Colloid Polym Sci (2012) 290:349–357
DOI 10.1007/s00396-011-2558-x

ORIGINAL CONTRIBUTION

Fluorine-containing pH-responsive core/shell microgel particles: preparation, characterization, and their applications in controlled drug release

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Received: 20 October 2011 / Revised: 13 November 2011 / Accepted: 17 November 2011 / Published online: 2 December 2011
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Abstract A kind of novel fluorine-containing pH-responsive core/shell microgels poly(DMAEMA-*co*-HFMA)-*g*-PEG were prepared via surfactant-free emulsion polymerization using water as the solvent. The well-defined chemical structure of the copolymers was characterized by FTIR, ^{1}H-NMR, ^{19}F-NMR, and elemental analysis. The microgel particles were studied by florescence probe technique, dynamic light scattering, and zeta potential measurement; the microgels displayed a significant pH-responsive behavior. Furthermore, the cytotoxicity assay indicated that the copolymer microgels had low toxicity, and 5-FU-loaded microgels offered a certain killing potency against cancer cells. In addition, the drug loading and in vitro drug release demonstrated that 5-FU was successfully incorporated into polymeric microgels, and the drug-loaded microgels showed a marked pH-dependent drug release behavior. This study suggests that the poly(DMAEMA-*co*-HFMA)-*g*-PEG microgels play an important role in the release mechanism stimulated by the change in the pH and have potential applications as a controlled drug release carrier.

Keywords Fluorine-containing · pH-responsive · Core/shell · Microgel · Controlled drug release

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Introduction

Stimuli-responsive microgels show a sharp change in properties upon a modest change in environmental condition, e.g., temperature, pH, light, electric field, or magnetic field [1–3]. This behavior can be utilized for the preparation of so-called "smart" drug delivery systems, which mimic biological response. As compared with the bulk hydrogels, microgels exhibit faster response to external changes due to their colloidal particle nature [4]. It is well known that different organs, tissues, and cellular compartments may have large differences in pH, which makes the pH a suitable stimulus. Therefore, pH-responsive microgels have attracted much attention for its pharmaceutical applications in treating chronic diseases, immunodeficiency, genetic disorders, and cancer [5].

Poly[2-(dimethylamino) ethyl methacrylate] (PDMAEMA) is a special polymer containing tertiary amino groups and exhibits pH-sensitivity which can be protonized in acidic solution [6–8]. Due to the unique property, a series of pH-responsive copolymers have been synthesized from PDMAEMA for application as controlled drug delivery system [9–11].

Poly(ethylene glycol) (PEG) is widely used as the hydrophilic side chains in graft copolymers. It is a low-cost commercial product that possesses some unique and outstanding properties such as hydrophilicity, biocompatibility, nontoxicity, lack of immunogenicity, metal complexing ability, as well as solubility in water and organic solvents [12–14]. If PEG is used as the graft chain of microgels, both the biocompatibility and the dimensional stability will be improved to a certain extent.

In recent years, fluoropolymers have attracted considerable interest because of many unique characteristics such as high hydrophobicity, high thermal and mechanical stability, gas-dissolving capacity, high fluidity, low dielectric constants, oil and water repellency, and very interesting surface properties [15–18]. If the microgel particles contain fluorinated segments, they will show some special properties, which cannot be achieved by non-fluorinated copolymers.

The pH-responsive polymers have been extensively reported [19–22], but the fluorine-containing pH-responsive core/shell graft microgels have not been studied. In this study, the poly(DMAEMA-*co*-HFMA)-*g*-PEG microgels comprising poly[2-(dimethylamino) ethyl methacrylate] as pH-responsive segment, poly(hexafluorobutyl methacrylate) as fluorinated segment and poly(ethylene glycol) as graft chains were prepared via emulsifier-free emulsion polymerization.

The pH-sensitive behavior of the copolymer microgels was studied by the fluorescence probe technique, the dynamic light-scattering instrument, and the zeta potential measurement. The results showed that the change of pH values had a significant impact on the microgel particles. Furthermore, the TEM image demonstrated that poly(DMAEMA-*co*-HFMA)-*g*-PEG copolymer mainly form hexagonal microgel particles. In addition, MTT viability assay showed that the copolymer microgels had low toxicity, and 5-FU-loaded microgels offered a certain killing potency against cancer cells. Importantly, the drug loading and in vitro drug release were studied in detail. The results demonstrated that 5-FU was successfully incorporated into polymeric microgels, and drug-loaded microgels displayed a significant pH-dependent release behavior. Our study suggests that poly(DMAEMA-*co*-HFMA)-*g*-PEG microgels have a potential application as an “intelligent” drug carrier for controlled drug release.

