed that the release of IND through the hydrogel followed non-Fickian release mechanism. This confirms that swelling/erosion plays a vital role in diffusion of IND through the copolymer hydrogels. It has been reported earlier that if a particular drug has less water solubility, as in case of IND, the possibility of drug release by diffusion is practically zero and release takes place by surface erosion.[48,49]

Table II. Analysis of the Release Data from the Copolymer Hydrogels

Sample identity	n	$k \times 100$	R^2
PMAPTACMAAc-1	0.52	2.25	0.954
PMAPTACMAAc-2	0.55	2.92	0.965
PMAPTACMAAc-3	0.64	3.35	0.978
PMAPTACMAAc-4	0.78	4.43	0.986
PMAPTACMAAc-5	0.98	4.97	0.982

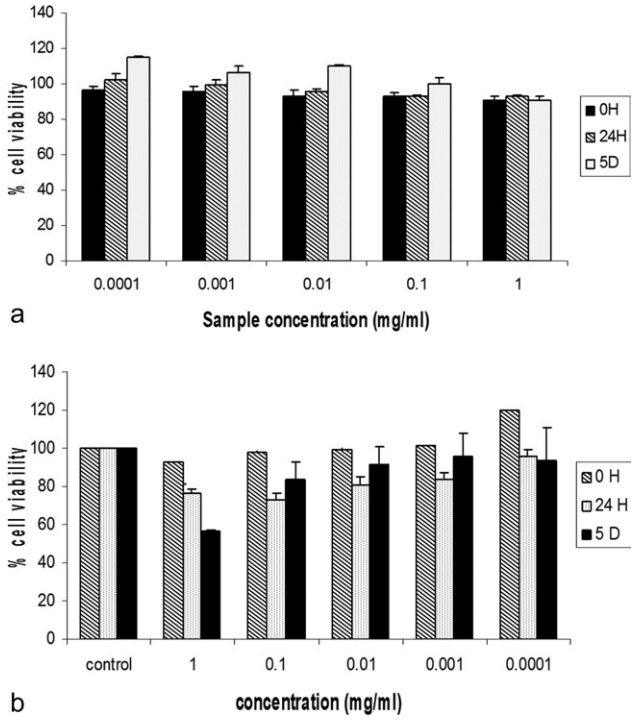

Figure 9. Cytotoxicity of PMAPTACMAAc-5 hydrogel at various concentrations (0.0001–1 mg mL^{-1}) without (a) or with IND (b) at 0 h, 24 h, or 5 days.

Cytotoxicity

The cytotoxicity of the PMAPTACMAAc-5 hydrogel was measured by MTT assay (Figure 9) using RAW 264.7 murine macrophage. The results showed that at all the tested concentrations PMAPTACMAAc-5 without the drug maintained their viability, indicating that the hydrogel itself was not cytotoxic. In contrast, when hydrogel was loaded with the drug, at high concentrations (1 mg mL^{-1}) the cells were killed ($P < 0.05$) by about 34 and 44% after 24 h and 5 days of incubation, respectively. The results showed that the drug released from PMAPTACMAAc-5 hydrogel remained biologically active. In addition, the developed hydrogels were not toxic.

CONCLUSIONS

We designed poly 3-[(methacryoylamino) propyl trimethylammonium chloride-*co*-methacrylic acid] (MAPTACMAAc) hydrogels by the aqueous free radical polymerization method for controlled delivery of IND. Chemical interaction between the comonomers (MAAc and MAPTAC) is confirmed by FTIR spectrum indicating that MAPTAC moieties were incorporated in

the hydrogel. Wide-angle X-ray diffraction pattern of the polymamholyte hydrogels confirmed the amorphous nature of the copolymers. The WAXD analysis of the IND loaded hydrogel showed no crystalline fractions possibly due to complete dispersion of IND at the molecular level in the gel. The DSC study showed increase in glass transition temperature of the copolymer gel with increase in MAAc content in the hydrogels and showed a single T_g in the gels which confirmed the formation of random copolymers. The increase of T_g in the copolymer hydrogels may be due to increase in segmental motion of the MAPTAC and MAAc molecules in the gel. The TGA analysis of the copolymer hydrogels showed a reduction in thermal stability. The FESEM observation of the PMAPTACMAAc hydrogels exhibits the three dimensional porous structure which relates with higher water uptake of the gel at SBF. The temperature sensitive nature is highly dependent on copolymer composition and MAAc improves the volume phase transition nature of the hydrogels. The IND release study showed the sustained release nature of the hydrogel formulations and it was highly dependent on the composition of polymers. The burst release was evident in all formulations and PMAPTACMAAc-5 showed maximum IND cumulative release (82%) after 12 h of release study. The calculated values of diffusional exponent from IND release data show that hydrogels followed non-Fickian release kinetics. *In vitro* cytotoxicity of the hydrogel (PMAPTACMAAc-5) against RAW 264.7 murine macrophages showed that the hydrogel was biocompatible and not toxic. The results suggest that PMAPTACMAAc hydrogel formulations can be interesting candidates for colon targeted delivery of IND.

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