Synthesis of Hydroxyethylcellulose-*graft*-Poly(*N, N*-dimethylacrylamide) Copolymer by ATRP and as Dynamic Coating in Capillary Electrophoresis

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ABSTRACT: Hydroxyethylcellulose-*graft*-poly (*N, N*-dimethylacrylamide) was synthesized by successive atom transfer radical polymerization (ATRP) of *N,N*-dimethylacrylamide (DMA) monomer using HEC-Br as initiator, CuBr and 5,5,7,12,12,14-hexamethyl-1,4,8,11-tetraazamacrocyclotetradecane (Me_6[14]aneN_4) as catalyst and ligand, with molar ratio DMA: HEC-Br (C—Br): CuBr: Me_6[14]aneN_4 = 100 : 1 : 1 : 3. HEC–Br macroinitiator was synthesized by esterification of HEC with 2-bromoisobutyryl bromide. GPC and ^{1}H NMR studies show that the molecular weight of the resulting PDMA increased linearly with the conversion. Within 6 h, the polymerization can reach almost 60% of conversion. The copolymer is applied for the separation of basic proteins in capillary electrophoresis. The results show that this medium has a powerful capability in resisting basic proteins adsorption because the polymer forms noncovalent coating in silica capillaries. With a broad range of pH 2–7, proteins were separated with sufficient efficiencies above 200,000 plates/m. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 116: 3468–3472, 2010

Key words: atom transfer radical polymerization; graft copolymers; hydroxyethylcellulose; separation techniques; surface modification

INTRODUCTION

Water-soluble macromolecules have attracted much attention because of their applications in medicine and biotechnology. Hydroxyethylcellulose (HEC) and poly (*N, N*-dimethylacrylamide) (PDMA) are two of the most frequently investigated classes of hydrophilic polymer, which have many potential applications.[1,2] The properties of aqueous solutions of hydroxyethyl cellulose (HEC) can be controlled by modifying the polymer backbone with side chain attachments, such as grafting hydrophobic groups or grafting polymer. In general, modifications are tailored to cause an increase in property that improves the use of the polymer in food, cosmetic products, waterbased paints, and other industrial applications. Hydroxyethylcellulose-*graft*-poly (*N, N*-dimethylacrylamide) (HEC-*g*-PDMA) is an efficient multifunction separation medium for biomolecules by capillary electrophoresis (CE).[3] To optimize the separation performance, the effect of copolymer composition is important to tailor the separation in CE. The conventional synthesis method using initiator in aqueous solution is difficult to characterize the copolymer, such as the grafting density and grafting length. The recent development of controlled/'living' radical polymerizations, especially atom transfer radical polymerization (ATRP), has opened a new route to synthesize functional and architectural polymers with well-defined structures.[4] This method has more advantages than other conventional polymerization method. In 2002, Carlmark and Malmstrom first grafted methyl acrylate (MA) onto cellulose fibers with controlled molecular weight by using ATRP conditions.[5,6] Their research works gave us a new idea to synthesize the graft copolymer as the protein separation medium by using ATRP. Our group also successive synthesized hydroxyethylcellulose-*graft*-polyacrylamide by using ATRP.[7] Although both *N, N*-dimethylacrylamide (DMA) and PDMA are strong coordinating ligands that can compete for the binding of the metal catalysts in polymerization system, PDMA can be successfully polymerized with the molecular weight distributions.[8–11] The ligand is very important for controlled radical polymerization of DMA.

In this article, we synthesized graft copolymer HEC-*g*-PDMA by using ATRP. First, a HEC–Br macroinitiator for ATRP was synthesized by reacting HEC with 2-bromoisobutyryl bromide. Then DMA was grafted from the HEC–Br macroinitiator in the presence of catalyst and ligand. We select a macrocyclotertraamine (Me_6[14]aneN_4), which is synthesized easily,[12] as ligand in the ATRP of HEC-*g*-PDMA, and investigate

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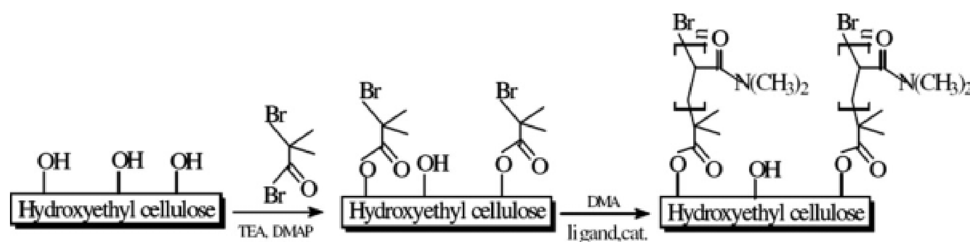

Figure 1 Formation of HEC-*g*-PDMA.

the nature of polymerization. HEC-*g*-PDMA with well defined structures was applied as a coating for the separation basic proteins. The results of the HEC-*g*-PDMA coating in protein separation runs have demonstrated the suitability of the coating for electrophoretic protein separations.

