PMMA-Based Microgels for Controlled Release of an Anticancer Drug

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ABSTRACT: Methyl methacrylate (MMA), methoxy poly (ethylene glycol) monomaleate (MPEG), and acrylamidoglycolic acid (AGA) terpolymeric microgels (MGs) have been synthesized by free-radical surfactant-free emulsion polymerization. MPEG was synthesized from maleic anhydride and methoxy poly(ethylene glycol). The MGs were crosslinked with ethylene glycol dimethacrylate, and the chemical crosslinking was confirmed by Fourier transform infrared spectroscopy. 5-Fluorouracil (5-FU), a model anticancer drug, has been loaded into the MGs by *in situ* and adsorption methods. Empty as well as drug-loaded MGs were then characterized by transmission electron microscopy (TEM), differential scanning calorimetry (DSC), and X-ray diffraction (XRD). DSC and XRD studies indicated a molecular level dispersion of the drug in PMMA MGs during *in situ* loading. TEM images showed the formation of spherical MGs. *In vitro* release of 5-FU from the crosslinked poly(MMA-*co*-AGA-*co*-MPEG) MGs were investigated at both pH 7.4 and 1.2 buffer medium that controlled release of the drug up to $\sim$ 18 h. Both the encapsulation efficiency and the release patterns were dependent on the amount of crosslinking agent and the amount of drug loaded. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 111: 845–853, 2009

Key words: methyl methacrylate; microgels; 5-fluorouracil; controlled release

INTRODUCTION

The success of using synthetic polymers as biomaterials[1–4] primarily relies on their wide range of mechanical properties and transformation processes that allow a variety of shapes to be easily obtained at low production costs. On the contrary, biological polymers possess good biocompatibility, although their mechanical properties are often poor. In this sense, functionalized crosslinked polymers have attracted attention as carrier networks in a wide variety of medical and biological applications such as affinity immobilization technologies and drug-delivery systems, though the necessity of preserving biological properties complicates their processability.

The polymeric colloids, micro- and nanoparticles, have been investigated in recent years in controlled release (CR) applications in the biomedical and pharmaceutical fields.[5–7] In addition to biocompatibility, one of the important requirements for any material used in the production of microgels (MGs) is that the matrix should be biodegradable. In the preparation of polymeric colloids, many methods are based on emulsions formed by mixing an organic phase and an aqueous phase.[5–7] However, organic solvents are known to affect the stability of a bioactive agent.[7,8] The most frequently used polymers for the production of particles used in the CR of drugs are acrylic derivatives. To increase the hydrophilicity of the particle surface, attempts have been made to employ copolymerization of alkylmethacrylate with various acrylic acid derivatives such as acrylamide and acrylic acid.[9,10] However, poly(methyl methacrylate)-based micro- and nanoparticles have been prepared using Eudragit E-100, dextran, and others, as stabilizers, for CR application.[11,12] A microparticulate system or any other carrier designed for drug delivery not only preserves its original physical and chemical properties during the formulation and storage, but also maintains the content of drug achieved at the end of the loading, and guarantees an effective release of the drug in the body.[13] Nevertheless, physical and chemical stability are the primary limitations of these carriers that may hamper the development of colloidal drug carriers.

Gels are the three-dimensional network polymers in which individual hydrophilic/hydrophobic polymeric chains are connected by physical and/or

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chemical bonds, which offer the required dimensional stability depending upon the surrounding media. Gels are generally considered to be biocompatible because of their high water-absorbing tendencies. These characteristics make them as alternative materials for biomedical applications such as drug-delivery devices,[3] among others. Moreover, the mechanical properties of a gel have to permit it to maintain its structure without fast degradation or cracking.

Biodegradable and biocompatible matrices derived from block and graft copolymers have recently attracted much attention, not only because of their potential for CR applications, but also due to their biocompatible and biodegradable properties.[4] Polymeric networks containing side-chain poly(ethylene glycol) (PEG) molecules and ionic moieties are considered to be high-value polymeric materials, because of their potential use as biocompatible materials in medical applications.[14,15] These materials render the system responsive to pH changes in the surrounding environment and also aid in the formation of intramolecular hydrogen bonding between oxygen in the PEG chain and hydrogen atom of ionic moieties. The pK_a of poly(acrylamidoglycolic acid) (PAGA) is 3.1,[16] so that carboxylic groups of PAGA are nonionized under lower acidic conditions, and the system is able to form hydrogen bonds within the network. The crosslinked network is collapsed following complex formation in acidic conditions. When a system becomes alkaline, the nature of complexation will reverse such that the system will swell. This type of behavior has been studied by Peppas and coworkers.[17,18] The systems are not only sensitive to pH environments but also inhibited the proteolytic enzymes of the gastrointestinal tract and opened the tight junctions present in the intestinal wall, allowing absorption. Therefore, it is a promising candidate for the delivery of environmentally susceptible bioactive agents that are characterized by poor absorption through the intestinal wall.[19,20]

Maleic acid copolymers have been known to possess antimicrobial and antitumor activity, in particular.[21,22] Stover and coworkers used maleic anhydride copolymers to investigate the temperature and pH sensitivity of PEG-grafted poly(styrene-*alt*-maleic anhydride) in aqueous solutions.[23] The advantages of these copolymers include their regular alternating structure and the possibility of varying the hydrophobicity.

