Preparation of Comb-Type Grafted Hydrogels Composed of Polyacrylamide and Chitosan and Their Use for DNA Adsorption

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ABSTRACT: Comb-type grafted hydrogels composed of polyacrylamide (PAAm) and chitosan (CT) were prepared and used for DNA adsorption. Instead of direct grafting of the acrylamide monomer onto the CT chain, semitelechelic PAAm with carboxylic acid end groups (PAAm–COOH) was synthesized by free-radical polymerization with mercaptoacetic acid as the chain-transfer agent, and it was grafted onto CT with amino groups. The synthesis of telechelic PAAm–COOH and the formation of comb-type grafted hydrogels were confirmed by attenuated total reflectance/Fourier transform infrared spectroscopy and scanning electron microscopy measurements. The prepared comb-type grafted hydrogels were used as sorbents in DNA adsorption experiments conducted at +4°C in a tris(hydroxymethyl)aminomethane/ethylenediaminetetraacetic acid solution of pH 7.4. DNA adsorption capacities as high as 2.0×10^3 μg of DNA/g of dry gel could be achieved by the comb-type hydrogels with higher PAAm contents. This value was approximately 6 times higher than that of CT alone. In addition, the comb-type hydrogels showed a high adsorption/desorption rate depending on the PAAm content in the hydrogel. As a result, these comb-type hydrogels carrying higher amounts of DNA may be considered good candidates for achieving higher removal rates for anti-DNA antibodies and for effective gene therapy systems. J Appl Polym Sci 111: 1862–1868, 2009

Key words: adsorption; gels; graft copolymers

INTRODUCTION

Chitosan (CT), the *N*-deacetylated derivative of chitin, is a well-known abundant natural polymer. Its structure basically consists of D-glucosamine units, with the content of *N*-acetyl-D-glucosamine in the range of 0–50%.[1] Because of its biocompatibility, biodegradability, antibacterial properties, and remarkable affinity to proteins, it has been increasingly applied in areas such as hematology, immunology, wound healing, drug delivery, gene therapy, and cosmetics.[2–5] However, CT also exhibits some shortcomings, such as hydrophobicity, lower mechanical properties, and a high pH dependence for its physical properties. For these shortcomings of CT, a main approach has been applied, such as chemical modification by the grafting of a suitable monomer or polymer onto the CT backbone and then the crosslinking of this modified CT.[6,7] Many graft copolymers of CT and vinyl monomers have been synthesized and evaluated as flocculants, paper strengtheners, drug releasers, adsorbent resins, and so on.[8,9] For example, Ohya et al.[10] reported that because of the high hydrophilicity and biocompatibility of poly(ethylene glycol), grafting it onto CT is considered to be a convenient route to the synthesis of drug carriers. Kim et al.[11] prepared temperature- and pH-sensitive comb-type grafted hydrogels composed of CT and poly(*N*-isopropylacrylamide) (PNIPAAm) by the direct grafting method using the *N*-isopropylacrylamide monomer and ceric ammonium nitrate as a grafting agent. However, it was shown that the direct grafting method had the disadvantage of excessive formation of the homopolymer of PNIPAAm.

In this study, polyacrylamide (PAAm) chains were grafted by the formation of an amide linkage with CT after acrylamide (AAm) monomers were polymerized with carboxylic acid groups in the chain ends to graft efficiently and control the grafted regions. The chemical structures and internal morphologies of the hydrogels before and after the grafting process were characterized with attenuated total reflectance/Fourier transform infrared (ATR–FTIR) and scanning electron microscopy (SEM) measurements. Moreover, the prepared comb-type hydrogels with different PAAm contents were used as sorbents in DNA adsorption experiments conducted at +4°C in a tris(hydroxymethyl)aminomethane/ethylenediaminetetraacetic acid (TE) solution of pH 7.4.

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EXPERIMENTAL

Materials

CT (weight-average molecular weight = 600×10^3 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 with a glass filter. AAm (Aldrich), ammonium persulfate (APS; Aldrich), *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED; Aldrich), 1-ethyl-(3,3-dimethylaminopropyl) carbodiimide hydrochloride (EDC; Aldrich), *N*-hydroxysuccinimide (NHS; Aldrich), and glutaric dialdehyde (GDA; Aldrich) were used as received. Mercaptoacetic acid (MAA; Aldrich) was purified by distillation under reduced pressure. Deoxyribonucleic acid (DNA) was isolated from plants by Clark's method.[12] Deionized water was used in all experiments.

