pH-Responsive Poly(DMAPMA-*co*-HEMA)-Based Hydrogels for Prolonged Release of 5-Fluorouracil

R. K. Mishra,[1,2] K. Ramasamy,[3] A. B. A. Majeed[1,2]

[1]*Brain Science Research Laboratory, Faculty of Pharmacy, Universiti Teknologi MARA, 42300, Puncak Alam, Selangor, Malaysia*
[2]*Biopharmaceutics and Pharmacokinetics Laboratory, Faculty of Pharmacy, Universiti Teknologi MARA, 42300, Puncak Alam, Selangor, Malaysia*
[3]*Collaborative Drug Discovery Research Group, Faculty of Pharmacy, Universiti Teknologi MARA, 42300, Puncak Alam, Selangor, Malaysia*

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ABSTRACT: This work reports the synthesis and characterization of pH-sensitive hydrogel (PSHs) system composed of *N*[-3(dimethylamino)propyl] methacrylamide (DMAPMA) and 2-hydroxyethyl methacrylate (HEMA). This hydrogel was prepared efficiently by solution copolymerization method using *N*,*N*-methylene bisacrylamide as crosslinker and sodium persulfate/ammonium persulfate as joint initiator system. The chemical structure of the hydrogel was confirmed by Fourier transform infrared spectroscopy and proton nuclear magnetic resonance spectroscopy. Thermal stability and morphology of the hydrogels were assessed by thermogravimetric analysis and field emission scanning electron microscopy. 5-Fluorouracil (5-FU), an anticancer drug was loaded in the hydrogels to investigate their drug release properties using a human colon cancer cell line (DLD-1). The pH (pH 1.2–7.4) as well as temperature-sensitive swelling of the hydrogel was also determined. The morphological analysis of the resulting hydrogel revealed a highly interconnected macroporous interior with pore size ranging from 10 to 100 μm in size. The swelling of the hydrogel was highly influenced by pH of the surrounding medium and higher swelling ratio was observed at simulated intestinal fluid (SIF). The 5-FU release was found to be more efficient in SIF as compared with simulated gastric fluid. The results showed that the 5-FU released from the hydrogel remained biologically active and the developed hydrogel was not cytotoxic. The hydrogel with a 50 : 50 feed ratio of DMAPMA and HEMA was better than the other developed hydrogels. The PSH is furthermore safe for colon-targeted delivery of 5-FU. © 2012 Wiley Periodicals, Inc. J Appl Polym Sci 000: 000–000, 2012

Key words: pH-sensitive hydrogel; HEMA; DMAPMA; colon cancer; 5-fluorouracil

INTRODUCTION

Cancer is the leading cause of death worldwide; however, the existing chemotherapeutic agents have clear limitations because of their toxicity. Colon cancer is still the major cause of deaths in developed world, second only to heart diseases.[1] In the case of colon cancer, conventional chemotherapy is not as effective as compared with other cancers because drugs do not reach the target site in effective concentration, leading to increased dose size causing enormous toxicity. Therefore, the opportunity to deliver cancer drugs efficiently to the colon to improve safety and efficacy is of great importance. The high mortality associated with colorectal cancer demands effective prevention along with surgery, radiation, and chemotherapy. The various methodologies used for colon-targeted delivery of drugs include pro-drug approach, time dependent system, pH dependent formulations, and microbial triggered systems.[2–8] The design and development of new formulations or oral dosage forms for controlled drug-delivery system (CTDDS) has gained significant attention over the years. Polymers have been widely used in the pharmaceutical industry as encapsulants and vehicles for drug carriage. Polymeric hydrogels, in particular, have shown tremendous assurance in formulations for controlled drug-delivery applications. pH-sensitive hydrogels (PSHs) are emerging tailor made materials that demonstrate excellent structural and compositional features as well as unique tunable time-dependent swelling behavior. Smart or stimuli responsive hydrogels are a special class of materials that can quickly respond to slight changes in external stimuli such as pH, temperature, ionic strength, light, electric and magnetic field.[9–14] They can, therefore, be used as a biological on–off switches for different biomedical applications.

Poly(2-hydroxyethyl methacrylate) (PHEMA) is a widely used polymer in various hydrogel formulations.

Correspondence to: A. B. A. Majeed (abubakar@salam.uitm.edu.my).

Significant attention has been paid to PHEMA-based macroporous hydrogels because of their potential use in cell and tissue engineering-based applications.[15–17] However, PHEMA hydrogels are not believed to be pH responsive in nature so attempts have been made to improve it by copolymerizing with some co-monomers.[18–21] Poly(*N*[-3(dimethylamino)propyl] methacrylamide) (PDMAPMA), on the other hand, is believed to be temperature responsive in nature but mechanical properties of the gels are not good enough to be used in drug release formulations.[22] Recently, a few reports have been published on PDMAPMA hydrogels for various drug and protein release applications.[23–26]

In this study, 5-fluorouracil (5-FU), a widely used anticancer drug in clinical treatment of different solid tumors, including colorectal cancer[27] is used as a model drug to study the feasibility of using poly (DMAPMA-*co*-HEMA) hydrogels as a carrier. We therefore, synthesized poly(DMAPMA-*co*-HEMA) hydrogels by simple and efficient aqueous copolymerization method for colon cancer delivery. The developed hydrogels were investigated for water uptake and 5-FU release efficiency in different physiological fluids. The hydrogels were extensively characterized by Fourier transform infrared spectroscopy (FTIR), proton nuclear magnetic resonance (^{1}H-NMR), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), and field emission scanning electron microscopy (FESEM) analysis. The cytocompatibility of the hydrogels was also assessed under *in vitro* conditions with a human DLD-1 colon cancer cell line.

