DNA Adsorption on Poly(*N*,*N*-dimethylacrylamide)-Grafted Chitosan Hydrogels

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ABSTRACT: In this study, poly(*N*,*N*-dimethylacrylamide) grafted chitosan (PDMAAm-*g*-CT) hydrogels were prepared for deoxyribonucleic acid (DNA) adsorption. Instead of directly grafting the *N*,*N*-dimethylacrylamide (DMAAm) monomer onto the chitosan (CT) chains, poly(*N*,*N*-dimethylacrylamide) with carboxylic acid end group (PDMAAm-COOH) was firstly synthesized by free-radical polymerization using mercaptoacetic acid (MAAc) as the chain-transfer agent and then grafted onto the CT having amino groups. The synthesis of PDMAAm-COOH and its grafting onto the CT chains were confirmed by attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy. From gel permeation chromatography measurements, the number-average molecular weight ($\overline{M}_n$) and polydispersity index of PDMAAm-COOH were found as 2400 g/mol and 2.3, respectively. The PDMAAm-*g*-CT hydrogels were utilized as the adsorbents in DNA adsorption experiments conducted at +4°C in a trisEDTA solution of pH 7.4. The hydrogels produced with higher PDMAAm-COOH content exhibited higher DNA adsorption capacity. The DNA adsorption capacity up to 4620 μg DNA/g dry gel could be achieved with the PDMAAm-*g*-CT hydrogels prepared in 80.0 wt % PDMAAm-COOH feed concentration. This value is approximately seven times higher than that of CT alone. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 120: 1420–1425, 2011

Key words: hydrogels; *N*,*N*-dimethylacrylamide; chitosan; DNA adsorption

INTRODUCTION

The adsorption of deoxyribonucleic acid (DNA) to polymeric supports provides convenient tools in a variety of fields including biotechnology and medicine. Especially, DNA-adsorbed polymeric supports are used in the therapy of systemic lupus erythematosus (SLE) involving the removal of anti-DNA antibodies from plasma.[1,2] The amount of anti-DNA antibodies correlates well with the disease activity, such as cerabritis and nephritis. Moreover, antibody adsorption capacity and antibody removal rate are also proportional to the amount of adsorbed DNA on the polymeric supports. The presence of a higher amount of DNA adsorbed on the polymeric support usually provides a higher anti-DNA antibody removal rate.

Chitosan (CT), obtained from deacetylation of chitin, is one of the most facile natural polymers that can be altered structurally to give useful solid-phase supports for DNA adsorption.[3] However, CT exhibits some shortcomings, such as hydrophobicity, lower mechanical properties, and a high pH dependence of its physical properties. For these shortcomings of CT, a main approach is applied such as chemical modification by the grafting of a suitable monomer onto CT backbone and then the crosslinking of this modified CT.[4,5] The grafting of polymer chains onto CT may be more accessible for the adsorption of DNA than the ungrafted CT matrix.

PDMAAm is a one of the most typical nonionic water-soluble polymer that has received considerable attention because of its hydrogel forming property.[6,7] Because of its hydrophilic and biocompatible[8] nature, it finds numerous applications in the biomedical fields.[9,10]

To our knowledge, there have been only a few attempts to prepare PDMAAm-*g*-CT hydrogels according to the grafting process. Tripathy et al.[11] reported the preparation of PDMAAm-*g*-CT hydrogels using peroxymonosulphate/mandelic acid redox pair, but they mainly attributed the grafting to hydroxyl groups and amino groups, which is supported by a tentative mechanism. Yuan et al.[12] produced thermosensitive smart chitosan-*g*-poly(*N*-isopropylacrylamide-co-*N*,*N*-dimethylacrylamide) and showed significant changes in swelling ratio and drug release behavior below and above the lower critical solution temperature, demonstrating the preservation of responsive properties. Moreover, Babu et al.[13] fabricated semi-interpenetrating networks of DMAAm and CT in the form of microspheres by water-in-oil emulsion technique for *in vitro* release of chlorothiazide.