Synthesis of semitelechelic PAAm

Semitelechelic PAAm with carboxyl group termination (PAAm–COOH) was synthesized by free-radical polymerization with MAA as the chain-transfer agent and APS and TEMED as the redox initiator system. AAm (0.2 mol), MAA (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 reactant was precipitated into an excess of acetone and dried in a vacuum oven at 60°C.

Preparation of PAAm–COOH-grafted CT hydrogels

CT and PAAm–COOH (35, 45, 55, 65, or 75 wt %) were simultaneously dissolved in a 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 carboxyl groups of PAAm–COOH. The solutions had a CT/EDC/NHS molar ratio of 2 : 2 : 1 with reference to the CT amino group. The mixed solutions were continuously stirred at 22°C for 24 h. After that, unreacted amino groups of CT were crosslinked with GDA. The resulting comb-type grafted hydrogels composed of AAm and CT were purified by immersion in deionized water for 1 week to remove unreacted chemicals. The water was replaced three to four times every day, and the purified hydrogel was stored in deionized water for characterization and DNA immobilization.

ATR–FTIR measurements

ATR–FTIR measurements were made with a Nicolet (Madison, WI) 6700 FTIR spectrometer equipped with a smart orbit accessory in the range of 4000–500 cm^{-1}. Before the measurements, the originally swollen hydrogels were freeze-dried in a Virtis freeze drier (Lobconco, Kansas City, MO) for 2 days to completely remove the water

Internal morphology observations

SEM (JSM-6360 LV SEM instrument, JEOL, Tokyo, Japan) was used to study the internal or cross-section morphology of the hydrogels. To prepare samples for SEM, the swollen hydrogels at 22°C were first freeze-dried and then fractured and sputter-coated with gold.

DNA adsorption

To prepare the adsorption medium, a certain amount of DNA was dissolved in a TE buffer solution (50 mL, pH 7.4, ionic strength = 0.1*M*) by magnetic stirring at 200 rpm and 4°C for 24 h. Then, the hydrogels (approximate dry weight = 0.2 g), equilibrated in a TE buffer solution, were transferred into the adsorption medium including DNA at a certain concentration. The adsorption process was conducted at +4°C for 24 h. To determine the equilibrium DNA adsorption capacity of the hydrogels, the initial and final DNA concentrations in the adsorption medium were measured at both 260 and 280 nm in an ultraviolet–visible spectrometer (Hitachi, Tokyo, Japan). By the use of the calibration curve between known DNA concentrations, the concentration of DNA in the assayed sample was determined. The equilibrium DNA adsorption capacity [Q (mg of DNA/g of dry gel)] was calculated as follows:

$$Q = \frac{(C_0 - C_e)V}{m_0} \tag{1}$$

where C_0 and C_e are the concentrations of DNA in the adsorption medium before and after the adsorption process, respectively (mg of DNA/mL); V is the volume of the DNA solution (mL); and m_0 is the dry mass of the hydrogel.

RESULTS AND DISCUSSION

Synthesis and spectral characterization of the hydrogels

The comb-type grafted hydrogels with free and mobile end chains were prepared in three steps. As mentioned in the Experimental section, first, semitelechelic PAAm–COOH was synthesized by a free-radical polymerization method with MAA as a chain-transfer agent. Second, semitelechelic PAAm–