EXPERIMENTAL

Materials

HEMA (Sigma Aldrich, St. Louis) and DMAPMA (Sigma Aldrich, St. Louis) were used as monomers in the study. *N*,*N*-methylene bisacrylamide (MBA), sodium persulfate (SPS) ammonium persulfate and *N*,*N*,*N*,*N*-tetramethyleneethylene (TEMED) were purchased from Sigma Aldrich, (St. Louis). 5-FU a model anticancer drug was purchased from Kyowa Hakko Bio Singapore Pte. Ltd (Singapore). Deionized water was used for all copolymerization reactions and in the preparation of buffer solutions.

Synthesis of copolymer hydrogels

The copolymer hydrogels were prepared by free radical aqueous copolymerization of DMAPMA and HEMA using MBA, SPS/APS, and TEMED as cross-linker, initiator, and accelerator respectively. The reactions were carried out with the slight modification in the technique as described earlier.[25]

In a typical reaction, predetermined amount of HEMA and DMAPMA were taken in a 100-mL three-necked flask equipped with nitrogen inlet system and stirred continuously on a magnetic stirrer for 10 min at 37 ± 1°C. Predetermined amount of deionized water was added into the flask followed by addition of MBA (1 mol %) and SPS/APS (0.5 mol %). The nitrogen was continuously purged inside the flask and after 5 min TEMED (3 mol %) was added to the reaction mixture under continuous stirring. Further, the reaction mixtures were transferred to PVC molds and nitrogen gas was purged for 5 min each, gelation was found to occur in the molds after 10 min. The obtained hydrogels were cut into small disks and placed in deionized water (3 days) to remove the unreacted monomers. The water was changed daily. The disks were dried at room temperature to xerogels. The synthesized hydrogels were designated as GEL-1, −2, −3, and −4. The homopolymers PDMAPMA and PHEMA were also synthesized under the same reaction conditions used for the copolymer hydrogels. The PHEMA was obtained as cylindrical gel and PDMAPMA was obtained by precipitation in excess of 2-butanone. The feed composition of poly (DMAPMA-*co*-HEMA) hydrogels and other synthetic parameters are listed in Table I.

TABLE I
Composition of Poly(HEMA-*co*-DMAPMA) Hydrogels[a]

Sample ID	HEMA (mol %)	DMAPMA (mol %)	Water (mol %)[b]
GEL-1	50	50	200
GEL-2	60	40	150
GEL-3	70	30	100
GEL-4	80	20	50

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

Characterization

FTIR-ATR spectroscopy

The infrared spectra of homopolymers (PHEMA and PDMAPMA) and copolymer hydrogels were recorded with Varian 640-IR FT-IR spectrophotometer.

^{1}H-NMR spectroscopy

^{1}H-NMR of the hydrogel was performed with Ultrashield Plus 500 MHz Bruker NMR spectrometer with 5-mm BBO probe using DMSO-d_6 as the solvent and TMS as the internal standard.

Field emission scanning electron microscopy

A JEOL JSM-670 IF field emission scanning electron microscope (FESEM) was used to investigate the

interior morphology of the PSHs. All the hydrogels (GELs 1–4) were allowed to swell in simulated intestinal fluid (SIF) 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.

Thermogravimetric analysis

Thermal stability of the homopolymer and copolymer networks were analyzed with NETZSCH TG 209 F3 at a heating rate of 20°C/min with nitrogen flushed at 100 mL/min.

Differential scanning calorimetry

DSC of the polymeric gels and homopolymers were recorded with PerkinElmer Differential scanning calorimeter with Hyper DSC at a 10°C/min heating rate.

Swelling studies

To investigate the pH-sensitive swelling behavior, the dry hydrogel disks of 5 mm were weighed and placed in buffered solution of various pH (1.2, 5.8, 6.8, and 7.4) at room temperature. Swelling ratio (SR) was determined by gravimetric method. The temperature-sensitive swelling of the hydrogels was measured at 20–70°C in a pH 7.4 buffer (SIF) solution. At predetermined time intervals, the swollen hydrogel disks were removed, excess water was blotted from the surface with tissue paper, and the sample was weighed. The SR was calculated from eq. (1), correlating the weight of the swollen hydrogel (W_s) to the weight of the dried hydrogel (W_d). The swelling experiments were repeated three times and the average values were reported:

$$Q_S = (W_s - W_d)/W_d \quad (1)$$

where Q_S is the SR of the hydrogel.

5-FU loading and encapsulation efficiency

5-FU was incorporated into copolymer networks by a swelling equilibrium method. The hydrogel disk (GEL-1) was allowed to swell in the drug solution of known concentration in SIF and simulated gastric fluid (SGF) for 3 days at room temperature. During this process, drug in the solvent was adsorbed into the hydrogels. To determine the actual drug entrapped in the hydrogel, the samples were placed in 30-mL buffer solution and stirred for 48 h. The solution was filtered and assayed by UV–vis spectrophotometer at 266 nm.[28] The percent drug loading and encapsulation efficiency (EE) were calculated using eqs. (2) and (3), respectively.

$$\text{Drug loading (\%)} = \text{Weight of drug in gel/weight of gel} \times 100 \quad (2)$$

$$\text{Encapsulation efficiency (\%)} = \text{Actual loading/Theoretical loading} \times 100 \quad (3)$$

5-FU release experiment

The 5-FU loaded dried hydrogel disk (GEL-1) was placed into a conical flask containing 50-mL physiological fluid (SIF and GIF). 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 in order to maintain the same volume of solution. The percentage cumulative release of 5-FU was analyzed at 266 nm by using UV visible spectrophotometer. Each sample experiment was repeated three times and the final results were calculated as an average.[29]

Anticancer effect of released 5-FU

The human colon cancer cell line DLD-1 was obtained from American Type Culture Collection (Manassas, VA) and was cultured in Roswell Park Memorial Institute (RPMI) 1640 Medium (Sigma, Germany) supplemented with 10% heat-activated fetal bovine serum (PAA Laboratories, Austria) and 1% penicillin/streptomycin (PAA Laboratories, Austria). Cultures were maintained in a humidified incubator at 37°C in an atmosphere of 5% CO_2.