Experimental

Materials

2-(Diethylamino)ethyl methacrylate (DMAEMA) was purchased from Acros Organics and passed through an alumina column to remove inhibitor prior to use. 2,2,3,4,4,4-Hexafluorobutyl methacrylate (HFMA) was purchased from Xeogia Fluorine-Silicon Chemical Company (Harbin, China, chemical purity) and distilled under reduced pressure before use. Poly(ethylene glycol) methyl ether methacrylate (PEGMA) (average molecular weight of 1,100 g/mol) was purchased from Sigma–Aldrich and used as received. *N*,*N*′-Methylenebisacrylamide (MBA) was purchased from Shanghai Chemical Reagents Co. (Shanghai, China, analytical grade) and was used as received. Ammonium persulfate (APS) was purchased from Shanghai Chemical Reagents Co. (Shanghai, China, chemical purity) and purified by recrystallization in ethanol before use. Ultrapure water was used in all the preparation and characterization processes.

Preparation of the fluorinated pH-sensitive graft microgels

The poly(DMAEMA-*co*-HFMA)-*g*-PEG microgels were prepared via surfactant-free emulsion polymerization. In a typical reaction, DMAEMA (0.253 g), HFMA (0.454 g), PEGMA (0.301 g), and H_2O (25 mL) were added into a 50-mL round bottom flask containing a magnetic stirrer. After pre-emulsification for 30 min, MBA (0.021 g) and APS (0.012 g) were added into the flask. The flask was then deoxygenated under reduced pressure and backfilled with nitrogen several times. Polymerization was carried out at 75 °C for 24 h. After polymerization, the product was put into a dialysis bag (molecular weight cut-off (MWCO)= 8,000–14,000) and dialyzed against 1,000 ml ultrapure water at 25 °C for 3 days. The polymerization conversion and solid content were measured by gravimetric analysis. The theoretical contents of DMAEMA, HFMA, and PEGMA in the copolymers were calculated and listed in Table 1.

Structural characterization

Solid samples of the latexes were obtained from precipitation in ethanol. The filtered latexes were dispersed in a small

Table 1 The composition, conversion, and solid content of the copolymer microgels

Sample	DMAEMA (%)	HFMA (%)	PEGMA (%)	Conversion (%)	Solid content (%)
Poly(DMAEMA-*co*-HFMA)-*g*-PEG	25.1[a] 26.35[b]	45.04[a] 44.59[b]	29.86[a] 29.06[b]	92.7	3.74

[a]Theoretical composition

[b]Elemental analysis results

amount of THF. Then the mixture was precipitated in *n*-hexane. After filtration, the precipitate was purified by re-precipitation repeatedly in *n*-hexane. The obtained product was dried under vacuum at 30 °C for 48 h. FTIR spectrum of the copolymer was recorded on the Perkin-Elmer Spectrum one Transform Infrared Spectrometer (Perkin-Elmer, USA). ^{1}H NMR and ^{19}F NMR spectra were recorded using a UNITY INOVA 600-MHz spectrometer (Varian, USA) with $CDCl_3$ as the solvent at room temperature. The content of carbon, hydrogen, oxygen, nitrogen, and fluorine were determined with an Elementary Vario EL III elemental analyzer (Germany).

Fluorescence spectra

Nile red was chosen as the fluorescence probe to study the effect of pH on the hydrophilic/hydrophobic properties of the microgels. The samples for spectroscopic analysis were prepared as follows. The Nile red/acetone solution (25 μg/mL) was prepared and placed into some dry volumetric flasks (10 mL). After evaporation of the acetone, the microgel suspensions (1.0 mg/mL) at different pH values were added into the flasks to produce a final Nile red concentration of 0.5 μg/mL. The fluorescence emission spectra were obtained using the RF-540 spectrofluorimeter (Hitachi High-Technologies Corporation, Tokyo, Japan) at 25 °C. The fixed excitation wavelength was 535 nm.

Dynamic light scattering

The average hydrodynamic diameter and size distribution of the microgel particles (1.0 mg/mL) were measured by dynamic light scattering (DLS) (Autosize Loc-Fc-963, Malvern Instrument) at different pH values (3.2, 4.1, 5.0, 6.2, 7.4, 8.0, 9.2, and 10.1, respectively). Measurements were carried out at a scattering angle of 90° at 25 °C. Sample cell was thermostated for 5 min prior to measurements.

Zeta potential

The variation of zeta potential of the microgel particles in phosphate buffer solution (1.0 mg/mL, ionic strength= 0.01 M) was determined by Malvern Zetasizer Nano ZS (Malvern Instruments, Malvern, UK) at different pH values from 2.0 to 10.0.

Transmission electron microscopy

The morphology of the microgel particles was characterized by transmission electron microscopy (TEM) (Tecnai G20, FEI Corp.USA) in phosphate buffer solution (pH 7.4). The drops of the emulsion (1 mg/mL) were placed on Formvar-coated 400-mesh copper grid. The sample was dried at room temperature. The TEM images were obtained at an electron acceleration voltage of 200 kV.