In our previous study, we prepared poly(acrylamide) grafted chitosan [PAAm-*g*-CT] hydrogels and

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used for DNA adsorption.[14] The results of this and other studies indicated that higher anti-DNA antibody adsorption capacity could be obtained with polymeric supports carrying higher amounts of adsorbed DNA on their surfaces.[14–17] Moreover, it was also shown that more quantitative and stable adsorption of DNA could be achieved by hydrophobic interactions between the polymeric support and DNA molecules.[14–19]

The goal of this study is to prepare the hydrogels composed of PDMAAm and CT with higher DNA adsorption capacity than those obtained in earlier studies. The chemical structures of the PDMAAm-COOH and the hydrogels before and after grafting process were characterized by attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy. The $\overline{M}_n$ and PDI of PDMAAm-COOH were determined by GPC measurements. Furthermore, the difference of adsorption capacities was discussed by comparing CT alone with the PDMAAm-*g*-CT hydrogels.

EXPERIMENTAL

Materials

CT ($M_w = 600 \times 10^3$ g/mol, degree of deacetylation = 85%) was purchased from Sigma Chemicals (Aldrich Chemical, Milwaukee, WI) and used after being dissolved in a 2 wt % acetic acid aqueous solution and filtered using a glass filter. DMAAm (Aldrich), ammonium persulfate (APS, Aldrich), *N,N,N′,N′*-tetramethylenediamine (TEMED, Aldrich), 1-ethyl-(3-3-dimethylaminopropyl) carbodiimide hydrochloride (EDC, Aldrich), *N*-hydroxy-succinimide (NHS, Aldrich), and glutaric dialdehyde (GDA, Aldrich) were used as received. MAAc (Aldrich) was purified by distillation under reduced pressure. DNA was isolated from plants by Clark's method.[20] Deionized water was used in all experiments.

Synthesis of end-functionalized PDMAAm

PDMAAm with carboxylic acid end group (PDMAAm-COOH) was synthesized by free-radical polymerization using MAAc as the chain-transfer agent and APS and TEMED as the redox initiator system. In a typical experiment, DMAAm (0.2 mol), MAAc (6.5 mmol), and APS (5 mL, 0.056 *M*) were dissolved in deionized water (50 mL) and purged with nitrogen gas for 10 min. Then, 5 mL (0.32 *M*) of TEMED was added to accelerate the polymerization. The polymerization was carried out at 22°C for 24 h. The product was precipitated into an excess of acetone and dried in vacuum oven at 60°C.

Preparation of PDMAAm-*g*-CT hydrogels

CT and PDMAAm-COOH (28.6, 37.5, 44.4, 50.0, 60.0, 66.7, 75.0, and 80 wt % in feed mixture) were simultaneously dissolved in 2 wt % acetic acid solution at 22°C. EDC and NHS were added to the solutions to form amide bonds between the amino groups of CT and the carboxylic acid groups of PDMAAm-COOH. The mixed solutions were continuously stirred at 22°C for 24 h. After that, unreacted amino groups of CT were crosslinked using GDA. The resulting hydrogels were purified by immersing in deionized water for 1 week to remove unreacted chemicals and contaminated homopolymers. The water was replaced three to four times every day. The purified hydrogels were stored in deionized water for characterization and DNA immobilization.

GPC measurements

The molecular weights of PDMAAm-COOH were determined by GPC using a Waters 510 HPLC pump with a Waters 410 differential refractometer and a Waters Styragel HR1 + HT6E column system, THF being the eluting solvent at 40°C. Monodisperse polystyrene standards were used to calibrate the molecular weights. The molecular weights of polystyrene standards were between 500 and 6000 g/mol.

ATR-FTIR measurements

ATR-FTIR measurements were made with a Nicolet 6700 FTIR (USA) spectrometer equipped with a smart orbit accessory in the range of 4000–525 cm^{-1}. Before the measurements, the originally swollen hydrogels were freeze-dried in a Virtis freeze drier (Lobconco Company, Kansas City, MO) for 2 days to completely remove water.

Equilibrium swelling ratio measurements

For the swelling ratio measurements, preweighed dry, freeze-dried samples were immersed in the pH buffer solutions at 22°C for 24 h. After the excess water was wiped off the sample surface with moistened filter paper, the mass of the swollen samples was measured in the pH range of 2–9. The equilibrium swelling ratio (SR) was determined as follows:

$$\mathrm{SR} = \frac{m_s - m_d}{m_d} \quad (1)$$

where m_s and m_d are the mass of the hydrogels at swelling state and dry state, respectively. All the reported swelling values are an average of at least three separate measurements.