One of the most common antineoplastic drugs used for the treatment of several malignancies is 5-FU. Owing to its high toxicity, it is a good candidate for controlled-release technology to obtain a therapeutic effect *in situ* and minimize collateral effects of the drug. Different gels have been used to release 5-FU. Although some microparticles have been designed as a polymeric matrix, 5-FU release and 5-FU delivery from these polymeric devices has been studied *in vitro*. Thus, 5-FU was included in polyacrylamide, crosslinked with ethylene glycol dimethacrylate (EGDMA). Total 5-FU release took place in about 14 h in *in vitro* experiments.[24] Kawashima et al. developed 5-FU encapsulated Eudragit-based microspheres by an oil/oil emulsification process; these released the 5-FU within 8 h.[25] Poly(acrylamide-methylmethacrylate) copolymeric microspheres were able to release around 95% within 14 h, which were crosslinked by MBA.[26] Poly (ethylene glycol diacrylate)-based hydrogels showing the encapsulation of about 61% of the drug were released in the first few hours. By the end of 5 h, about 98% of the drug entrapped was released.[15] In the present work, we prepared MGs composed on PMMA, PAGA, and methoxy PEG monomaleate (MPEG) [poly(MMA-*co*-AGA-*co*-MPEG)] crosslinked by EGDMA. The *in vitro* release studies of MGs were performed at 37°C (pH, 7.4 and 1.2).

EXPERIMENTAL

Materials

Methyl methacrylate (MMA), acrylamidoglycolic acid (AGA), ethylene glycol dimethacrylate (EGDMA), 5-fluorouracil (5-FU), potassium persulfate (KPS), dichloromethane (DCM), maleic anhydride, methoxy poly(ethylene glycol) (M_n = 750 g/mol), and triethylamine were purchased from Aldrich (Milwaukee, WI). All the chemicals were used as received, and all experiments were carried out with double-distilled water.

Synthesis of methoxy PEG monomaleate

Methoxy PEG monomaleate was synthesized as reported previously.[27] Typically, 7.3 mL (0.0133 mol) of methoxy poly(ethylene glycol) was dissolved in 50 mL of dry acetone. Next, 3 g (0.03 mol) of maleic anhydride and 4.12 mL of triethylamine were added. The resulting solution was refluxed for 12 h. At the end of the reaction, acetone was evaporated under reduced pressure, and the product was dissolved in water and extracted twice using DCM, which was then dried over anhydrous Na_2SO_4 and decolored using activated active carbon powder. The resulting MPEG product was obtained as a brown orange viscous liquid by removing the solvent under vacuum.

Synthesis of poly(MMA-*co*-AGA-*co*-MPEG) microgels

The ratio of monomer to water was 1 : 10 by weight (w/w), and the initiator concentration was 6.79 $\times$ 10^{-3} mol/L. Typically, 50 mL of double-distilled water was added to a three-necked flask equipped

TABLE I
Results of % Encapsulation Efficiency and Mean Size of PMMA MGs Loaded with 5-Fluorouracil

Sample code	MMA : MPEG : AGA (wt/wt)	EGDMA (wt %)	5-FU (%)	Encapsulation efficiency (%)	Mean particle diameter (nm)
MMA-1	1 : 0.1 : 0	10	8	62.34 ± 0.48	168 ± 1.86
MMA-2	1 : 0.2 : 0	10	8	64.08 ± 0.35	173 ± 2.13
MMA-3	1 : 0.2 : 0.1	10	8	68.71 ± 0.26	189 ± 0.78
MMA-4	1 : 0.2 : 0.2	10	8	70.23 ± 0.43	200 ± 2.67
MMA-5	1 : 0.2 : 0.2	5	8	71.78 ± 0.79	214 ± 1.25
MMA-6	1 : 0.2 : 0.2	20	8	66.50 ± 0.56	182 ± 1.11
MMA-7	1 : 0.2 : 0.2	10	4	64.09 ± 0.75	191 ± 1.45
MMA-8	1 : 0.2 : 0.2	10	12	74.18 ± 0.55	206 ± 0.65
MMA-9	1 : 0.2 : 0.2	10	Ad	76.32 ± 0.68	214 ± 2.01

Ad = adsorption (or adsorbed).

with a mechanical stirrer, a condenser, and a gas inlet to maintain the inert nitrogen atmosphere. The flask was immersed in an oil bath with thermostatic control to maintain a constant temperature of 70°C ± 1°C, and the solution was stirred at 600 rpm. Calculated amounts (see Table I) of MMA, MPEG, AGA, and the crosslinking agent (EGDMA) were added to the reaction flask. The mixture of 5-FU and KPS in 20 mL of water was added to the reaction mixture dropwise using a dropping funnel, after which the reaction proceeded for 6 h at 70°C to obtain the maximum yield (92%). Particles were then isolated by freezing and washing with water and methanol repeatedly, to remove the unreacted initiator and monomer. The formed MGs were then dried under vacuum at 40°C for 24 h.

Loading of 5-FU

5-FU was loaded into MGs using two methods. In the first method (method-I), 5-FU was added during *in situ* polymerization, i.e., the drug was mixed with the monomer, crosslinking agent, initiator, and the mixture was then added to the polymerization medium. The solubility of 5-FU in water is very low (13 mg/mL), although the solubility of the sodium salt is as high as 65 mg/mL.[28] In the second method (method-II), an aqueous solution of drug neutralized with 0.1*N* NaOH was used in the feed mixture to load the maximum drug into the polymeric network. During this process, the drug was adsorbed onto the MGs. The formula of the sodium salt of 5-FU can be illustrated as follows (Scheme 1).

FTIR analysis

FTIR spectra were recorded on a Jasco, FTIR-430 (Japan). Approximately 2 mg of the samples was ground thoroughly with KBr, and the pellets were prepared using a hydraulic press under a pressure of 600 kg/cm^2. Spectra were scanned between 500 and 4500 cm^{-1}.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) thermograms were recorded on a Rheometric scientific DSC (model-DSC SP, UK). The instrument was calibrated using indium as a standard. Samples were heated in sealed aluminum pans between 50 and 400°C at a heating rate of 10°C/min under inert nitrogen purge gas at the rate of 20 mL/min.