In vitro cytotoxicity of microgels and drug-loaded microgels

In vitro cytotoxicity of the poly(DMAEMA-*co*-HFMA)-*g*-PEG microgels was measured by an MTT viability assay against Caco-2 cells. The cells were seeded at a density of 1×10^{4}cells/well in 96-well plates in a standard growth medium at 37 °C for 24 h. Then the culture medium was removed and the microgel suspension was added into the plates at different concentrations. To evaluate the killing efficacy of 5-FU-loaded microgels, the cells were also treated with the same amount of 5-FU-loaded microgels (200 μg/mL) for different periods of 12, 24, 36, 48, 60, and 72 h. Meanwhile, the wells containing only the cell medium were also prepared as untreated controls. After the cells were incubated for another 48 h, the medium was removed and 20 μL MTT solutions (5 mg/mL) were added into each well. Then the cells were incubated for an additional 4 h. The supernatant was discarded, followed by addition of dimethyl sulfoxide (DMSO, 150 μL/well) and agitation for 30 s to completely dissolve the crystals. The absorbance of each well was measured at a wavelength of 570 nm by a microplate reader, and the cell viability was calculated by the following formula:

Cell viability (%)=OD_{570}(sample)/OD_{570}(control)× 100, where OD_{570}(sample) is the optical density (OD) of the treated cells measured at a wavelength of 570 nm and OD_{570}(control) represents that of the untreated control cells.

Drug loading and in vitro release

5-Fluorouracil (5-FU) was chosen as the hydrophobic drug model to study the in vitro drug release from poly (DMAEMA-*co*-HFMA)-*g*-PEG microgels in the phosphate buffer solution (PBS) under different pH conditions (pH 3.0, 7.4, and 10.1, respectively). Five milliliters 5-FU aqueous solution (1 mg/mL) was added into 5 mL microgel suspension (2 mg/mL). After 20-min vortex, the mixture was stirred for 24 h and put into a dialysis bags (MWCO= 8,000–14,000). Then the mixture was dialyzed against 1,000 mL ultrapure water at 25 °C for 24 h (water changed every 6 h) to remove the drugs unloaded in the microgel particles. Afterwards, the drug-loaded microgels were obtained. The drug loading content (DLC) and encapsulation efficiency (EE) were determined by measuring the UV absorbance at 265 nm. The mass of 5-FU loaded in the

microgels was calculated by the standard calibration curve experimentally obtained with 5-FU/H_2O solutions. The DLC and EE were calculated based on the following formulas:

$$\mathrm{DLC(wt.\%)} = (\text{Mass of 5} - \text{FU loaded in microgels/Mass of drug} - \text{loaded microgels}) \times 100\%$$

$$\mathrm{EE(wt.\%)} = (\text{Mass of 5} - \text{FU loaded in microgels/The initial mass of 5} - \text{FU before dialysis}) \times 100\%$$

To study in vitro drug release under different pH conditions, the drug-loaded microgels in the dialysis bag (A), (B), and (C) were dialyzed against 1,000 mL phosphate buffer solutions for 48 h at pH 3.0, 7.4, and 10.1, respectively. Periodically, aliquots of 5 ml of phosphate buffer solution outside the dialysis bag were removed for UVvis analysis and replaced with the same volume fresh PBS in order to hold the volume of solutions constant. The amount of drug released from microgels at different temperatures was still measured by UV absorbance at 265 nm, using the standard calibration curve experimentally obtained with 5-FU/H_2O solutions.

Results and discussion

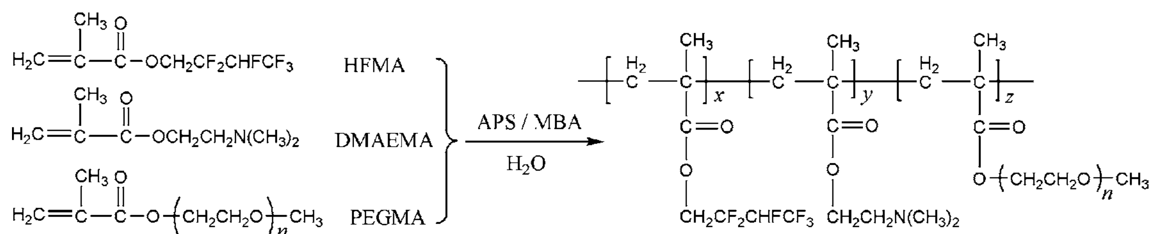
Synthesis and characterization of poly(DMAEMA-co-HFMA)-g-PEG copolymers

The fluorine-containing graft copolymers poly(DMAEMA-*co*-HFMA)-*g*-PEG was synthesized via emulsifier-free emulsion polymerization in order to obtain controlled and narrow size core/shell microgel particles. Scheme 1 illustrates the polymerization process of the graft copolymer. The chemical structure of the obtained product was analyzed by FTIR, ^{1}H-NMR, ^{19}F-NMR and elemental analysis.