Adsorption of DNA on hydrogels

DNA adsorption studies were done using both ungrafted CT and PDMAAm-*g*-CT hydrogels with

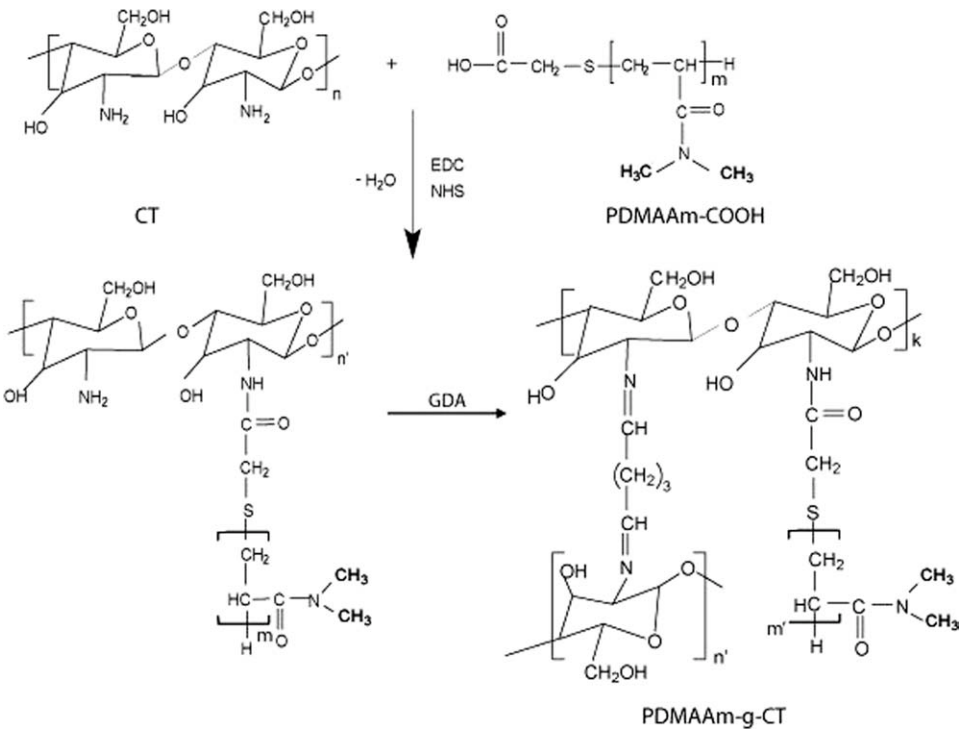

Figure 1 Molecular scheme for preparation of PDMAAm grafted CT hydrogels.

different PDMAAn content as adsorbents. The initial DNA concentration in the adsorption medium was varied between 2.0 and 35 μg/mL. A typical DNA adsorption experimental procedure can be described as follows. A known amount of DNA was dissolved in trisEDTA buffer solution (40 mL, pH 7.4, ionic strength 0.1 *M*) by stirring magnetically with 200 rpm at + 4°C for 24 h. Then, the hydrogels (approximate dry weight: 0.5 g) equilibrated in a trisEDTA buffer solution were transferred into the adsorption medium containing DNA with a different initial concentration. The adsorption process was conducted at +4°C for 24 h. After this period, the hydrogels were removed from DNA solution, and the DNA concentration in the adsorption medium was measured.

The DNA adsorption capacity of the hydrogels was determined by measuring the initial and final DNA concentrations in the adsorption medium according to the method developed by Spirin.[21] To digest DNA into nucleotides, 0.1 mL of DNA solution was transferred into perchloric acid solution (4 mL, 0.24 *M*) and incubated in a water bath at 90°C for 20 min. After cooling to 22°C, the adsorbance of the solution was measured at both 260 and 280 nm in a UV/vis spectrometer (Hitachi, Japan). By using the calibration curve between known DNA concentration, the concentration of DNA in the assayed sample was determined. The equilibrium DNA adsorption capacity (Q, mg DNA/g dry gel) was calculated as follows:

$$Q = \frac{(C_o - C_e)V}{m_o} \quad (2)$$

where C_o and C_e (mg DNA/mL) are the concentration of DNA in the adsorption medium before and after the adsorption process, respectively. V (mL) is the volume of DNA solution, and m_o is the dry mass of the hydrogel.

RESULTS AND DISCUSSION

Synthesis and spectral characterization of hydrogels

In previous studies, to prepare the grafted hydrogel with polysaccharide containing the carboxyl groups as pendent groups, the amino-terminated poly(*N*-isopropylacrylamide) has been synthesized by using 2-aminoethanethiol hydrochloride as a chain-transfer agent.[22–24] In this study, to covalently graft the PDMAAm on the amino groups of CT, PDMAAm-COOH was synthesized by free radical polymerization using MAAc as a chain-transfer agent. The chemical structure of the grafted hydrogels is shown in Figure 1.