X-ray diffraction

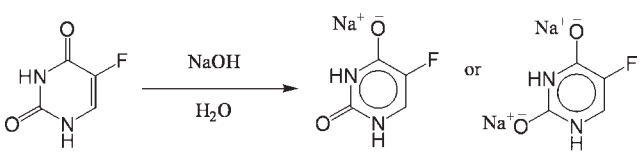
Wide-angle X-ray diffraction (XRD) patterns of the placebo MGs, pure 5-FU and 5-FU-loaded MGs were recorded on a Rigaku diffractometer (model Rigaku Miniflex) equipped with a Ni-filtered Cu Kα radiation (λ = 1.5418 E). The dried gels of uniform size were mounted on a sample holder, and XRD patterns were recorded in an angle range of 5–60° at a speed of 5°/min to estimate the crystallinity of the samples.

Transmission electron microscopy

The size and shape of PMMA MGs were investigated by transmission electron microscopy (TEM). TEM measurements were performed on a HITACHI H-7500 TEM. Samples for TEM were prepared on 300-mesh copper grids coated with carbon.

Particle size analysis

Size distribution of the MGs were determined using a particle size analyzer (Mastersizer 2000, Malvern Instruments, UK) equipped with a dry accessory system.



Scheme 1 Schematics of 5-FU salt.

Estimation of drug loading and encapsulation efficiency

The loading efficiency of 5-FU in the MGs was determined spectrophotometrically. Approximately 10 mg of the drug-loaded MGs were placed in 10 mL of buffer solution and stirred vigorously for 48 h to extract drug from the MGs. The solution was filtered and assayed by UV spectrophotometer (model Anthelie, Secomam, Dumont, France) at fixed λ_{max} value of 270 nm. The results of % drug loading and encapsulation efficiency were calculated using eqs. (1) and (2), respectively. These data are compiled in Table I.

$$\% \text{ Drug loading} = \left(\frac{\text{Amount of drug in microgels}}{\text{Amount of microgels}}\right) \times 100 \quad (1)$$

$$\% \text{ Encapsulation efficiency} = \left(\frac{\text{Actual loading}}{\text{Theoretical loading}}\right) \times 100 \quad (2)$$

In vitro release study

Dissolution was performed using a fully automated dissolution coupled UV spectrophotometer (Logan System 888J, NJ), equipped with six baskets. Dissolution rates were measured at 37°C under 100 rpm speed. Drug release from the microspheres was studied both in simulated the gastrointestinal tract (phosphate buffer pH 7.4 and 0.1*N* HCl pH 1.2) fluid. The release of drug was automatically measured by UV, which was further used to estimate drug loading and encapsulation efficiency.

Statistical analyses

Statistical analyses were done by SPSS statistical package. Analysis of variance followed by the least significant difference procedure, was used for monomer amount, amount of crosslinking agent, and the drug content, for the comparison of drug release rates from different formulations, by considering $P < 0.05$ as significant value.

RESULTS

FTIR analysis

The copolymeric MGs, monomers, and drug were characterized by FTIR spectra. FTIR spectra of (a) PMMA MGs, (b) PMMA MGs with drug, (c) MMA, (d) MPEG, (e) AGA, and (f) 5-FU are reproduced in Figure 1. In the FTIR spectra of MMA, MPEG, and AGA, we have observed the characteristic absorption bands at 3450, 2616, 1700, 1665 cm^{-1}, which are assigned to hydroxyl stretching vibrations, carbonyl stretching vibrations, carboxylic acid groups, and amide, respectively. Additional characteristic absorption bands of monomers appear at 1449 and 1301 cm^{-1} due to C—C multiple bond stretching and C—H bending vibrations, respectively. From the spectrum of MGs, we observed the peak at 1665 cm^{-1} corresponding to —NH— and the peak at 2955 cm^{-1} corresponding to aliphatic —CH stretching vibration. The strong peak at 3450 cm^{-1} is due to the stretching vibrations of the —OH group. The peak at 1733 cm^{-1} is characteristic for the —C=O group of —COOH and ester groups. The peak bands at 1245, 1153, and 1070 cm^{-1} (C—O—C ether and CH_2—O groups) correspond to the branched PEG. Schematic representation of crosslinking chemistry of PMMA MGs is presented in Scheme 2.

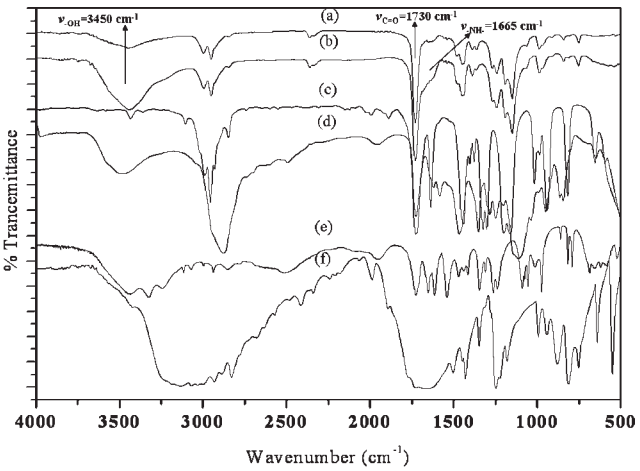
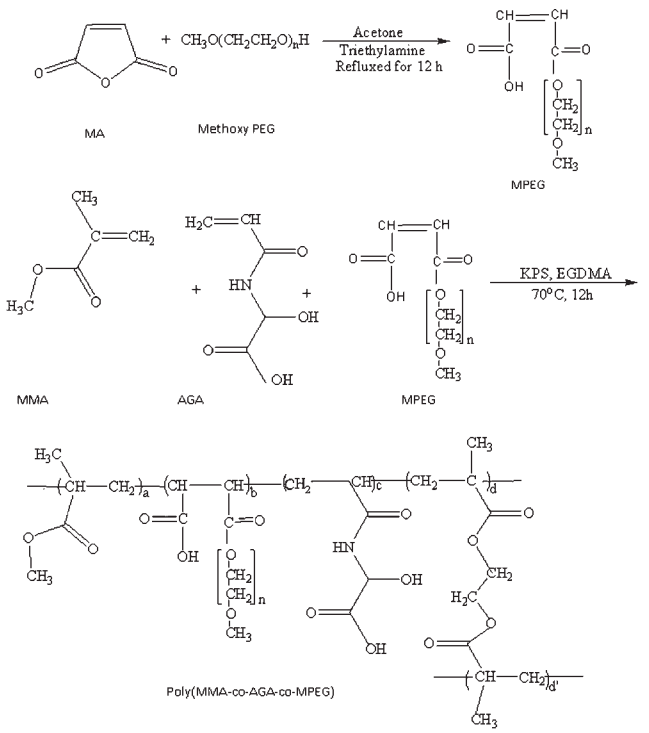