EXPERIMENTAL

Materials

Hydroxyethylcellulose (QP-300, Dow Chemical Company, US, brookfield viscosity: 300–400 mPa s, 2% water solution, 25°C) was dried at 50°C in a vacuum with P_2O_5 for 24 h, 2-bromoisobutyryl bromide (98%) was used as received from Aldrich. N, N-dimethylacrylamide (DMA) was purchased from Aldrich Chemical Company (Milwaukee, WI) and distilled under reduced pressure before use. CuBr (Sinopharm Chemical Reagent Co., Ltd) was purified by stirring in acetic acid, washing with ethanol, and then drying under vacuum. 2-dimethylaminoprydine (DMAP), triethylamine (TEA) and $CuBr_2$ (Sinopharm Chemical Reagent Co.) were purified before used. $Me_6[14]aneN_4$ was synthesized according to our previous works.[12]

Synthesis of macroinitiator

In a 100 mL three-neck flask, 8.6 g (34.5mmol hydroxyl-group) hydroxyl-ethylcellulose was dissolved in DMF/THF (50 mL, 10/90, v/v). After stirring for 0.5 h, 0.22 g TEA and a catalytic amount of DMAP were added. The flask was then cooled in water/ice bath. Then 0.46 g 2-bromoisobutyryl bromide was diluted in 5 mL THF and added dropwise to the solution while stirring. The reaction proceeded at 25°C for 12 h. The residue was filtered and thoroughly washed with THF, dried under vacuum at 40°C.

General procedure for polymerization from macroinitiator

The graft copolymer HEC-*g*-PDMA was synthesized by ATRP of DMA with the molar ratio of DMA: macroinitiator (C-Br): CuBr: $Me_6[14]aneN_4 = 100 : 1 : 1 : 3$. Into a dried glass tube with a magnetic bar, $CuBr/CuBr_2$ (10/1) and ligand were added, then monomer, macroinitiator and DMF/Toulene (10/90, v/v) solvent were added. The mixture was degassed by three freeze-pump-thaw cycles. The tube was sealed under vacuum, and then immersed in a water bath stated at 30°C. After the reaction was carried out for a prescribed time, a small amount of sample was taken out, the conversion was measured based on ^1H NMR data. The crude copolymers were placed in the extraction thimber. The soxhlet extraction was run for 24 h in methanol, which is a good solvent for PDMA block, to remove any ungrated PDMA. The polymer was filtered and dried in a vacuum for 24 h at 50°C. The synthesis route is displayed in Figure 1.

Characterization

^1H NMR spectra were performed in an AV-300 NMR spectrometer, using D_2O as solvents. The IR spectra were recorded in solid state using KBr pellet. A MAGNA-IR 750 (Nicolet Instrument Co.) was used to record the spectra in the range of 400–4000 cm^{-1}. Molecular weights and molecular weight distributions were determined by gel permeation chromatography (GPC) equipped with a Waters 1515 pump and Waters 2414 differential refractive index detector (set at 30°C). It used a series of three linear Styragel columns HT2, HT4, and HT5 at an oven temperature of 35°C. The eluent was DMF at a flow rate of 1.0 mL/min. A series of low polydispersity polystyrene (PS) standards were employed for the GPC calibration.

Separation of basic proteins

Analyses of basic proteins were carried out on a P/ACE MDQ system equipped with a UV–Vis detector working at 214 nm and 25°C. Polymer solutions were prepared by dissolving the polymers in deionized water to the desired concentrations (i.e., 0.2% w/v). The capillary was treated prior to its first use by flushing with 0.1 M NaOH for 10 min, and then with water for another 10 min. A very simple coating strategy was used: rinsing the capillary at 20.0 psi with the above diluted polymer solution for 10 min and then standing with the polymer solution for another 10 min and next replacing this solution by flushing the