Cytotoxic activity of GEL-1 loaded with or without 5-FU at various concentrations (0.1–1000 μg mL^{-1}) following 72 h of incubation was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (Sigma, Germany) assay, as described by Mosmann[30] but with minor modifications. Assay plates were read using a spectrophotometer at 520 nm. Data generated were used to calculate the percentage of viable cells.

RESULTS AND DISCUSSION

FTIR-ATR spectroscopy

Figure 1(a) shows the FTIR spectra of the respective homopolymers (PDMAPMA and PHEMA). The IR spectrum of PDMAPMA showed peaks at $\nu = 3335$, 1626, and 1537 cm^{-1} corresponding to N—H stretching, amide-I and amide-II peaks, respectively.[25] The spectrum showed peaks at $\nu = 2962$ and 1042 cm^{-1} that can be attributed to —C—H stretching and

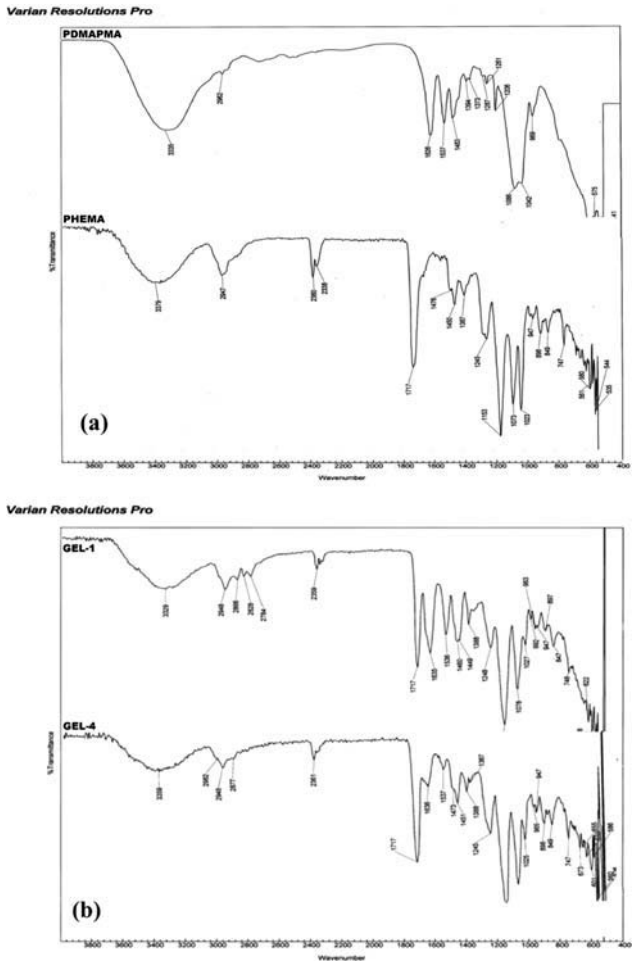

Figure 1 (a) IR spectra of PDMAPMA and PHEMA; (b) IR spectra of hydrogels (GEL-1 and 4).

—O—H bending vibration peaks. Intense peaks were observed in the IR spectrum of PHEMA at $\nu = 3379$, 2947, and 1717 cm^{-1}, which corresponds to —O—H stretching, —C—H stretching and ester group (C=O) stretching.[31] The peak observed at $\nu = 1023$ cm^{-1} can be attributed to —O—H bending vibration peak. The chemical interaction between HEMA and DMAPMA was evident from the IR spectrum of GEL-1 and −4 [Fig. 1(b)]. The spectrum of GEL-1 showed intense peaks at $\nu = 1717$, 1635, and 1536 cm^{-1}, which confirm the incorporation of ester (C=O) and amide groups (amide-I and amide-II) in the copolymer hydrogel. The other important absorption peaks observed were at $\nu = 3329$, 2948, 1027, and 1460 cm^{-1}, which relates to —N—H stretch, —C—H stretch, —O—H bending and CH_2 deformation vibration peak, respectively. The weak band at $\nu = 2359$ cm^{-1} can be due to vibrations of associated bound water (band of H—O—H bending) in the PHEMA and copolymer hydrogel samples.[32,33] The IR spectra of GEL-4 evinces absorption at $\nu =$ 1717, 1636, and 1537 cm^{-1} which relates to ester group (C=O) and amide groups (amide-I and amide-II) in the gel. There was a regular decrease in intensity of amide groups peaks from GEL-1 to GEL-4 which was associated with decrease in DMAPMA units in the gels.

^{1}H-NMR spectroscopy

^{1}H-NMR was applied to study the formation of poly(DMAPMA-*co*-HEMA) hydrogels (Fig. 2). The proton NMR spectra of the gel in DMSO-d_6 showed a chemical shift at $\delta = 0.7$–1.5 ppm that is attributed to inner methylene signals (CH—CH_2 and C—CH_2) and methyl proton signals (CH—CH_3). The chemical shifts in the region of $\delta = 1.8$–2.6 ppm can be associated with methylene (N—CH_2) and methyl signal (N—CH_3) protons of the DMAPMA units. The chemical shifts in the region of $\delta = 3.6$–4.1 ppm attributes to methylene protons (adjacent to oxygen moieties of the ester linkages (CH_2—O—C=O) and to the terminal hydroxyl group of HEMA units (CH_2—OH) and the amide protons (—NH).[34] The chemical shift at 2.5 ppm corresponds to the residual proton signal of DMSO-d_6 in the ^{1}H-NMR spectrum.