Figure 1 FTIR spectra of (a) PMMA MGs, (b) PMMA MGs with drug, (c) MMA, (d) MPEG, (e) AGA, and (f) 5-FU.

The structure of 5-FU illustrates that 5-FU can potentially act as either proton acceptor (through the carbonyl groups, —C=O) or proton donor (through the amine group, —NH). MGs has proton acceptor (carbonyl group, —C=O), methoxy groups (—C—O—C—) functioning as the proton donor or acceptor. The stretching vibration bands attributed to the carbonyl (1706 cm^{-1}) and amine groups (3354 and 3374 cm^{-1}) of the molecularly dispersed 5-FU associating with MGs via H-bonds. However, further investigation using complementary techniques, such as DSC, should be carried out.

Differential scanning calorimetry

To examine the interaction of PMMA MGs with 5-FU, DSC and FTIR spectroscopy were employed. Figure 2 shows that the DSC curves of pure drug, drug-loaded PMMA MGs, and pristine PMMA MGs. 5-FU shows a sharp peak at 285°C because of



Scheme 2 Schematics of the crosslinking chemistry.

polymorphism and melting, while the 5-FU-loaded MGs show no such peaks, but rather, broad melting peaks were observed over the ranges of 285–400°C, suggesting that 5-FU is widely dispersed through the PMMA networks.

X-ray diffraction studies

XRD had been used to identify crystalline phase and to determine drug maximum amorphous drug loading in polymeric matrix at room temperature. To aid the analysis of drug–polymer interactions of this study, XRD was used to evaluate the crystallinity of 5-FU, MGs, and to identify possible formation of 5-FU polymorphs in the MGs. 5-FU exhibits a characteristic crystalline peaks at 20 of 29° and 32° (Fig. 3). The X-ray diffractograms of drug-loaded MGs together with those of crystalline 5-FU and pristine MGs without drug loading were shown in Figure 3. As indicated by the X-ray diffractograms, MG was an amorphous material in nature. For 5-FU-loaded MGs, the characteristic peaks of crystalline 5-FU were not detected noticeably in the X-ray diffractograms, indicating that 5-FU is dispersed in the polymer matrix as a monomer.

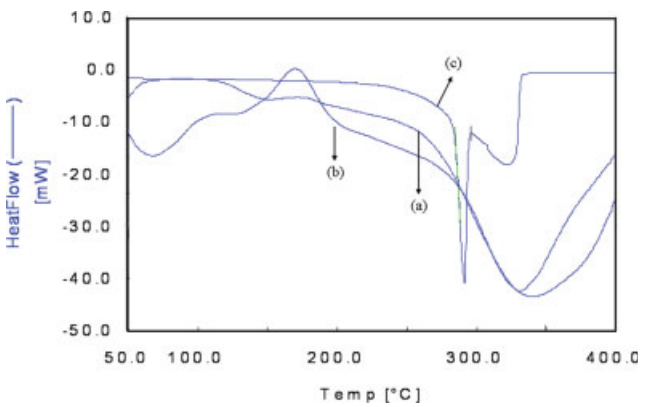


Figure 2 DSC thermograms of (a) placebo PMMA MGs, (b) 5-FU loaded PMMA MGs, and (c) pure 5-FU. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com].

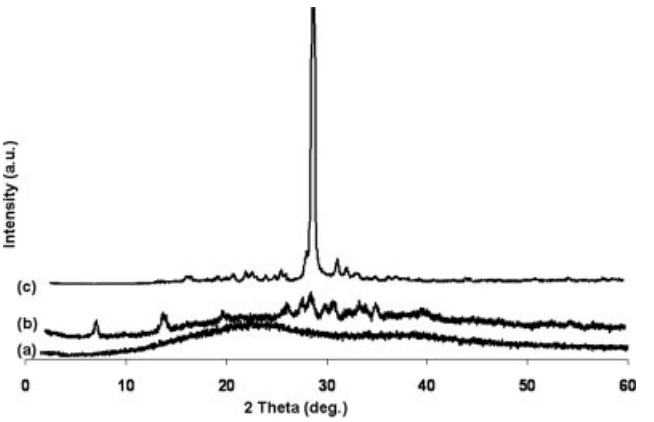


Figure 3 XRD patterns of (a) placebo PMMA MGs, (b) 5-FU-loaded PMMA MGs, and (c) pure 5-FU.