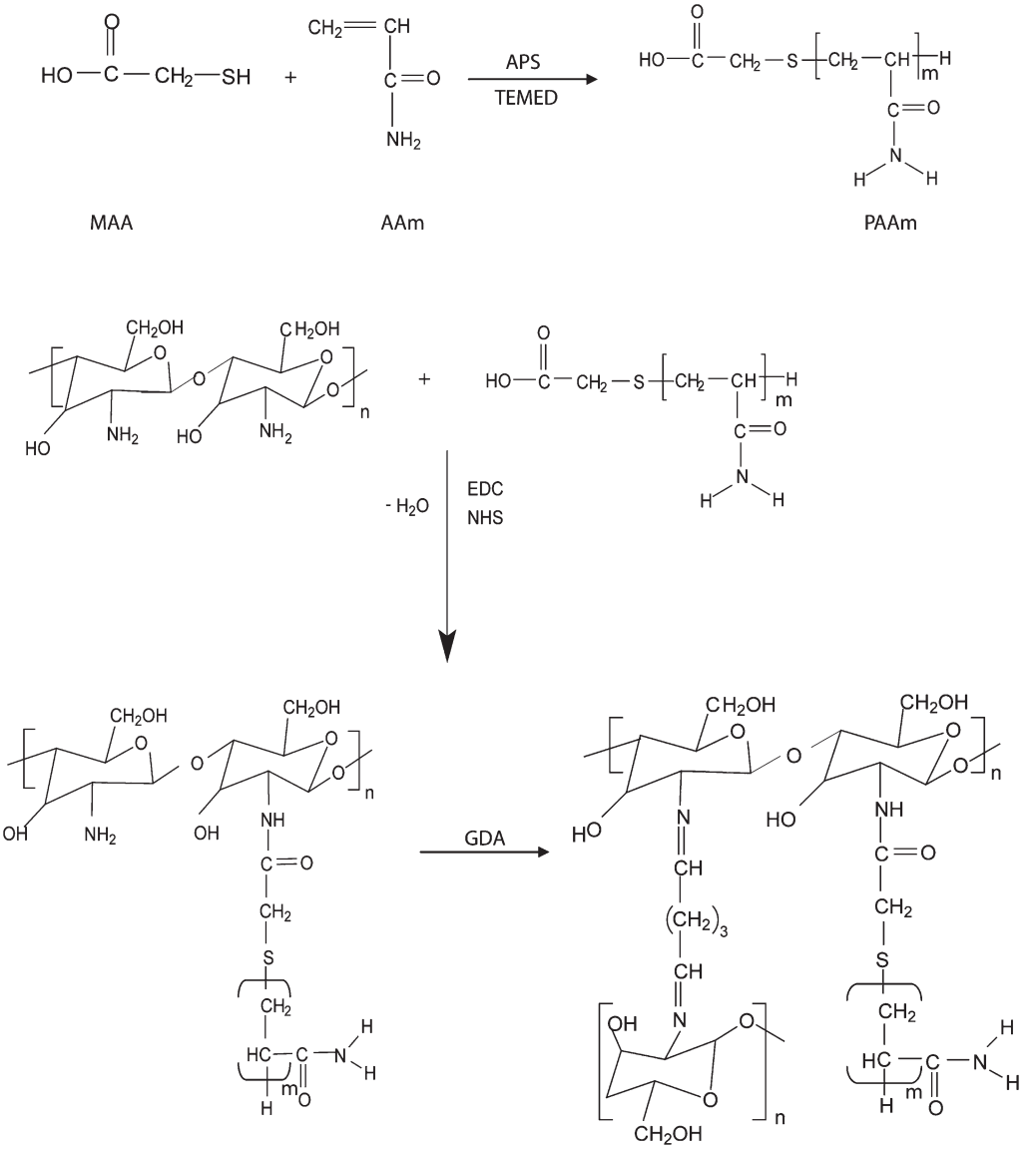

Figure 1 Molecular scheme for the preparation of the carboxylic acid terminated PAAm and comb-type grafted hydrogel.

COOH was grafted onto CT by the formation of the amide linkage. Finally, unreacted amino groups of CT were crosslinked with GDA. A possible reaction mechanism is shown in Figure 1.

ATR–FTIR spectroscopic measurements were carried out to confirm the comb-type formation on the basis of the changes in the chemical structure of the PAAm-grafted CT (PAAm-*g*-CT) hydrogels. Figure 2(a–c) shows the ATR–FTIR spectra for PAAm–COOH, CT, and PAAm-*g*-CT. The ATR–FTIR spectrum of CT with an 85% deacetylation degree indicated that bands appearing 1653 and 1598 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. In addition, Figure 2(a), obtained from a linear PAAm–COOH homopolymer, shows characteristic bands at 1654 and 1542 cm^{-1}, which can be attributed to the characteristic bands of amide I and amide II, respectively. Thus, in the case of the PAAm-*g*-CT hydrogel [Fig. 2(c)], the formation of amide groups was confirmed by the band intensity increase of amide I in PAAm–COOH at 1654 cm^{-1} and almost disappearance of free amino groups of CT at 1598 cm^{-1}, which can be attributed to the formation of amide bonds, in comparison with CT itself and PAAm–COOH.