DSC study

The DSC curves of respective homopolymers (PDMAPMA and PHEMA) and copolymer hydrogels (GEL-1 and −4) are shown in Figure 3. The DSC curve of PDMAPMA homopolymer showed an endothermic peak at 69.98°C which is associated with bound water elimination and another inflexion at 124°C which may be due to glass transition temperature (T_g) of PDMAPMA network. On the other hand, PHEMA showed only one broad endothermic peak at 146°C corresponding to the melting temperature inflexion of the homopolymer network. DSC curves of the copolymer gels did not show any glass transition peak. The DSC curve of GEL-1 showed two broad endothermic inflexions at 80°C and 191°C that is associated with bound water and melting temperature peak of the gel. Contrary to GEL-1, the GEL-4 DSC curve showed a sharp endothermic inflexion at 65°C due to elimination of bound water and a second peak was observed at 160°C corresponding to melting transition in the gel network.

Thermogravimetric analysis

The respective TGA thermograms of GEL-1 and −4 are shown in Figure 4(a,b). The thermogram of GEL-1 showed a three step thermal degradation pattern. The TGA thermogram of GEL-1 showed initial decomposition temperature (IDT) at 240°C, with a sample loss of 7.80% of its weight at this temperature. The second onset temperature in the thermogram was observed at 293°C. The maximum weight

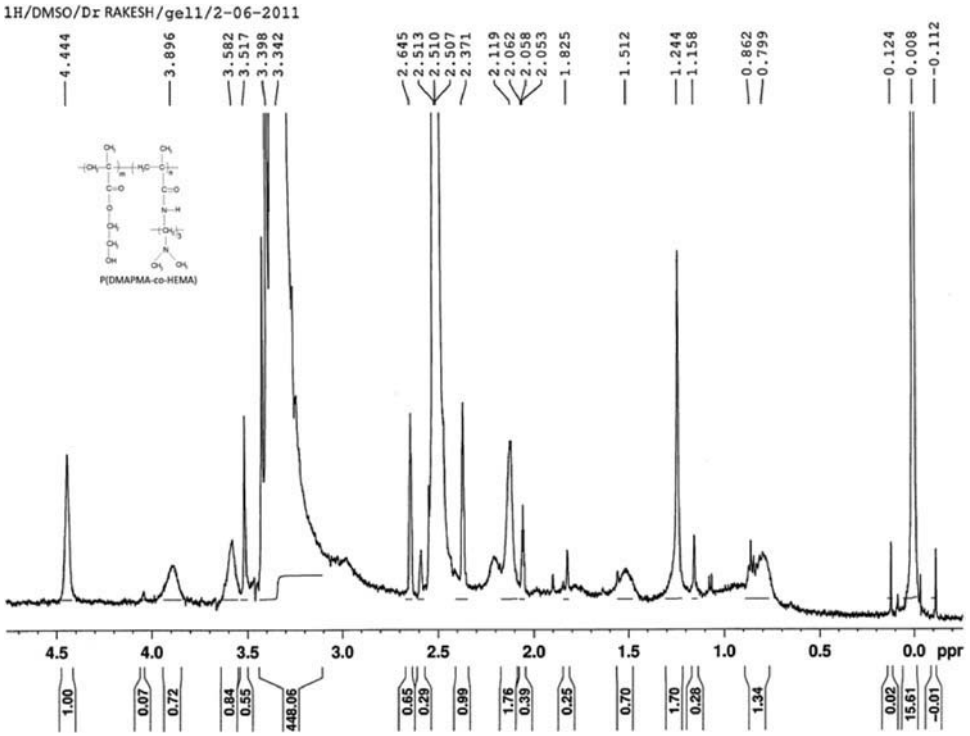

Figure 2 Proton NMR (^{1}H-NMR) spectra of the copolymer hydrogel (GEL-1).

loss (T_{max}) was observed at 450°C that is evident from the DTG curve of the gel. The third degradation step was noticed at 380–520°C, which is associated with the degradation of the polymeric network. At this temperature range, the sample rapidly lost 55.18% of the original weight and residual char yield was 11.35%. The TGA thermogram of GEL-4 [Fig. 4(b)] also confirmed the three step thermal degradation mechanism. The sample started to thermally degrade at 193°C (IDT) and lost almost 6.08% of its original weight. The thermogram of GEL-4 evinces second weight loss step at 289–390°C, which can be explained by the random chain scission of the PHEMA and PDMAPMA network. At this temperature range, the total weight loss was almost 18.43% that is faster when compared to GEL-1. The third thermal degradation was at 415–520°C, where sample rapidly lost 56.33% of its original weight. The maximum thermal degradation (T_{max}) peak was observed at 451°C. It was noticed that IDT was much higher in GEL-1 (240°C) as compared to GEL-4 (193°C). The total residual mass or char yield was also higher in GEL-1 as compared to GEL-4 at 798°C, which confirm that GEL-1 is thermally more stable than GEL-4. The higher thermal stability of GEL-1 may be ascribed to more pronounced intermolecular association among ester group of PHEMA and —NH group of PDMAPMA molecules that require more energy to break the bonds, and in turn enhances the thermal stability of the gel.

pH/temperature-induced swelling

The swelling properties of the developed hydrogels were investigated in buffer from pH 1.2 to 7.4. It is already an established fact that swelling or water uptake of the hydrogels correlates directly with drug release properties. Figure 5(a) shows the pH dependent swelling of GEL-1 in which SR was plotted against time (min). It is evident (from the figure) that an increase in the pH of the solution has a significant effect on SR of the gel. We observed that GEL-1 showed maximum SR (12.99) at SIF and

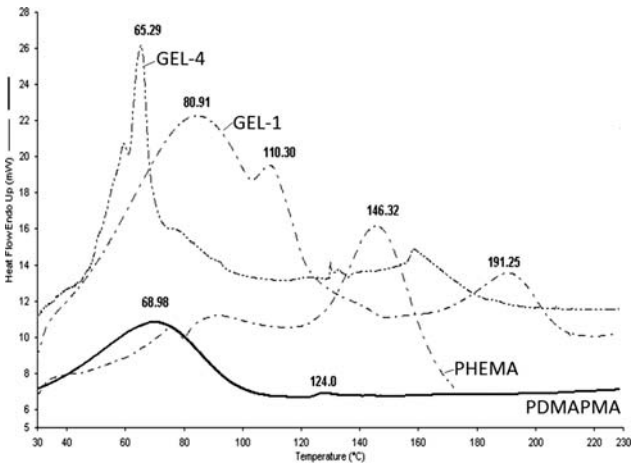

Figure 3 DSC of homopolymers (PDMAPMA and PHEMA) and hydrogels (GEL-1 and 4).