Morphology and particle size

Figure 4 shows that MGs are monodispersed and spherical in nature. Results of mean particle size are presented in Table I. The size distribution curve for

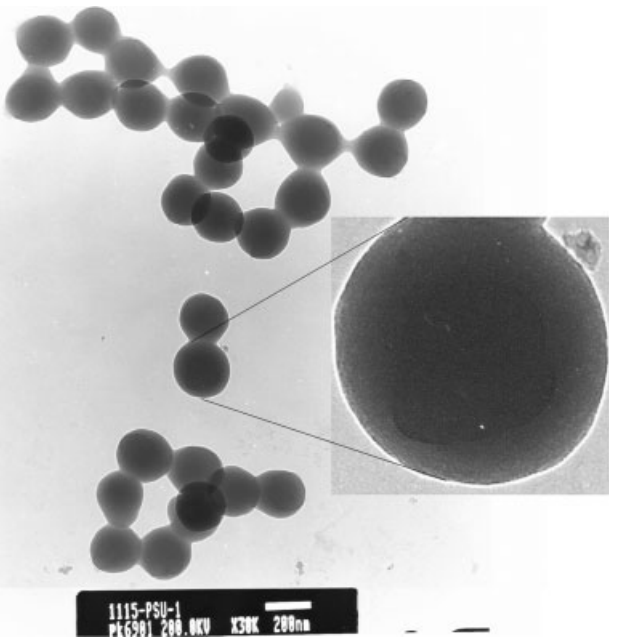


Figure 4 TEM image of PMMA MGs.

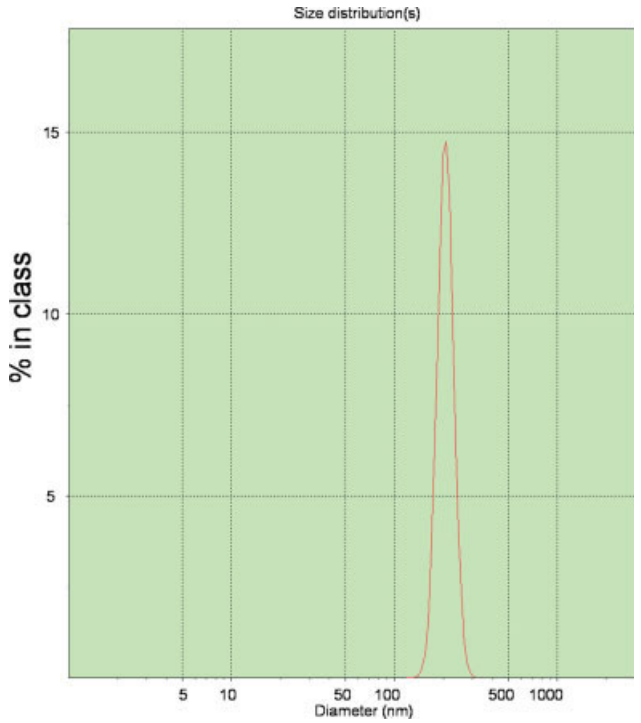


Figure 5 Particle size distribution curve of MGs. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com].

typical formulation containing MMA : MPEG : AGA in the ratio of 1 : 0.2 : 0.2 loading, with 8% 5-FU and 10 wt % of EGDMA (MMA-4) presented in Figure 5. The observed size distribution is bell-shaped (normal distribution) with a mean particle diameter of 200 nm. Ninety percent of the population ranges in size between 160 and 208 nm.

Drug release kinetics

In many cases, the use of simple empirical or semi-empirical models such as classical Higuchi equation and the so-called power law is fully sufficient.[29] Drug release kinetics were analyzed by plotting the cumulative release data, (M_t/M_∞), versus time by fitting the data to a simple exponential Peppa's model.[30]

$$\left(\frac{M_t}{M_\infty}\right) = kt^n \tag{3}$$

where M_t corresponds to the amount of drug released in time t, M_∞ is the total amount of drug that must be released at infinite time, k is a constant, and "n" is the release exponent indicating the type of drug release mechanism. For example, $n \leq 0.45$ for case I or the Fickian diffusion, which is characterized by a dependence on the square root of time in both the amount diffused and the penetrating diffusion front position; $n \geq 0.89$ for case II transport, which is completely governed by the rate of polymer relaxation and exhibits a linear time dependence in both the amount diffused and penetrating swelling front position; $0.45 < n < 0.89$ for anomalous behavior or non-Fickian transport, which is exhibited whenever the rates of Fickian diffusion and polymer relaxation are comparable.[27] Table II shows values in the range of 0.083 and 0.305, as calculated from the empirical equation, which indicates that the drug release is swelling controlled (i.e., case I transport). These results, along with correlation coefficients "r", are presented in Table II.

Encapsulation efficiency

Three different concentrations of drug (4, 8, and 12 wt %) were loaded during crosslinking. The % encapsulation efficiency was also included in Table I, and these values were increased with increasing drug loading. In the case of MMA-4, MMA-7, and MMA-8 MGs, the encapsulation efficiency increases from 64.09% to 74.18% as the drug content increases from 4 to 12 wt %. The % encapsulation efficiency also followed the same trend with an increasing amount of MPEG and AGA in the MGs. For example, to study the effect of MPEG and AGA in the MGs [e.g., for MGs containing different ratios of MPEG and AGA with 8% of 5-FU (MMA-1, MMA-2, MMA-3, and MMA-5)], encapsulation efficiencies were found to be 62.34, 64.18, 68.71, and 70.03%, respectively. The effect of crosslinking on size and entrapment efficiency of the microspheres using the ratio 1 : 0.2 : 0 : 2 containing MMA MGs is also represented in Table I. With an increasing degree of crosslinking, the % encapsulation efficiency was decreased, e.g., for microspheres crosslinked with 5, 10, and 20 wt % of EGDMA (MMA-5, MMA-4, and MMA-6), entrapment efficiencies were 72.16, 70.03, and 66.50%, respectively. This may be due to the increasing degree of crosslinking, which leads to MGs becoming more rigid and, thus, reducing the free volume space within the polymeric network to yield reduced encapsulation efficiency.