Figure 1 shows the FTIR spectrum of poly(DMAEMA-*co*-HFMA)-*g*-PEG copolymers. The peaks at 2,945, 2,872, 2,826, and 2,773 cm^{-1} are attributed to the stretching vibration absorption of –CH_3, –CH_2–, and CH groups. The characteristic strong absorption of the ester carbonyl (C=O) bands appears at 1,729 cm^{-1}. The peak at 1,455 cm^{-1} is assigned to the bending vibration of –CH_2– groups. The characteristic absorption of C–F bands appears at 1,187 and 1,145 cm^{-1}. The strong peak at 1,101 cm^{-1} is attributed to the characteristic absorption of ether bands (C–O). Importantly, the characteristic absorption of C=C bands disappears at 1,620 cm^{-1}. The FTIR results demonstrate that HFMA, DMAEMA, and PEGMA all participate in the polymerization and those unreacted monomers have been already removed completely.

To further confirm the structure of the product, ^{1}H-NMR was carried out in $CDCl_3$. Figure 2 shows the ^{1}H-NMR spectrum of poly(DMAEMA-*co*-HFMA)-*g*-PEG copolymers. The characteristic signals of PEG graft chains appear as follows: –CH_2CH_2O– (h, δ=3.58 ppm), –OCH_3 (i, δ=3.31 ppm). The characteristic signals of fluorinated HFMA segments appear as follows: –$CHFCF_3$ (d, δ=4.96 ppm), –CH_2CF_2– (c, δ=4.01 ppm). The characteristic signals of DMAEMA segments appear as follows: –CH_2–N (f, δ=2.49 ppm), –$N(CH_3)_2$ (g, δ=2.21 ppm). Besides, the signal at 4.28 ppm (e) is attributed to –$COOCH_2$– in both PEGMA and DMAEMA, the signals at 0.80~1.42 ppm (a) are assigned to –CH_2– of the copolymer backbones, and the signals at 1.76~1.95 ppm (b) are assigned to –CH_3 linked with the backbones.

In addition, ^{19}F-NMR was employed to determine the fluorine moiety in the copolymers. Figure 3 shows the ^{19}F-NMR spectrum of the copolymers. There are three different kinds of fluorine resonances originating from the HFMA segments in the copolymers. The signals at −210 and −75.3 ppm are attributed to –CF_3 (a) and –CHF– (b), respectively. Specially, due to the influence of –OCH_2–, the signal of –CF_2– (a) displays the characteristic double peaks at −114.3 and −119.6 ppm.



Scheme 1 Schematic diagram illustrating the polymerization process of the graft copolymer

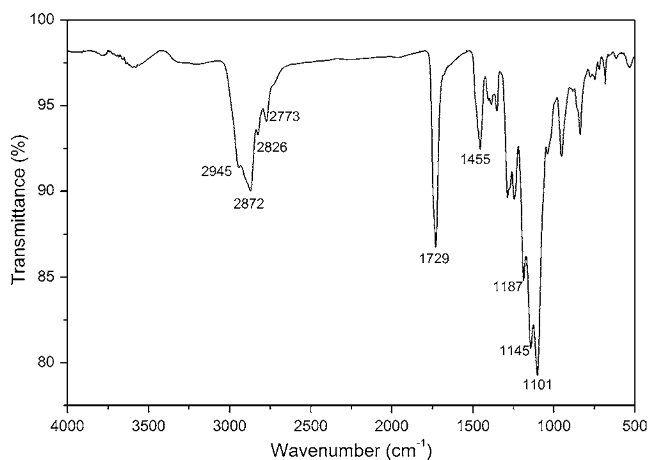


Fig. 1 FTIR spectrum of poly(DMAEMA-*co*-HFMA)-*g*-PEG

The contents of C, H, O, N, and F in the copolymer determined by elemental analysis are 49.26%, 6.55%, 21.52%, 2.35%, and 20.32%. Due to the elemental analysis, the contents of DMAEMA, HFMA, and PEGMA can be calculated, and the data was listed in Table 1.

The FTIR, ^{1}H-NMR, ^{19}F-NMR, and elemental analysis results confirm that the copolymers have been successfully prepared.

Fluorescence spectroscopy

The effect of pH on the hydrophilic/hydrophobic properties of poly(DMAEMA-*co*-HFMA)-*g*-PEG microgels is studied by the fluorescence probe technique. Due to the distinct polarity dependence of the fluorescence emission, Nile red is often employed to probe micellar microenvironments. In particular, the changes in the fluorescence intensity are useful in determining whether the Nile red is located within a hydrophobic micelle core. Importantly, the fluorescence of Nile red is negligible in an aqueous solution, whereas the