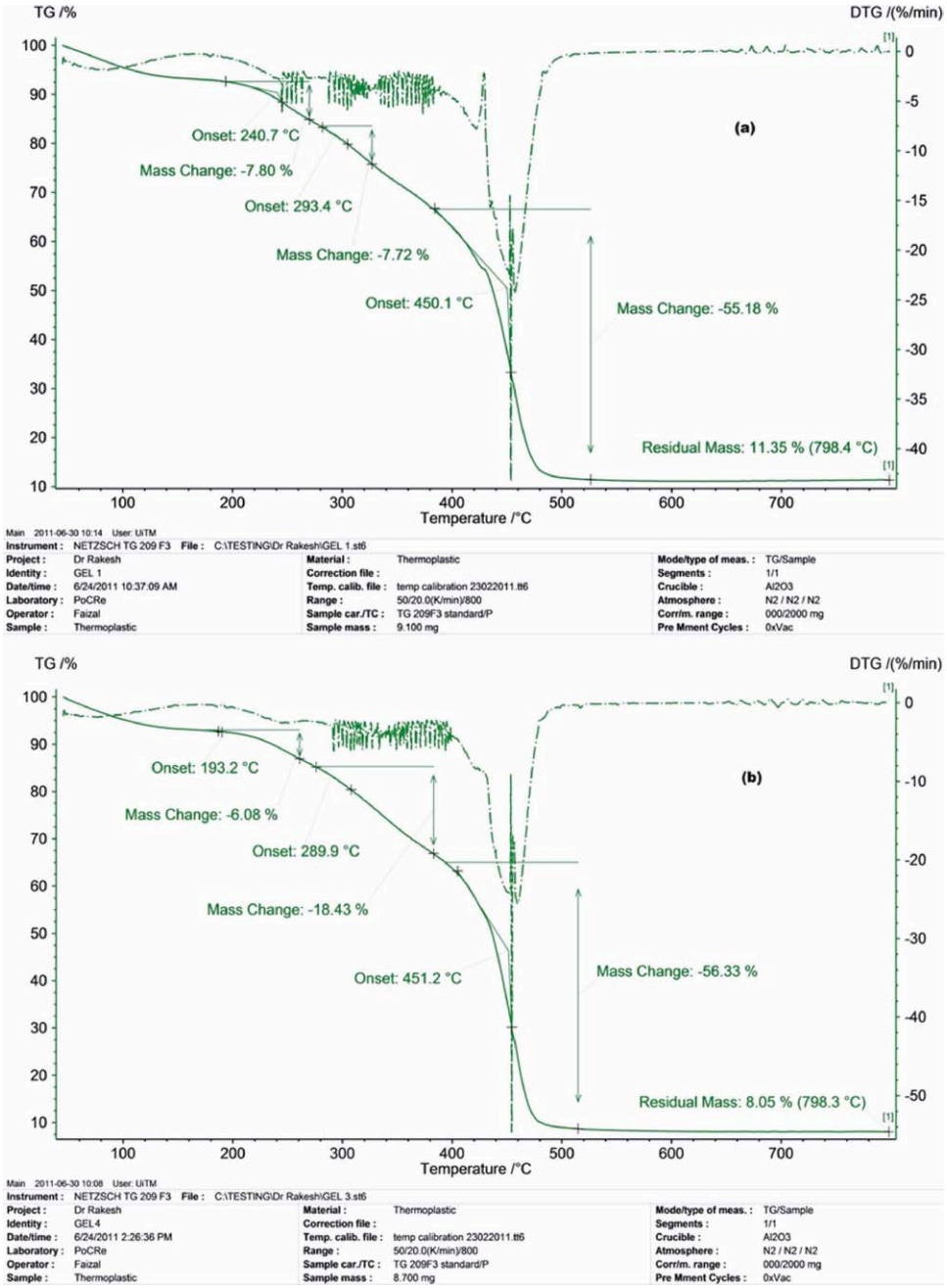

Figure 4 TGA and DTG thermograms of (a) GEL-1 and (b) GEL-4. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

minimum SR (3.71) at SGF. It is evident from the swelling curve of GEL-1 that almost similar SR was noticed up to 9 h of swelling study ($P > 0.05$) at pH 5.8 and 6.8. The swelling kinetics of GEL-4 [Fig. 5(b)] showed less pH-sensitive nature as compared to GEL-1. This might be attributed to the less water and higher HEMA concentration in the hydrogel feed. Swelling of hydrogel involves large segmental motion, resulting ultimately, in increased separation of hydrogel chains.[35] The $(CH_3)_2$ N $(CH_2)_3$ group of DMAPMA is believed to be more hydrophobic[36] than HEMA which has free —OH groups, imparting a hydrophilic nature to the hydrogel system. The maximum swelling of GEL-1 at SIF can be attributed to the fact that the —OH groups of HEMA is involved in H-bond interaction between hydrogel and water. The strong H-bonding with water and hydroxyl group of HEMA also enhances the hydrophilicity of the system.[37,38] The maximum swelling of GEL-1 can also be very likely due to the partial hydrolysis of HEMA at alkaline pH.[36,39] In contrast, under acidic conditions (SGF), the —OH group of HEMA does not have much intermolecular attraction toward water, which resulted in a reduction in