TABLE II
Release Kinetics Parameters of Different Formulations

Sample code	k	n	r
MMA-1	0.21	0.199	0.9929
MMA-2	0.19	0.200	0.9859
MMA-3	0.16	0.213	0.9814
MMA-4	0.15	0.217	0.9714
MMA-5	0.07	0.305	0.9944
MMA-6	0.21	0.188	0.9790
MMA-7	0.06	0.303	0.9963
MMA-8	0.29	0.164	0.9851
MMA-9	0.10	0.083	0.9711

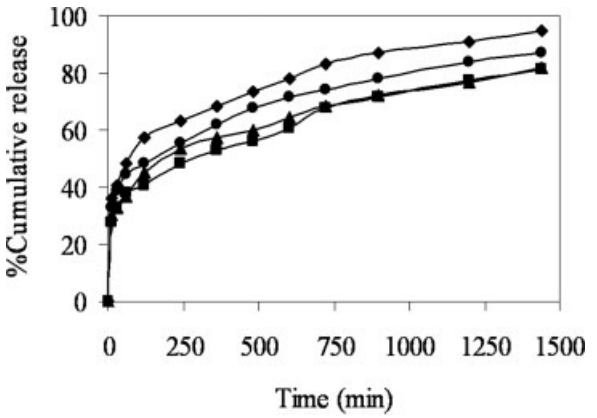


Figure 6 % Cumulative release of 5-FU through PMMA MGs containing different ratios of MPEG and AGA: (■) MMA-1, (▲) MMA-2 (●) MMA-3, (◆) MMA-4.

In vitro release study

To understand the drug release mechanism from the 5-FU loaded MGs based on MMA, *in vitro* release experiments were performed at intestinal pH conditions. These results exhibit a phenomenal effect of the network crosslinking on the drug release characteristics for all the formulations. Drug release rates from different formulations were statistically evaluated by ANOVA method. For formulations MMA-1 to MMA-4, F value was 1.393 (df = 35, $P < 0.05$), indicating that a significant difference in the release rates of 5-FU from PMMA MGs. Figure 6 displays the cumulative % release data of PMMA microspheres for different amounts of MPEG and AGA containing 8% 5-FU and 10 wt % of MMA-1, MMA-2, MMA-3, or MMA-4, respectively, in pH 7.4 media with respect to time. Increased cumulative release is observed as the amount of MPEG and AGA in the MGs increases. This is due to the increased —COOH groups resulting from increasing MPEG and PAGA content, which leads to a higher water-uptake capacity of the MGs, subsequently increasing the network swelling.

In case of formulations MMA-4 to MMA-6, the F value was found 9.343 (df = 35, $P < 0.05$), indicating insignificant differences in the 5-FU release rates when varying amounts of crosslinking agent were added to form the PMMA MGs. Figure 7 displays the release profiles of MGs crosslinked with different amounts of EGDMA containing 8 wt % 5-FU (MMA-4, MMA-5 and MMA-6) in pH 7.4 media with respect to time. The % cumulative release was higher in case of MGs crosslinked with 5 wt % of EGDMA (MMA-5), while the lowest % cumulative release was observed with MGs crosslinked with 20 wt % of EGDMA (MMA-6). Intermediate values were observed for MGs crosslinked with 10 wt % of EGDMA (MMA-4). This was due to a decrease in

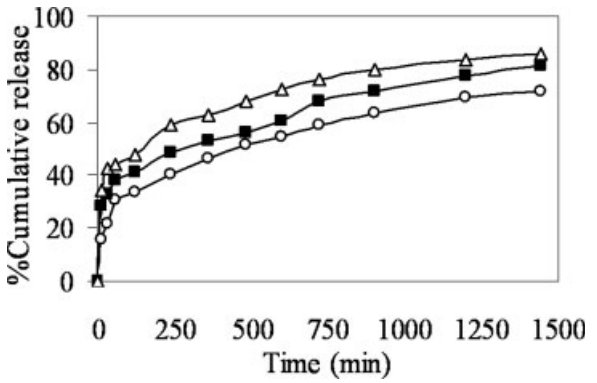


Figure 7 % Cumulative release of 5-FU through PMMA MGs containing different amounts of crosslinking agent: (△) MMA-5, (■) MMA-4, (○) MMA-6.

swelling as the amount of EGDMA increases in MGs. Release data showed that formulations containing higher encapsulation efficiency displayed much faster and higher release rates than those formulations containing lower encapsulation efficiency. In case of formulations MMA-4, MMA-7, and MMA-8, the F value was found 0.304 (df = 35, $P > 0.05$), indicating insignificant differences in the 5-FU release rates when varying amounts of crosslinking agent were added to form the PMMA MGs. Thus, CR was observed for formulations containing lower amount of 5-FU, since its release from the MGs was sustained by the diffusion mechanism. Upon increasing the concentration of 5-FU in the MGs, the release rates increased. Likewise, the release rates were slower as the amount of 5-FU was decreased due to an increase in void spaces through which a smaller number of drug molecules had to move. These results are presented in Figure 8 for the formulations MMA-4, MMA-7, and MMA-8.