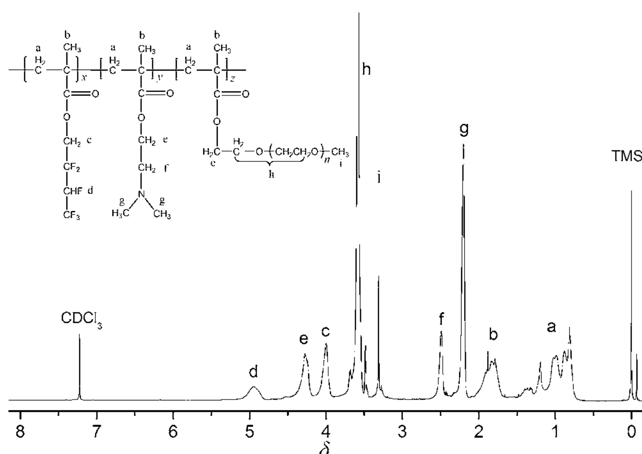


Fig. 2 ^{1}H-NMR spectrum of poly(DMAEMA-*co*-HFMA)-*g*-PEG

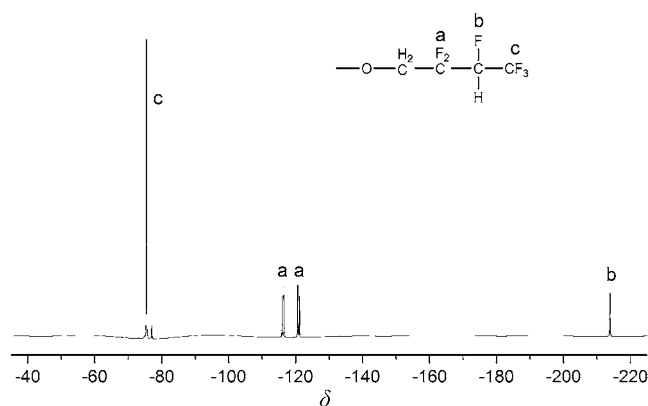


Fig. 3 ^{19}F-NMR spectrum of poly(DMAEMA-*co*-HFMA)-*g*-PEG

increase in fluorescence intensity indicates that the Nile red is located in a more hydrophobic environment [23].

Figure 4 shows the fluorescence spectra of Nile red in the copolymer emulsions at different pH values. When Nile red is added into the poly(DMAEMA-*co*-HFMA)-*g*-PEG emulsion, the fluorescence intensity increases significantly, indicating that the Nile red is located within the hydrophobic particle core. Specially, with increasing pH, the fluorescence intensity increases gradually, indicating that the hydrophobic interaction between Nile red and the microgels is enhanced gradually.

Due to the hydrophobic characteristic of the fluorinated HFMA segments, they have a high tendency to bury themselves in the interior of the microgels [24]. The formation of hydrophobic microenvironment leads to an increase in the fluorescence intensity. Under acidic conditions (pH< pK_a 8.0), the protonation of dimethylamino groups of DMAEMA enhances the hydrophilicity of microgels. But under basic conditions (pH>pK_a 8.0), the deprotonation of dimethylamino groups enhances the hydrophobicity of

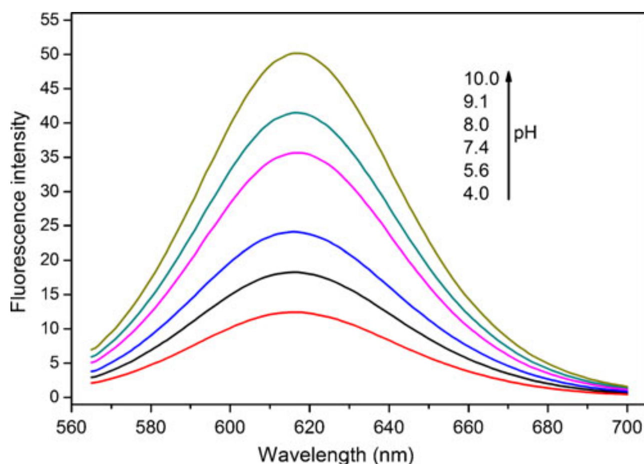


Fig. 4 Fluorescence spectra of Nile red in the copolymer emulsions at different pH values

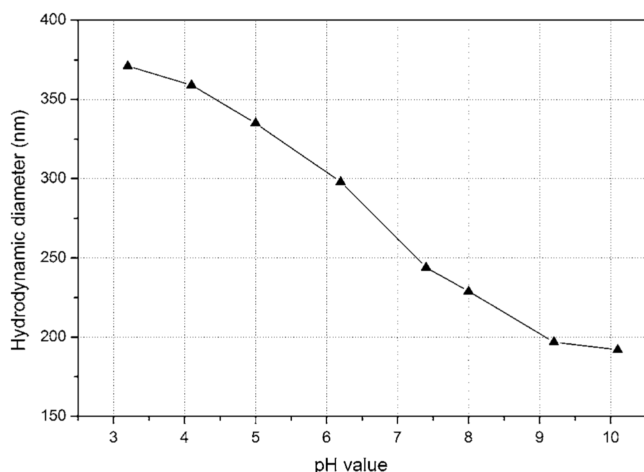


Fig. 5 The variation of average hydrodynamic diameters with pH values at 25 °C

microgels. Therefore, with increasing pH, the hydrophobic interaction between Nile red and the microgels becomes stronger, and the fluorescence intensity increases gradually.