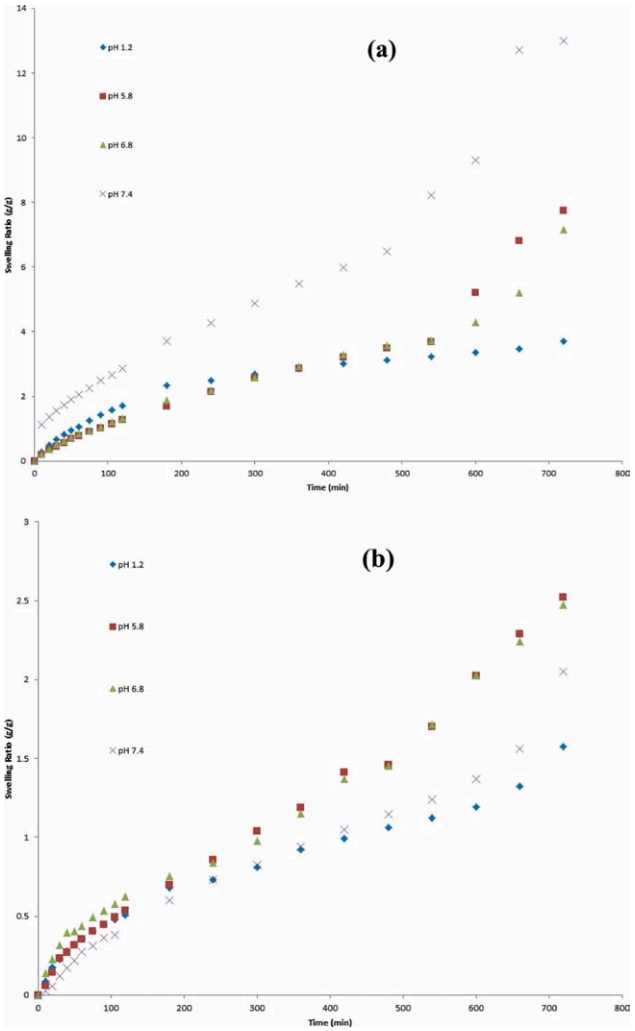

Figure 5 (a) Swelling curve of GEL-1 in different pH buffers; (b) GEL-4 in different pH buffers. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

SR of the gel. It is worthwhile to mention here that in the developed hydrogel formulations (GEL-1 to −4), the amount of water reduced but the HEMA content increased. Reduction in water content leads to a lesser phase separation behavior and probability of a lesser H-bond formation with hydroxyl group of HEMA that in turn reduces the SR of the hydrogel (GEL-4). The experimental results of SR of the hydrogels were in good agreement with the microstructure of the hydrogels (Fig. 7), which suggested that the SR of the poly(DMAPMA-*co*-HEMA) hydrogels also depends on the characteristic of their network structure.

Temperature-dependent swelling nature of the hydrogels were investigated in SIF at 20–70°C. All the hydrogels showed typical re-entrant conformational phase transition (RCPT) behavior. The swelling curve of GEL-1 (Fig. 6) showed a decrease in SR (7.52) for up to 30°C but after that a sharp increase in SR (13.99) was noticed, which confirm the RCPT nature of the gel. It was observed that after 50°C, there was a slight increase in SR of the hydrogels after which it leveled off. It is worthwhile to mention here that phase transition behavior is more prominent in GEL-1 as compared to other hydrogels (GEL-2 to −4). This may be attributed to the fact that as the temperature increased to the range below the RCPT, the more hydrophobic groups of the gel network become involved in hydrogen bond formation which lowers the entrance of water molecules inside the gel network, leading to a decrease in SR of the hydrogels. When temperature increased above RCPT the hydrophobic interactions due to hydrogen bonding among gel chains decreased leading to a dramatic increase in SR of the hydrogels.[40,41]

FESEM analysis (morphology)

The cross section or interior morphology of the poly(HEMA-*co*-DMAPMA) hydrogels was taken with the help of FESEM [Fig. 7(a–p)]. The photomicrograph of GEL-1 showed uniform pores all over the surface of the hydrogel with 100–200 μm in size (43×). On higher magnification (1000×), it revealed pores with lateral skin on the surface. GEL-2 showed a highly macroporous nature of the hydrogel as compared to GEL-1. It is evident from the photomicrograph of GEL-2 that a lower magnification (43×) revealed smaller pores interlinked with each other, but at a higher magnification (1000×), the gel evinces macroporous interior with pore size ranging from 10 to 50 μm. The interior morphology of GEL-3 showed big pores which are interconnected with each other like honeycomb architecture and interior morphology of the gels revealed big giant pores on higher magnification (1000×). In contrast, the interior morphology of GEL-4 showed very few

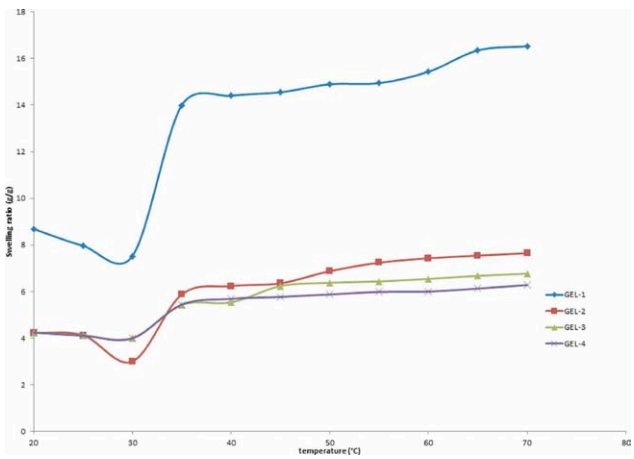

Figure 6 Temperature dependent swelling of the copolymer hydrogels (GEL-1 to −4). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