Figure 9 displays the *in vitro* release profiles of 5-FU from the MGs loaded both by the *in situ* (method-I) and adsorption (method-II) systems. The release data for method II indicated that more than

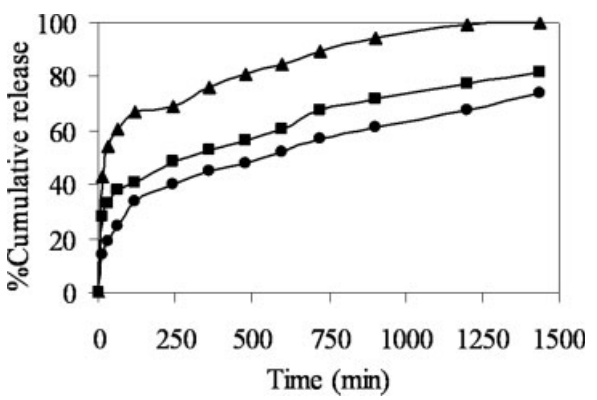


Figure 8 % Cumulative release of 5-FU through PMMA MGs containing different drug loadings: (▲) MMA-8, (■) MMA-4, (●) MMA-7.

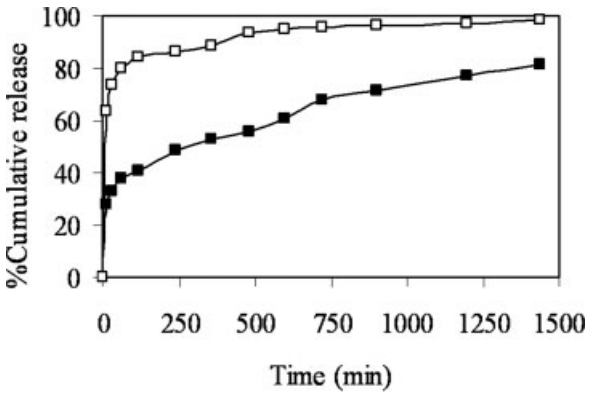


Figure 9 % Cumulative release of 5-FU through PMMA MGs; effect of the drug loading method: (■) MMA-4, (□) MMA-9.

98% of the drug was released within ~ 18 h, compared to a release rate of only 75% within ~ 18 h in the case of method I. However, the drug adsorbed on the surface of MGs exhibits a smaller interaction and binding efficiency with the polymeric matrix with a higher affinity to buffer solution used. The results showed that drug release rates are much faster when the drug was loaded by method-II than by method-I. For all the 5-FU-loaded formulations, the complete release of 5-FU was not observed even after 1250 min.

Figure 10 display the % cumulative release data of MMA-4, at pH 1.2 and 7.4 media with respect to time. A pronounced difference is observed in the release data at pH 1.2 and 7.4, which is attributed to the presence of COOH groups for higher swelling in higher pH media. This is due to the increased COOH groups with increasing AGA content, thereby inducing higher water-uptake capacity of the MGs. This consequently increases matrix swelling. Such a behavior was also observed earlier for chitosan blended with modified poly(vinyl alcohol) MGs.[31]

DISCUSSION

Drug release from a polymeric system that combined drug cleavage and diffusion was analyzed by mathematically.[14] The kinetics of cleavage and release of the therapeutic agent from solid polymer matrices are believed to be determined by a number of interdependent processes. These include diffusion of the external medium, water, protons, or hydroxide ions into the hydrogel, relaxation of the polymer chains in the medium including swelling (~ 6 h), hydrolytic cleavage of the conjugate linkage and diffusion of the cleaved agent from the polymer matrix (~ 18 h). The water-transport mechanism through anionic hydrogels was significantly dependent on the pH of the swelling medium, hydrogel composition, and PEG molecular mass. At a high pH, the water transport was controlled more by polymer relaxation than by penetrant diffusion. This resulted from the ionization of carboxylic acid groups on the poly(methacrylic acid) (PMAA) of the hydrogels. Increase in the degree of ionization contributed to the electrostatic repulsion between adjacent ionized groups, leading to chain expansion, which in turn, affected macromolecular chain relaxation. In the acidic environment of the stomach, these hydrogels are collapsed as a result of hydrogen bonding, thus holding and protecting the incorporated drug in the hydrogel. However, under the basic and neutral conditions of the intestine, the gels are swollen to a high degree, because of electrostatic repulsions, so the percent of released drug is 45%. Kim and Peppas[19] observed that PEG-PMAA based hydrogels could adhere more strongly to the mucosa of the intestine than to the mucosa of the stomach, and this can localize the delivery system in a site-specific manner. The poly(MMA-*co*-AGA-*co*-MPEG) MGs, which are stable for long time release applications because of their hydrophobic core and hydrophilic shell structure (see Fig. 4), have been prepared by copolymerizing hydrophilic and hydrophobic monomers by surfactant-free emulsion polymerization. The main advantage of core–shell type microspheres is that both hydrophilic and hydrophobic drugs can be incorporated. And also the MGs are pH sensitive is due to the presence of glycolic acid moiety.

The poly(MMA-*co*-AGA-*co*-MPEG) MGs allow 5-FU to be trapped in the polymerization feed mixture and a maximum of 74.18% for the MMA-8, but by the adsorption method, it is found that 76.32% of the drug can be encapsulated. The release of total drug takes place in 1440 min from MMA-8, which possesses 10 wt % of EGDMA and 12 wt % of the drug in pH 7.4. Hence, these copolymeric MGs allow more 5-FU to be trapped in the gel, but the release time of 100% drug decreases in comparison with other MGs. It is important to note that the pH and core–shell nature of these MGs increases with the