Interior morphology of the hydrogels

To examine the differences in the physical appearances of the PAAm-*g*-CT hydrogel and ungrafted CT and to see if there were any observable physical changes on CT that occurred during the grafting

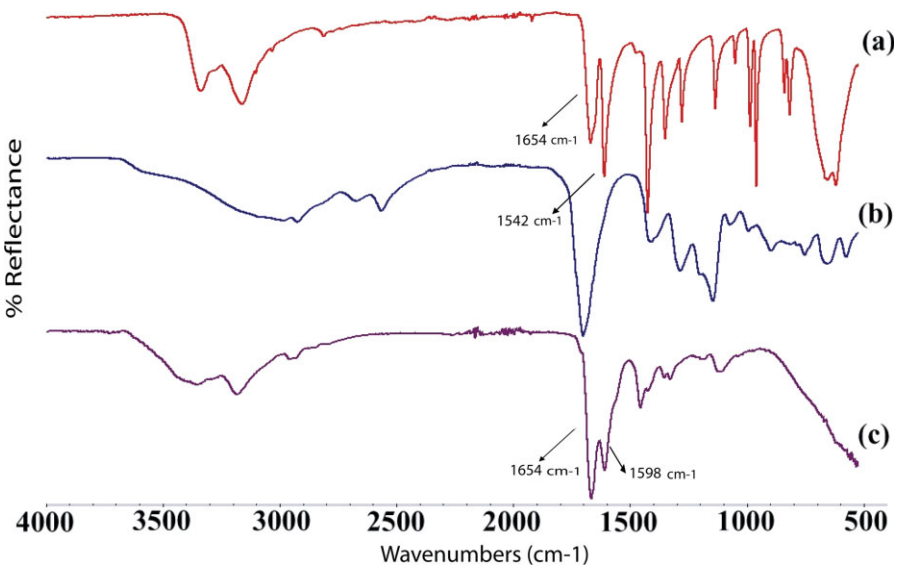

Figure 2 ATR–FTIR spectra of (a) PAAm–COOH, (b) CT, and (c) PAAm-*g*-CT. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

process, the internal morphologies of these freeze-dried hydrogels were investigated with the SEM technique. SEM micrographs of the PAAm-*g*-CT hydrogel and ungrafted CT are shown in Figure 3. The ungrafted CT was dense and smooth, whereas the PAAm-*g*-CT hydrogels exhibited a porous microstructure. These changes in the internal morphology of the ungrafted CT hydrogels were physical evidence for the grafting process.

Adsorption kinetics

DNA adsorption kinetics of the CT and PAAm-*g*-CT hydrogels prepared with 35 or 75 wt % AAm in a PAAm–COOH/CT feed mixture were studied in an initial 13.7 μg/mL DNA solution with a pH 7.4 TE buffer at +4°C as a function of time and are presented in Figure 4. The data show that the CT hydrogel had about 100 μg of DNA/g of dry gel within 3 h or 150 μg of DNA/g of dry gel within 96 h, whereas the hydrogel with 75 wt % AAm had about 700 and 1000 μg of DNA/g of dry gel within the same time frames. From these results for the adsorption kinetics, we could conclude that freely mobile chains with $-NH_3^+$ in comb-type grafted hydrogels are responsible for a fast rate of adsorption. However, although maximum adsorption rates for both the CT alone and comb-type grafted hydrogels were observed at the beginning of the adsorption, saturation values were gradually achieved in about 96 h. This behavior may be attributed to the decrease in the DNA concentration in the reservoir with time due to adsorption. As expected, when the DNA concentration in the mobile phase (i.e., the aqueous phase) decreased (and this also corresponded to an increase in the stationary phase, i.e.,