These results show that the changes in pH values have a obvious impact on the hydrophilic/hydrophobic properties of poly(DMAEMA-*co*-HFMA)-*g*-PEG microgels, and the dimethylamino [$-N(CH_3)_2$] groups from DMAEMA is the decisive factor for the response of the microgels to pH changes.

Dynamic light scattering

To investigate the effect of pH on the particle size of poly (DMAEMA-*co*-HFMA)-*g*-PEG microgels, DLS technique is used to measure the average hydrodynamic diameter in the pH range of 3–10. The measurement is carried out at 25 °C. The variation of average particle diameter with pH is shown in Fig. 5, and more specific values are listed in Table 2.

It is clear that the size of poly(DMAEMA-*co*-HFMA)-*g*-PEG microgel particles decreases gradually with increasing pH. This decrease should be attributed to the deswelling of microgel particles by the deprotonation of dimethylamino groups with increasing pH. Under acidic conditions, the protonation of dimethylamino groups both enhances the hydrophilicity of microgels and produces an electrostatic repulsion in the interior of microgels, leading to the swelling of microgels. Under basic conditions, the occurrence of deprotonation both enhances the hydrophobicity and causes the disappearance of electrostatic interaction, leading to the deswelling of microgels. Thus, the particle size of microgels varies with the swelling/deswelling process.

Zeta potential

It is well known that PDMAEMA is a weak polyeletrolyte (pKa≈8.0) and can be protonated in an acidic aqueous solution. Zeta potential is an important parameter to evaluate the protonation of the poly(DMAEMA-*co*-HFMA)-*g*-PEG microgel particles at different pH values.

As shown in Fig. 6, the zeta potential of the microgels is positive and decreases gradually from 47.3 to 3.3 mV when the pH value increases from 2.0 to 10.0. The variation of zeta potential can be attributed to the protonation and deprotonation of the PDMAEMA segment in poly(DMAEMA-*co*-HFMA)-*g*-PEG copolymers. Under acidic conditions, high degree of protonation leads to the positively charged microgels with high zeta potential. On the contrary, under neutral or alkaline conditions, due to the occurrence of deprotonation, the zeta potential becomes very low. The results indicate that the variation of pH values has a significant effect on the zeta potential of poly(DMAEMA-*co*-HFMA)-*g*-PEG microgels.

Transmission electron microscopy

In this work, TEM is employed to observe the morphology of poly(DMAEMA-*co*-HFMA)-*g*-PEG microgels in the dry state. Figure 7 shows a representative TEM image of the microgels in phosphate buffer solutions (PBS) at pH 7.4. The graft copolymers form core/shell hexagonal microgel particles of about 200 nm with reasonably narrow size distributions.

In a neutral pH of 7.4, due to the deprotonation of dimethylamino groups, DMAEMA segments in the copolymers become relatively hydrophobic. The formation of co-

Table 2 Hydrodynamic diameters and polydispersity index (PDI) for the microgels at different pH values

Microgel particles	pH values of phosphate buffer solutions							
	3.2	4.1	5.0	6.2	7.4	8.0	9.2	10.1
Hydrodynamic diameter (nm)	371.6	359.2	335.8	298.4	244.7	229.1	197.2	192.5
PDI	0.153	0.147	0.211	0.098	0.156	0.116	0.084	0.165

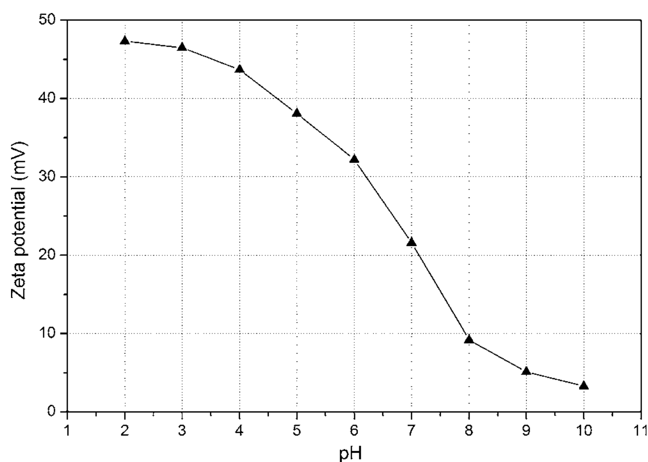


Fig. 6 The variation of zeta potential of the microgel particles at different pH values from 2.0 to 10.0

polymer micelles is virtually a kind of macromolecular self-assembled process, which is usually affected by the experimental temperature, the type of solvent, and the species of molecular chains, etc. In this work, because of the hydrophobic characteristic of the fluorinated HFMA segments and the DMAEMA segments, in the formation of micelle particles, they have a high tendency to bury themselves in the interior of the micelles to form the core of the microgels. Meanwhile, owing to the hydrophilic characteristic, the PEG graft chains migrate to the exterior of the micelles to form the shell of the microgels. Therefore, with the evaporation of the water, the copolymers mainly form regular self-assembled micelles with the relatively low free energy, displaying a hexagonal morphology with obvious core/shell structure in the TEM image. Moreover, the diameter of the entire core/shell microgels in pH 7.4 medium measured from the TEM image is in good agreement with the data measured by DLS.