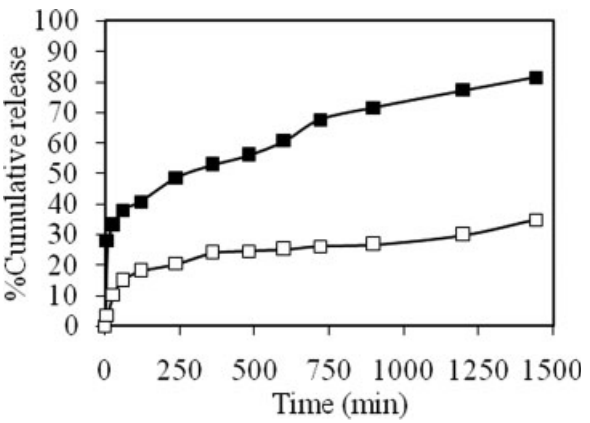


Figure 10 % Cumulative release of 5-FU through PMMA MGs (MMA-4); effect of pH: (□) pH = 1.2, (■) pH = 7.4.

release of 5-FU. This study demonstrated that by exploiting the relationship between the outer hydrophilic and pH-responsive shell–inner hydrophobic core combination and drug action will offer important information to design improved core–shell MGs for fine control of drug delivery of various bioactive molecules, as well as deeper understanding of drug-action mechanisms through core–shell structures.

CONCLUSIONS

PMMA-based MGs were prepared by free-radical polymerization and used for the CR of 5-FU. TEM observation showed that the MGs formed were spherical with smooth surfaces. DSC thermograms and XRD patterns implied that the drug was dispersed at the molecular level. The MGs exhibited good encapsulation efficiencies for the formation of single unit dosage forms, where the values varied between 62.34 and 76.32. Higher drug loadings and faster release rates were observed when the drug was loaded into MGs by the adsorption technique. Sustained and prolonged drug release rates have been observed from the *in situ* drug loaded MGs of this study. The release kinetics data some how exhibited Fickian transport behavior.

References

1. Lee, H. Y.; Yu, S. A.; Jeong, K. H. Macromol Res 2007, 15, 547.
2. Hiratania, H. Biomaterials 2004, 25, 1105.
3. Kim, H. Y.; Kim, S. W. J Controlled Release 1994, 28, 143.
4. Nivasu, V. M.; Reddy, T. T.; Shekharam, T. Biomaterials 2004, 25, 3283.
5. Byun, H.; Hong, B.; Nam, S. Y. Macromol Res 2008, 16, 189.
6. Edman, P.; Ekman, B.; Sjoholm, I. J Pharm Sci 1980, 69, 838.
7. Kim, S. Y.; Cho, S. H.; Lee, Y. M. Macromol Res 2007, 15, 646.
8. Lu, W.; Park, T. G. J Pharm Sci Technol 1995, 49, 13.
9. Kreuter, J.; Liehl, E.; Berg, U.; Soliva, M.; Speiser, P. P. Vaccine 1988, 6, 253.
10. Rolland, A.; Gibassier, D.; Sado, P.; Le Verge, R. J. J Pharm Belg 1986, 41, 83.
11. Sparnacci, K.; Laus, M.; Tondelli, L.; Magnani, L.; Bernardi, C. Macromol Chem Phys 2002, 203, 1364.
12. Zobel, H. P; Kreuter, J.; Werner, D. C.; Noe. R.; Kumel, G.; Zimmer, A. Drug Delivery 1997, 7, 483.
13. Schwarz, C.; Mehnert, W. Int J Pharm 1997, 157, 171.
14. Shantha, K. L.; Harding D. R. K. Int J Pharm 2000, 207, 65.
15. Pitt, C. G.; Shah, S. S. J Controlled Release 1995, 3, 391.
16. Faupel, M.; Barzaghi, B.; Gelfi, C.; Righetti, P. G. J Biochem Biophys 1987, 15, 147.
17. Peppas, N. A.; Bures, P. Polym Prepr 1999, 40, 506.
18. Peppas, N. A.; Little, M. D.; Huang, Y. In Handbook of Pharmaceutical Controlled Release Technology; Wise, D. L., Peppas, L. B., Klibanov, A. M., Langer, R. L., Mikos, A. G., Peppas, N. A., Trantolo, D. J., Wnek, G. E., Yaszemski, M. J. M., Eds.; Dekker: New York, 2000, p 255.
19. Kim, B.; Peppas, N. A. Polymer 2003, 44, 3701.
20. Kim, B.; Peppas, N. A. J Biomater Sci Polym Ed 2002, 13, 165.
21. Butler, G. B. Cyclopolymerization and Cyclocopolymerization; Marcel Dekker: New York, 1992.
22. Xu, X. D.; Zhang, X. Z.; Cheng, S. X.; Zhou, R. X. Colloid Polym Sci 2006, 285, 75.
23. Yin, X.; Stover, H. D. H. Macromolecules 2002, 35, 10178.
24. Sairam, M.; Babu, V. R.; Naidu, B. V. K.; Aminabhavi, T. M. Int J Pharm 2006, 320, 131.
25. Lamprecht, A.; Yamamoto, H.; Takeuchi, H.; Kawashima, Y. Eur J Pharm Biopharm 2005, 59, 367.
26. Babu, V. R.; Sairam, M.; Hosamani, K. M.; Aminabhavi, T. M. Int J Pharm 2006, 325, 55.
27. Krishna Rao, K. S. V.; Subha, M. C. S.; Sairam, M.; Halligudi, S. B.; Aminabhavi, T. M. Des Monom Polym 2006, 9, 261.
28. Garcia, O.; Trigo, R. M.; Blanco, M. D.; Teijon, J. M. Biomaterials 1994, 15, 689.
29. Siepmann, J.; Peppas, N. A. Adv Drug Delivery Rev 2001, 48, 39.
30. Ritger, P. L.; Peppas, N. A. J Controlled Release 1987, 5, 23.
31. Krishna Rao, K. S. V.; Naidu, B. V. K.; Subha, M. C. S.; Sairam, M.; Aminabhavi, T. M. Carbohydr Polym 2006, 66, 333.