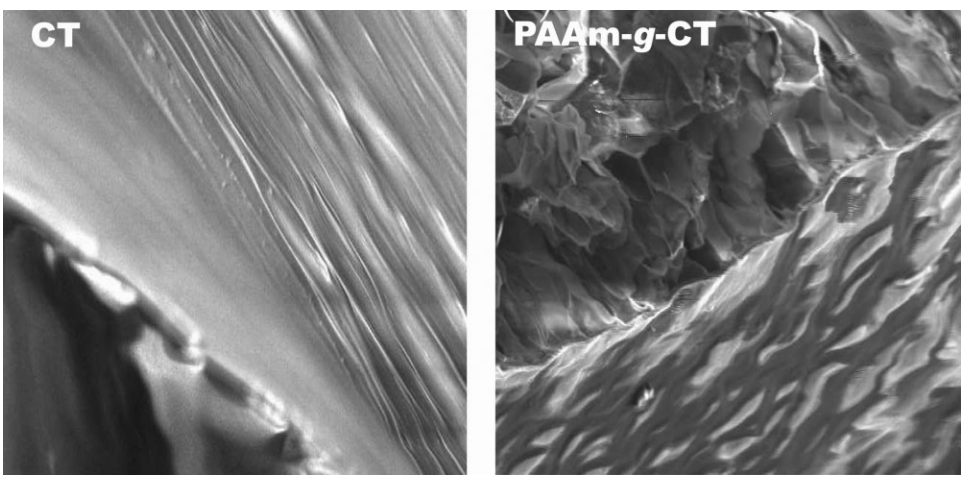

Figure 3 Cross-sectional SEM micrographs of CT and PAAm-*g*-CT hydrogels (magnification, 500×).

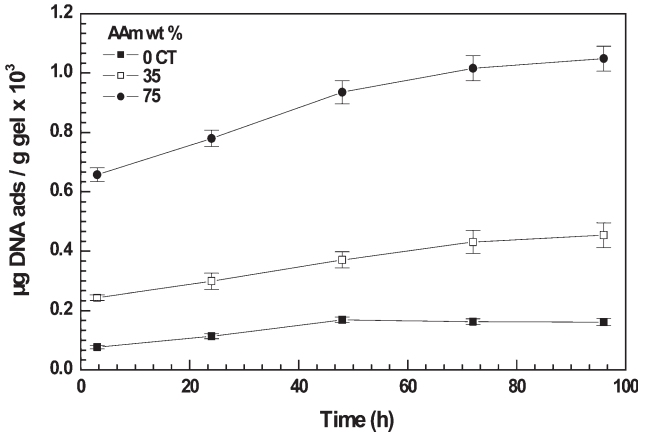

Figure 4 DNA adsorption kinetics of the CT and PAAm-*g*-CT hydrogels (initial DNA concentration = 13.7 µg/mL, pH = 7.4, temperature = +4°C).

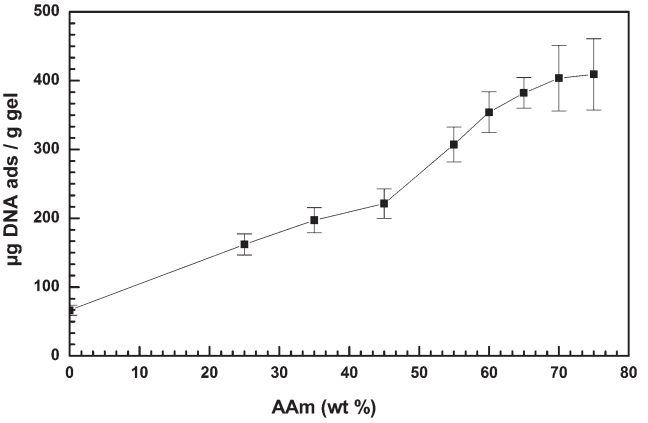

Figure 6 Effect of the PAAm content on the equilibrium DNA adsorption capacity of the PAAm-*g*-CT hydrogels (pH = 7.4, temperature = +4°C).

the sorbent hydrogels), the driving force (i.e., the concentration difference between the two respective phase) decreased, and this in turn resulted in a drop in the adsorption rate.[13,14]