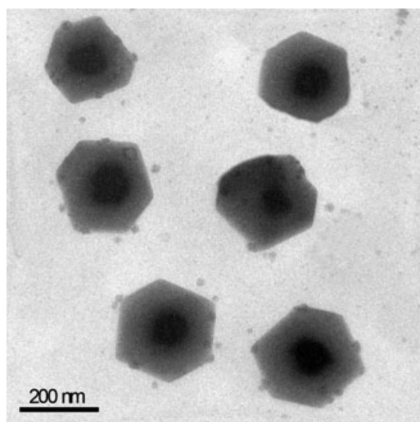


Fig. 7 TEM image of the microgels in phosphate buffer solution at pH 7.4

In vitro cytotoxicity of microgels

It is well known that the cytotoxixity of biomaterials is extremely important for their applications in drug delivery system. The in vitro cytotoxicity of poly(DMAEMA-*co*-HFMA)-*g*-PEG microgels is evaluated referenced to Caco-2 colon cancer cells using the MTT assay.

Figure 8 displays the cell viability after 48-h incubation in the microgel suspensions with different concentrations. The data demonstrates the microgels do not show significant cytotoxicity against Caco-2 cells. About 86.7% of cells are still viable even at a high concentration of 1,000 μg/mL. The results indicate that poly(DMAEMA-*co*-HFMA)-*g*-PEG microgels have good cytocompatibility.

Drug loading and in vitro drug release

To investigate the drug loading capacity of poly(DMAEMA-*co*-HFMA)-*g*-PEG microgels, 5-FU is used as a model hydrophobic drug [25, 26]. It is well known that hydrophobic drugs can be physically incorporated and stabilized in the hydrophobic core by the hydrophobic interaction [27]. Due to the strong hydrophobic HFMA segments in the copolymers, 5-FU can be absorbed and stabilized in the cores of microgel particles. In this work, the drug-loaded microgels were prepared using the dialysis technique. The mass of 5-FU loaded in the microgels was calculated by measuring the UV absorbance at 265 nm. The experimental results indicated that 5-FU was successfully incorporated into the microgels, and the DLC and EE were about 18.7% and 37.3%, respectively.

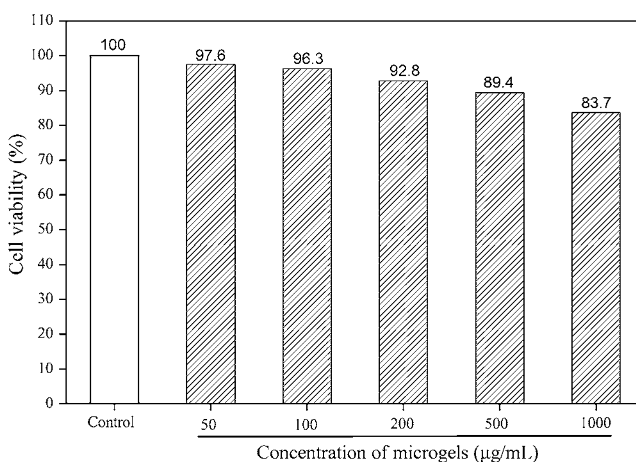


Fig. 8 Cell viability after 48-h incubation in the microgel suspensions with different concentrations

Furthermore, in order to investigate the pH-dependent release of the loaded drug from poly(DMAEMA-*co*-HFMA)-*g*-PEG microgels, the in vitro drug release studies are performed in PBS buffer solutions at different pH values (pH 3.0, 7.4, and 10.1). Figure 9 depicts the release profiles of 5-FU from the drug-loaded microgels. It is clear that the drug release behavior at different pH values (pH 3.0, 7.4, and 10.1) all undergoes two stages: the initial relatively rapid release in the first stage and the later relatively sustained release in the second stage.

The initial rapid release may be attributed to the release of unstable 5-FU in the shells of microgels. Moreover, the 5-FU release rate at pH 3.0 is faster than that at pH 7.4 and 10.1. At pH 3.0, the cumulative percentage of the drug release in the first stage is 22.8% within 6 h and the final cumulative release is 82.5% within 72 h. In comparison, at pH 7.4, only 10.2% of 5-FU is released in the first 6 h and the final cumulative release is 40.9% within 72 h. Even at pH 10.1, the percentage drops to 8.3% and 31.9%, respectively.