To confirm the preparation of PDMAAm-COOH, the existence of COOH groups on the end of chain and molecular weight of PDMAAm were investigated by using ATR-FTIR and GPC, respectively. Figure 2 shows ATR-FTIR spectra of DMAAm monomer and MAA chain-transfer agent to confirm

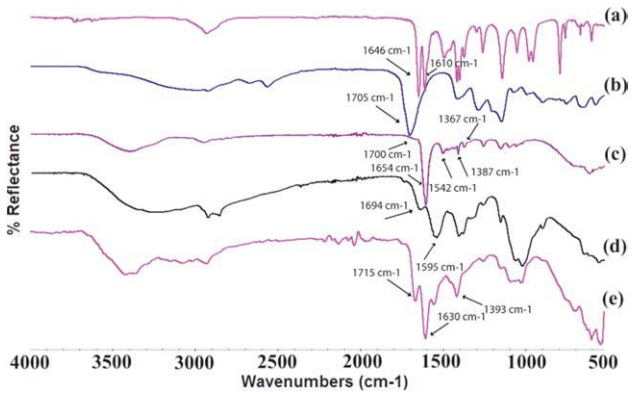

Figure 2 ATR-FTIR spectra of DMAAm (a), MAAc (b), PDMAAm-COOH (c), CT (d), and PDMAAm-*g*-CT (e). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

the formation of PDMAAm-COOH. The ATR-FTIR spectrum obtained from PDMAAm-COOH [Fig. 2(c)] shows three significant bands at 1654 cm^{-1} (amide I), 1542 (amide II), and 1387 cm^{-1} (methyl groups in $-N(CH_3)_2$) and a small shoulder at 1715 cm^{-1} due to the existence of a small amount of carboxylic acid group at the end of the PDMAAm. Moreover, the characteristic bands at 1610 cm^{-1} (C=C), 1450 cm^{-1} (CH_2=), and C—H vinyl out-of-plane bending vibrations, observed in the spectrum of monomer [Fig. 2(a)], disappeared in PDMMAm-COOH [Fig. 2(c)]. From this results, we could confirm the synthesis of PDMAAm-COOH.

The molecular weight distribution of PDMAAm-COOH, determined by GPC, showed number-average molecular weight ($\overline{M}_n$) and PDI of 2400 g/mol and 2.3, respectively. The ratio of PDMAAm having the carboxylic acid end groups in the entire PDMAAm chains was 96%, which was determined by comparing the molecular weight of PDMAAm measured from GPC with the carboxylic acid group contents obtained from titration assay. This result indicated that the PDMAAm-COOH had an average of one carboxylic acid-terminated group per polymer chain.

To form the PDMAAm-grafted hydrogels with free and mobile end chains, PDMAAm-COOH synthesized was grafted onto the CT amino groups, and then crosslinked with GDA. The formation of PDMAAm-*g*-CT hydrogels was confirmed by ATR-FTIR measurements. Figure 2 shows the ATR-FTIR spectrum for PDMAAm-COOH (c), CT (d), and PDMAAm-*g*-CT (e). The ATR-FTIR spectrum of CT with 85% deacetylation degree indicated that bands appearing 1694 and 1595 cm^{-1} could be assigned to a carbonyl stretching vibration (amide I) and *N*—H bending vibration (amide II) of a primary amino group, respectively. Thus, in the case of the PDMAAm-*g*-CT hydrogel [Fig. 2(e)], the formation of amide groups was confirmed by the band at 1694 cm^{-1} shifted to the lower wavenumbers, compared with CT itself and PDMAAm-COOH. However, the band intensity decreases of free amino groups of CT at 1595 cm^{-1} may also be ascribed to the crosslinking by GDA.