Effect of the initial DNA concentration

The effect of the initial DNA concentration on the equilibrium adsorption capacity of the CT and PAAm-*g*-CT hydrogels was studied in DNA solutions with a pH 7.4 TE buffer at +4°C. The initial DNA concentration was changed between 2.0 and 30 µg/mL. The variation of the equilibrium DNA adsorption capacity with the initial DNA concentration is given in Figure 5. In all the DNA solutions, the equilibrium adsorption capacity of the hydrogels increased almost linearly with increasing DNA concentration. In all the DNA solutions, the comb-type grafted hydrogels had a larger adsorption capacity than the CT hydrogels. For example, the adsorption capacity of the CT hydrogel was about 100 µg of DNA/g of dry gel in a 7.5 µg/mL DNA solution or 300 µg of DNA/g of dry gel in a 30 µg/mL DNA solution, whereas the adsorption capacity of the hydrogel with 75 wt % AAm was about 400 and 1800 µg of DNA/g of dry gel in the same DNA solutions. In this case, the high adsorption capacity for the comb-type hydrogels may be attributed to the increase in the number of grafting chains with $-NH_3^+$.

Effect of the PAAm content

The equilibrium DNA adsorption capacity of the PAAm grafted hydrogels fabricated with different PAAm–COOH/CT feed ratios was determined in a 5.8 µg/mL DNA solution with a pH 7.4 TE buffer at +4°C for 24 h and is given in Figure 6. The

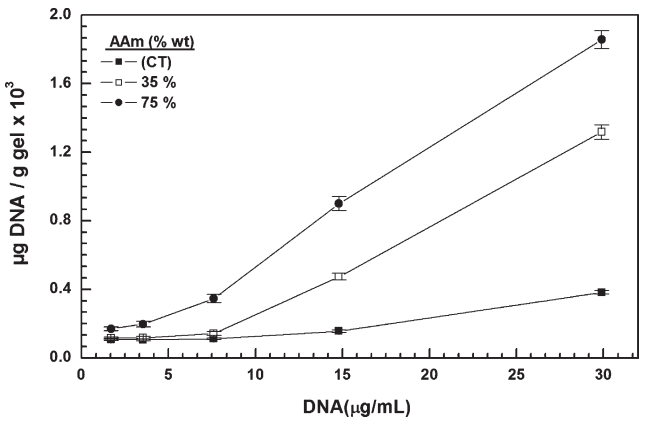

Figure 5 Effect of the initial DNA concentration on the equilibrium adsorption capacity of the CT and PAAm-*g*-CT hydrogels (pH = 7.4, temperature = +4°C).

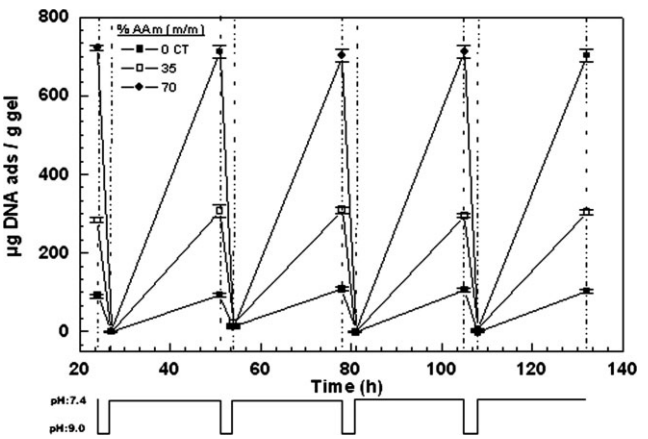

Figure 7 Pulsatile pH-dependent DNA adsorption/desorption behaviors of the CT and PAAm-*g*-CT hydrogels at +4°C.