The different 5-FU release behavior at different pH values should be attributed to the swelling and collapse of the poly(DMAEMA-*co*-HFMA)-*g*-PEG microgel particles. Under acidic conditions, the microgels become positively charged with the protonated dimethylamino groups. Due to the electrostatic repulsion, the molecular networks of the microgels are in the swelling state. Consequently, 5-FU can be release from the microgels more easily. On the contrary, in a neutral or alkaline medium, the dimethylamino groups are deprotonated, leading to the collapse of microgel particles. Thus, the molecular networks of the microgels become compact and restrict the release of 5-FU from microgels. These results demonstrate that the poly(DMAEMA-*co*-HFMA)-*g*-PEG microgels play an important role in the release mechanism stimulated by changes in the pH and have potential applications as a controlled drug release carrier.

In vitro assay of killing efficacy

The in vitro killing efficacy of 5-FU-loaded microgels was determined from Caco-2 colon cancer cells by MTT assay. Figure 10 shows the cell viability after incubation with drug-loaded microgels (200 μg/mL) for different time periods.

As shown in Fig. 10, the cytotoxicity is observed to increase gradually with time. The data shows that the drug-loaded microgels have high growth inhibition effect on Caco-2 colon cancer cells. After incubation for 72 h, only 47.5% of cells were still viable. The increased cytotoxicity may be attributed to the sustained release of the encapsulated 5-FU from the poly(DMAEMA-*co*-HFMA)-*g*-PEG microgels after a long incubation time. The results indicate that the microgels with 5-FU offer a certain killing potency against cancer cells.

Conclusions

We have successfully prepared the novel fluorine-containing pH-responsive core/shell microgels poly(DMAEMA-*co*-HFMA)-*g*-PEG. The copolymer microgels display a significant pH-sensitive behavior. Furthermore, not only the microgels had low toxicity, but also 5-FU-loaded microgels offer a certain killing potency against cancer cells. Importantly, drug loading and in vitro drug release demonstrated that 5-FU was successfully incorporated into polymeric microgels, and the change of pH values has a marked impact on the 5-FU release from the microgels. Our study show that the poly(DMAEMA-*co*-HFMA)-*g*-PEG microgels play an important role in the release mechanism stimulated by

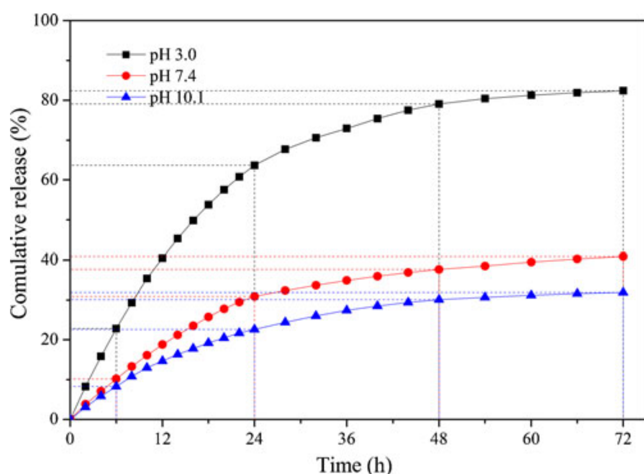


Fig. 9 The release profiles of 5-FU from the drug-loaded microgels at different pH values (3.0, 7.4, and 10.1, respectively)

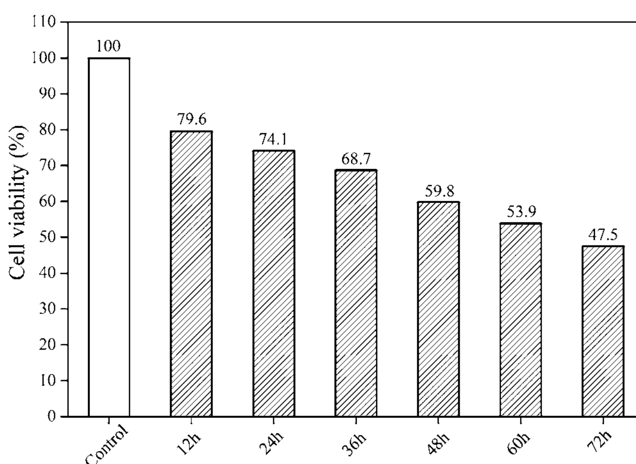


Fig. 10 Killing effects for different incubation time at the same concentrations (200 μg/mL) of drug-loaded microgels

changes in the pH and have potential applications as a controlled drug release carrier.

Acknowledgments The work was supported by the Key Projects in the National Science and Technology Pillar Program during the Eleventh Five-year Plan Period (no. 2008BAC32B03) and the National Natural Science foundation of China (no. 50973027).

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