Equilibrium swelling/deswelling behavior of hydrogels

Figure 3 shows pH-dependent swelling behaviors of PDMAAm-*g*-CT hydrogels prepared in different PDMAAm-COOH feed concentrations, which are investigated by swelling test under various pH values ranging between 2 and 9. The pH sensitivity is slightly affected by unreacted amine group of CT, which constitute a weak base with an intrinsic pK_a of about 6.5, that is, the hydrogels swelled at low pH because of the ionic repulsion of the protonated amine groups, and collapsed at high pH because the influence of unprotonated amine groups. As the pH value of the buffer solution increases, ionized $-NH_3^+$ groups become $-NH_2$ groups, and the resulting neutralization of ionic groups causes the hydrogels to be collapsed. As shown in Figure 3, the swelling ratio of the hydrogels continuously decreased with increasing pH values. On the other hand, the equilibrium swelling ratio difference between the swollen and collapse states decreased with an increase of feed concentration of PDMAAn component. Moreover, in pH $\geq$ 5 buffer solutions, the equilibrium swelling ratio of hydrogels prepared in the PDMAAm-COOH feed concentration of 50% or higher did not almost change. This is due to the fact that as the feed concentration of PDMAAm-COOH increases, hydrophobicity of the hydrogel formed could increase slightly due to the presence of methyl groups in DMAAm.

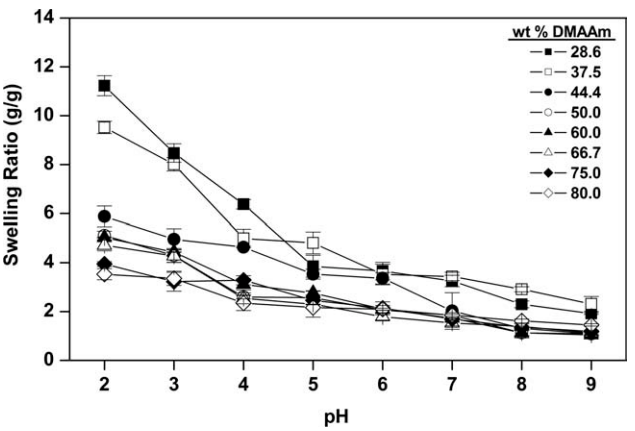

Figure 3 The variation of equilibrium swelling ratio with the medium pH for the PDMAAm-*g*-CT hydrogels produced in different PDMAAm-COOH feed concentrations.

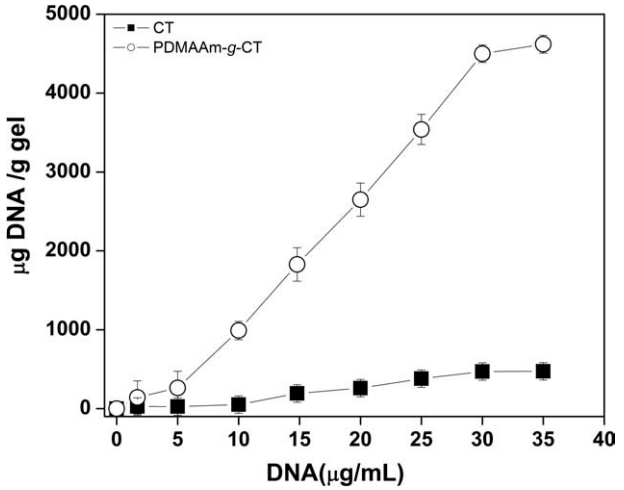

Figure 4 Effect of initial DNA concentration on the equilibrium adsorption capacity of the CT and PDMAAm-*g*-CT hydrogels produced in 80.0 wt % PDMAAm-COOH feed concentration. pH: 7.4, temperature: +4°C.

Effect of initial DNA concentration

DNA adsorption studies were carried out in DNA solutions with pH 7.4 TE buffer at +4°C by using CT and PDMAAm-*g*-CT hydrogels produced in 80.0 wt % PDMAAm-COOH feed concentration as adsorbents. The initial concentration of DNA in the adsorption medium was changed between 2.0 and 35 μg/mL. The ungrafted CT hydrogel was used a reference material. The variation of DNA adsorption capacity with initial DNA concentration was illustrated in Figure 4. An increase in DNA concentration led to an increase in the DNA adsorption capacity of the CT and PDMAAm-*g*-CT hydrogels, but this leveled off at a DNA concentration of 35 μg/mL. The maximum DNA adsorption capacity was 473 μg DNA/g dry gel for the ungrafted CT and 4620 μg DNA/g dry gel for the PDMAAm-*g*-CT hydrogel. These results indicated that the grafting of PDMAAm on CT chains provided a significant increase in the DNA adsorption capacity.