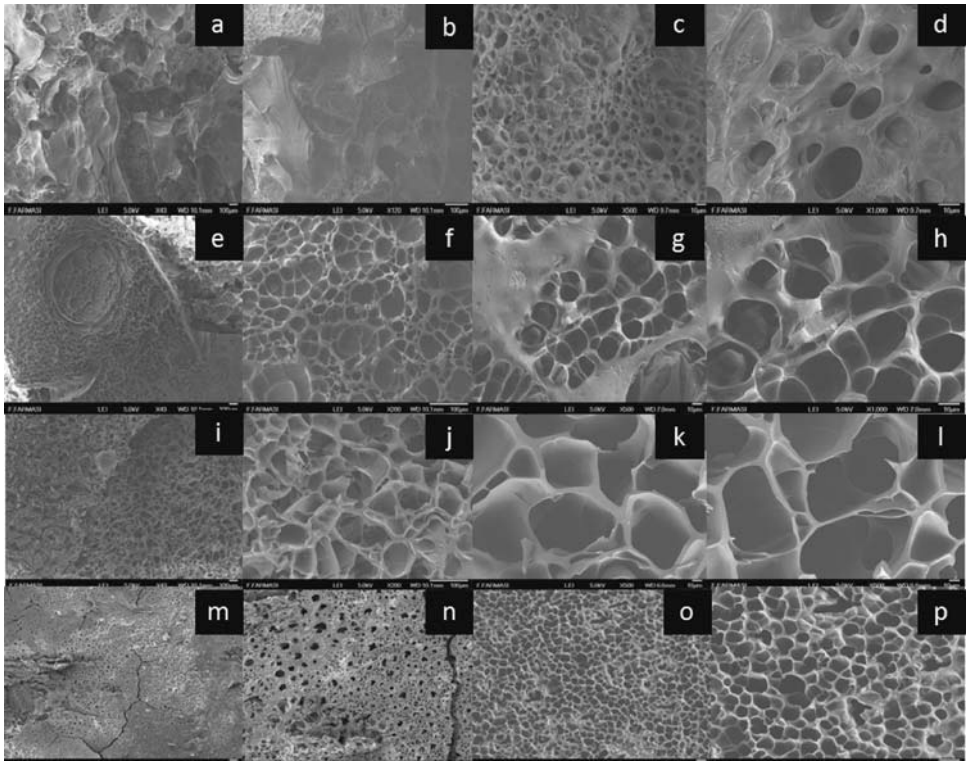

Figure 7 Interior morphology of copolymer hydrogels (a–d) GEL-1 (43×, 120×, 500×, and 1000×), (e–h) GEL-2 (43×, 200×, 500×, and 1000×), (i–l) GEL-3 (43×, 200×, and 500×), and (m–p) GEL-4 (43×, 200×, 500×, and 1000×).

narrower pores on the surface that are not interconnected and are smaller in comparison to other gels. The FESEM results can be correlated with the SR of the hydrogels. GEL-1 has higher SR in SIF due to the highly macroporous nature of the gel while GEL-4 has a lesser SR due to the less porous nature of the gel. The possible explanation for the highly macro porous nature of the hydrogels is the phase separation phenomenon. Since water is known to be nonsolvent for PHEMA, as the polymerization proceeds, the solubility of the PHEMA decreases dramatically. This result in phase separation of PHEMA rich DMAPMA phase into droplets which further join together to form an interconnected polymer network filled with large spaces by the end of polymerization.[42] It is worthwhile to mention here that in the developed hydrogel formulation we have increased the concentration of HEMA and decreased the concentration of DMAPMA in the reaction feed. We observed that an increase in the more hydrophobic PHEMA units in the gel led to a more pronounced phase separation behavior which eventually contributed toward the formation of big giant pores in the hydrogel network (GEL-1 to −3). Contrarily in GEL-4 the pores are small and not interconnected because of less phase separation of hydrophobic PHEMA molecules which restricts the entrance of water into the cross-linked copolymer network. It was suggested by Omidian et al. that due to a lesser solubility of HEMA in water, the amount of water should be reduced in the reaction feed in order to efficiently perform the polymerization.[43] Hence, in this work, we used lesser water and sodium/ammonium persulfate as a joint initiator system which may act as a foaming agent to enhance the porous nature of the poly(HEMA-*co*-DMAPMA) hydrogels.

In vitro 5-FU release study

The *in vitro* 5-FU release study from the hydrogel was carried out by immersing 5-FU incorporated gels in pH 1.2 and 7.4 buffers. The percentage cumulative release (R) of 5-FU from the hydrogel is calculated using eq. (4).

$$R = M_t/M_0 \times 100 \tag{4}$$

where M_t is the amount of drug released at time t, and M_0 is the initial drug loaded amount.

The EE of the hydrogel was calculated and it was found to be almost 60%. As shown in Figure 8, an initial burst was observed for GEL-1 for both the physiological fluids (SIF and SGF) within the first 30 min. This is attributed to crystallization of a small portion of 5-FU on the surface of xerogel. The fast dissolution of 5-FU has led to initial burst release of 20 and 30%, respectively. It was noticed that after 60

min the hydrogel served as diffusion barriers and 5-FU was released mainly by a diffusion phenomenon. As expected, the amount of 5-FU released from GEL-1 was slow in acidic conditions (pH 1.2). It was observed that 40% of the drug was released after 12 h of release experiment. It is worthwhile to relate with the comparatively low swelling response of GEL-1 at pH 1.2. We observed that under simulated intestinal conditions the 5-FU release increased considerably along with the swelling of the gel network. It is evident from the graph that 5-FU release in SGF was slow and steady whereas in SIF the release rate was quite efficient after 60 min. Almost 88% 5-FU was released from the hydrogel after 12 h at pH 7.4. The 5-FU release results of the gel showed that it can be used as potential drug carrier for prolonged release of 5-FU. 5-FU is clinically used as a potential chemotherapeutic agent for colorectal cancer therapy, and there is a need of colon-targeted drug carrier to ensure direct treatment at tumor site in colon.[44,45]

The release data was fitted in some mathematical models like Ritger and Peppas, eq. (5)[46]:

$$M_t/M_\infty = Kt^n \quad (5)$$

The value of n was calculated by linear regression of $\log(M_t/M_\infty)$ versus $\log t$, t is the time of fractional release. It was observed that the calculated value of n for the gel at both physiological fluids is 0.79 and 0.87, respectively, which confirms the non-Fickian release mechanism. The value of n revealed that swelling/chain erosion plays a significant role in diffusion of 5-FU through the hydrogel at both the pH.