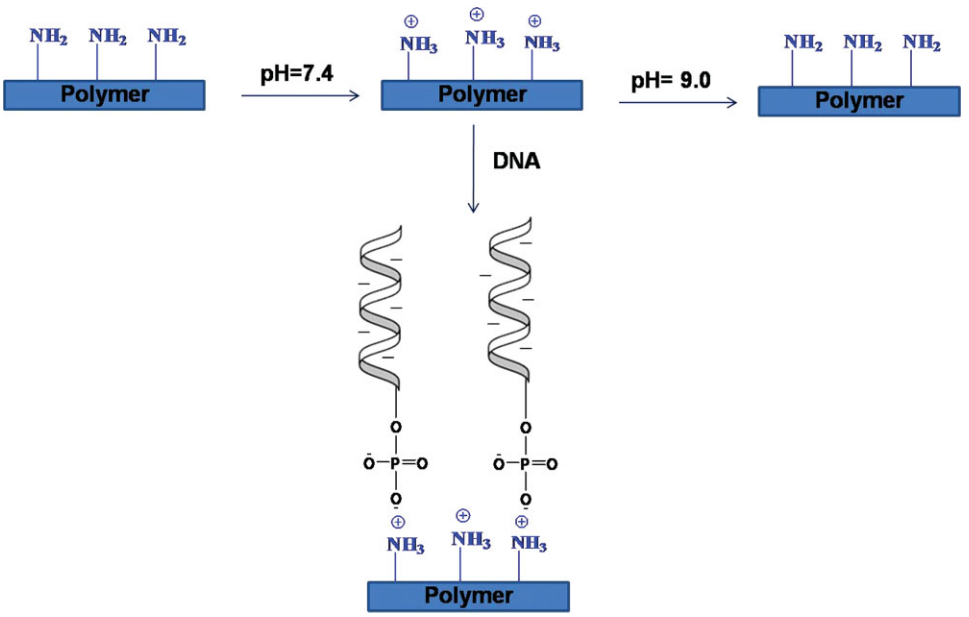

Figure 8 Schematic view of the interaction between the PAAm-*g*-CT hydrogel and DNA molecules at different pHs. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

equilibrium adsorption capacity of the PAAm-*g*-CT hydrogels increased almost linearly with increasing PAAm content in the hydrogel. The highest equilibrium DNA adsorption capacity was obtained by the hydrogel with 75 wt % AAm. On the other hand, the equilibrium DNA adsorption capacity of the ungrafted CT hydrogel was very low. The introduction of PAAm into the CT gel structure resulted in an appreciable increase in the equilibrium DNA adsorption capacity. This increase should be due to the complexation taking place between the phosphorus-containing groups of DNA and the amide groups of grafting chains in the protonated form.

Pulsatile DNA adsorption/desorption behavior of the hydrogels

Figure 7 shows the pulsatile adsorption/desorption behavior of the hydrogels at +4°C with DNA solution pH values alternating between 7.4 and 9.0. The amount of DNA adsorption was also measured in 24-h steps. The adsorption/desorption behavior of the PAAm-*g*-CT hydrogels responded to pH change more rapidly than that of the CT hydrogel, as shown in Figure 7. Moreover, the adsorption/desorption process proved to be repeatable and rapidly responded to changes in the pH. When the complex formation between phosphorus-containing groups of DNA and amino and/or amide groups was considered, the PAAm-*g*-CT hydrogel could adsorb DNA molecules at pH 7.4 because of the protonated amino and/or amide groups and desorb them at pH 9.0 because of the unprotonated amino and/or amide groups (Fig. 8). As a result, the PAAm-*g*-CT hydrogels carrying larger amounts of DNA may be considered good candidates for achieving higher removal rates for anti-DNA antibodies and for effective gene therapy systems.

CONCLUSIONS

To prepare comb-type grafted hydrogels composed of CT and PAAm, semitelechelic PAAm with a carboxyl end group was synthesized by free-radical polymerization and grafted with CT amino groups instead of direct grafting of the AAm monomer onto the CT chain. The synthesis of telechelic PAAm and its grafting onto CT were confirmed by ATR–FTIR and SEM measurements. By considering the complex formation between phosphorus-containing groups of DNA and amino and/or amide groups, we used the comb-type hydrogels as sorbents for DNA adsorption. Results from the adsorption revealed that the equilibrium DNA adsorption capacity of the comb-type hydrogels was higher than that of CT alone. The stepwise adsorption/desorption behavior confirmed that the adsorption/desorption process was repeatable, in accordance with the pH changes. As a result, comb-type hydrogels carrying larger amounts of DNA may be used for achieving higher removal rates for anti-DNA antibodies and for effective gene therapy applications.

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