Note that a variety of polymeric supports with a wide range of adsorption capacities have been reported in the literature for DNA adsorption. Denizli and Piskin[25] reported that the maximum equilibrium DNA adsorption capacity of activated poly(2-hydroxyethylmethacrylate) microbeads was 2750 μg DNA/g polymer. The adsorption capacity of polyethyleneimide attached poly(*p*-chloromethylstyrene) beads was found to be 2900 μg DNA/g polymer.[26] Moreover, the adsorption value obtained in this study was reasonably higher relative to our previously reported adsorption capacities of PAAm-*g*-CT hydrogels (2000 μg DNA/g dry gel).[14] This may be attributed to both the diminish of free amino groups of CT after the crosslinking by GDA and the higher $-N(CH_3)_2$ content of the PDMAAm-*g*-CT hydrogels.

Effect of weight percent of PDMAAm-COOH in CT/PDMAAm-COOH mixture

The hydrogels produced with different PDMAAm-COOH feed ratios were used as sorbents and their the equilibrium DNA adsorption capacities were determined in 35 μg/mL DNA solution with pH 7.4 TE buffer at +4°C for 24 h and given in Figure 5. As shown in Figure 5, the equilibrium adsorption capacity of the hydrogels increased almost linear with increasing the weight percent of PDMAAm-COOH in the feed mixture. Notice that during swelling experiments, the hydrogels have shown systematic deswollen trends with increasing amount of PDMAAm, probably due to the presence of methyl groups of DMAAm and the more number of residual $-CH_3$ groups available, which increases the hydrophobicity of the hydrogel, thereby increasing the DNA adsorption capacity for the PDMAAm-*g*-CT. The highest equilibrium DNA adsorption capacity (4652 μg DNA/g dry gel) was obtained by the PDMAAm-*g*-CT hydrogel (80.0 wt % PDMAAm-COOH in feed mixture). On the other hand, the equilibrium DNA adsorption capacity of the ungrafted CT hydrogel was very low (473 μg DNA/g dry gel). The introduction of PDMAAm into the CT gel structure resulted in an appreciable increase in the equilibrium DNA adsorption capacity in comparison with the DNA adsorption capacity of similar more hydrophilic hydrogels, such as PAAm-*g*-CT.[14] The high adsorption capacity for the PDMAAm-*g*-CT hydrogels may be attributed to the increase of the number of grafting chains with $-N(CH_3)_2$ on the hydrogels.

CONCLUSIONS

In this study, the hydrogels composed of CT and PDMAAm have been successfully prepared through

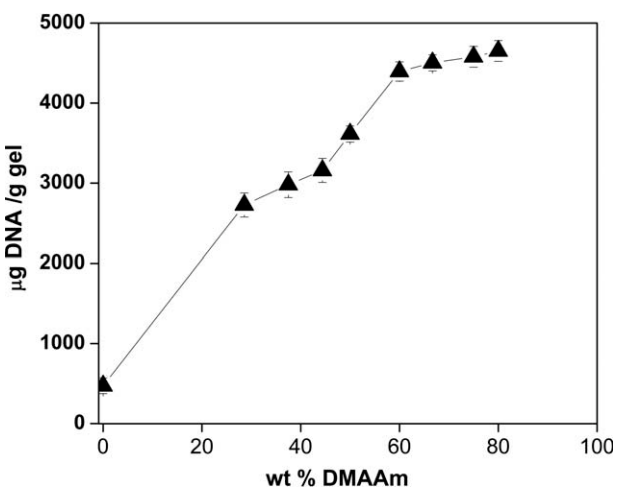

Figure 5 Effect of PDMAAm content on the equilibrium DNA adsorption capacity of the PDMAAm-*g*-CT hydrogels. pH: 7.4, temperature: +4°C.

grafting of PDMAAm with a carboxylic acid end group. The synthesis of PDMAAm-COOH and its grafting onto CT chains were confirmed by ATR-FTIR. The $\overline{M}_n$ and PDI values of PDMAAm-COOH by GPC measurements were determined as 2400 g/mol and 2.3, respectively. The PDMAAm-*g*-CT hydrogels were used as an adsorbent for DNA adsorption. The equilibrium DNA adsorption capacity of the PDMAAm-*g*-CT hydrogels is higher than that of CT alone. The low swelling and DNA adsorption of the hydrogels with high PDMAAm contents is the result of the rather increased hydrophobicity of these hydrogels, as a consequence of the methyl groups of PDMAAm. Moreover, the hydrophobic interactions between DNA and the PDMAAm-*g*-CT chains also provide more quantitative and stable adsorption of DNA in comparison with the covalent binding method. As a result, the hydrogels produced with higher PDMAAm-COOH feed concentration revealed good properties as an affinity hydrogel and will be find useful applications in the removal of anti-DNA antibodies from SLE serum.

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