Poly(DMAPMA-*co*-HEMA) hydrogels have several advantages in comparison to other pH-sensitive methacrylate hydrogels for effective colon cancer delivery. The PHEMA-based hydrogels are biodegradable and biocompatible[47] in nature which is beneficial in controlled oral drug-delivery applications. When taken orally, the formulation is able to reach the colon and after releasing the drug to colon they can easily be degraded inside the body. The PHEMA based hydrogels have good mechanical properties,[48,49] when compared with other hydrogel systems as the formulation can withstand the repeated peristaltic contractions in the human gastrointestinal tract[50] and release the drug in a sustained manner. The mechanical and *in vivo* studies are currently going on in our laboratory and these results will be in communication soon.

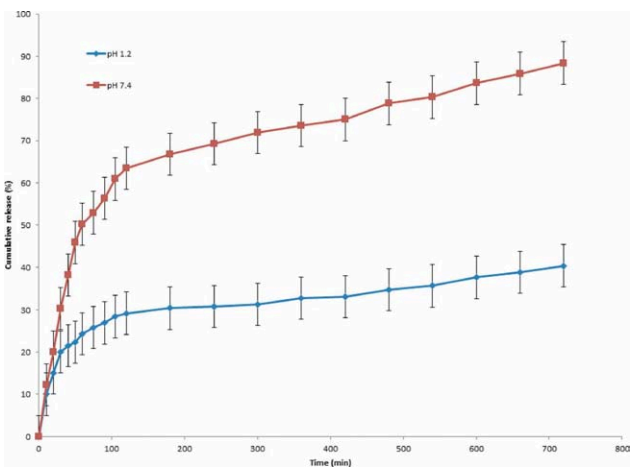

Figure 8 Cumulative release (%) of 5-FU through the hydrogel (GEL-1) at pH 1.2 and 7.4 buffer. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

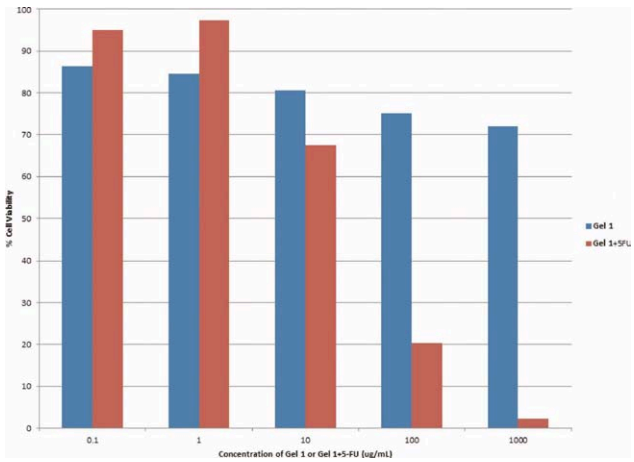

Figure 9 Dose-dependent anticancer effect of hydrogels loaded with or without (control) 5-FU after 72 h of incubation. (a) GEL-1 loaded with or without 5-FU. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Biological activity of 5-FU

The dose-dependent efficacy of 5-FU released from the hydrogels (GEL-1 agent and DLD-1) was measured by MTT assay (Fig. 9). The results showed that GEL-1 without 5-FU (control) maintained their viability, indicating that the hydrogel itself was not cytotoxic. In contrast, when GEL-1 was loaded with the drug, DLD-1 cells were killed in a dose-dependent manner. The results showed that the 5-FU released from the hydrogel remained biologically active. In addition, the developed poly(DMAPMA-*co*-HEMA) hydrogels were not toxic. The killing of the cancer cells in a dose-dependent manner by 5-FU loaded in GEL-1 correlates very well with the *in vitro* release study at pH 7.4.

CONCLUSIONS

In this study, poly(DMAPMA-*co*-HEMA) hydrogels were fabricated by aqueous copolymerization

method for controlled delivery of 5-FU. The chemical structure of the copolymer hydrogels were confirmed by FTIR and proton NMR spectroscopy. The FTIR analysis confirmed the formation of poly(-DMAPMA-*co*-HEMA) copolymer hydrogel. The proton ^{1}H-NMR spectrum of the hydrogel (GEL-1) showed the chemical shifts of both HEMA and DMAPMA. ^{1}H-NMR also showed the probable molecular interactions between ester group of HEMA and amide group of DMAPMA. The swelling investigation of the hydrogels revealed the dual sensitive (pH/temperature) nature of the hydrogels. The gel showed reentrant conformational phase transition behavior during temperature induced swelling study. The morphological analysis by field emission SEM revealed the highly macroporous nature of the hydrogels and that phase separation plays a crucial role in pore formation. The porous three-dimensional skeleton resulted from phase segregation of water from the growing amphiphilic polymer network during the progress of hydrogel fabrication. The thermal analysis of the hydrogels (GEL-1 and −4) showed that strong intermolecular association or hydrogen bonding plays a crucial role in increasing the thermal stability of GEL-1 as compared to GEL-4. The *in vitro* release of anticancer drug (5-FU) was investigated through the gel and almost 88% drug was released in 12 h of release study. However, initial burst effect was evident in the hydrogel formulation at both physiological conditions (SIF and SGF). The kinetic data treatment of the hydrogels in both pH showed non-Fickian release mechanism for drug release. The cytocompatibility evaluation of the hydrogels with human colon cancer cell lines (DLD-1) showed that the hydrogel is nontoxic in nature. It was observed that release of 5-FU from the gel was dose dependent and maximum cell killing was evidenced in GEL-1. The results showed that these smart hydrogels may have the potential to be used in formulations for colon cancer delivery.

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