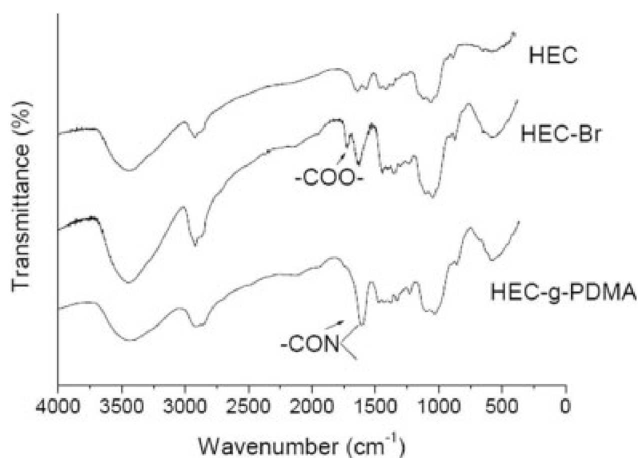

Figure 2 FTIR spectra of (a) HEC, (b) HEC–Br, and (c) HEC-*g*-PDMA.

capillary with the separation buffer for 2 min. Protein samples (cytochrome *c*, lysozyme and ribonuclease A, 0.5 mg/mL) were injected for 5.0 s with 0.5 psi. The applied voltage was 20 kV. Separations were all performed in phosphate-citrate buffers. The bare fused-silica capillaries with id/od of 75/365 mm and effective/total length of 30/40 cm were used without any coating in our experiments.[13–16]

RESULTS AND DISCUSSION

Synthesis of macroinitiator and HEC-g-PDMA copolymer

The IR spectra of HEC, HEC-Br, and HEC-*g*-PDMA are shown in Figure 2. From the IR spectra, the peak at 1064 cm^{-1} is indicative of C—O absorption of HEC. HEC-Br in Figure 2 revealed the C–Br stretching bands at 570 cm^{-1}, further confirming the presence of Br moieties with a peak at 1600 cm^{-1} and a peak around 1730 cm^{-1} which are not present in the HEC. These peaks originate from the carbonyl in the bromoester group. Figure 2 also shows the IR spectrum of HEC-*g*-PDMA. The —N stretching which appeared as a shoulder band around, 3200 cm^{-1} of the graft copolymer, has overlapped with a broad peak 3420 cm^{-1} of the hydroxyl group. The key band at 1634 cm^{-1} is because of the carbonyl group of the grafted PDMA chain, the presence of the above bands in the graft copolymer gives strong evidence of grafting.

The ^{1}H NMR spectrum of HEC-Br is shown in Figure 3. The peaks at $\delta = 2.1$ (b′) are ascribed to methyl protons of Br-C-CH_3. The peaks at $\delta = 3.0$–3.8 (a′) are corresponding to methylene and methine of HEC. From their integration ratio of peaks b′ and a′, the number of grafting point can be calculated. HEC-*g*-PDMA can be verified by its ^{1}H NMR spectrum shown in Figure 3. The resonances labelled c ($\delta = 1.4$–1.6 ppm) represent the methylene protons of PDMA, that labelled d ($\delta = 2.6$ ppm) is ascribed to methine protons of PDMA, and those labelled e ($\delta =$ 2.9–3.1 ppm) correspond to methyl protons of PDMA. The integration ratio of peaks c and e is 1 : 3 that indicates the formation of PDMA. Peaks labelled b ($\delta =$ 3.2–3.7 ppm) represent the methylene and methine of HEC. The peaks at $\delta = 2.1$ (a) are ascribed to methyl protons of C—CH_3. Assumed that all HEC-Br chains are initiating DMA, the number-average molecular weight M_n (NMR) can be calculated from the integration ratio of peaks c and a. The ^{1}H NMR analysis that HEC-*g*-PDMA was consistent with a PDMA graft length of different segments and one PDMA chain per 20 repeating units of backbone chain. The presence of the above peaks in the copolymer gives strong evidence of grafting.

Polymers synthesized for this study were characterized by GPC to determine polydispersity index (PDI) of each sample. The representative GPC of the HEC and HEC-*g*-PDMA shown in Table I. The curves are symmetrical and do not exist double peaks, which illustrated the homopolymer was initially removed from the reaction products. The molecular weights of the resulting PDMA increased linearly with conversion as shown in Table I, therefore the molecular weight can be controlled by the initial feed ratio of DMA to HEC-Br and conversion. After the fast polymerization, the polymerization slowed down and the PDI slightly increases. Probable reasons may be due to too much irreversible termination reactions between primary and chain radicals. Another reason may be the decrease of the concentration of DMA. NMR, IR and GPC data are supportive of the grafting reaction between DMA and

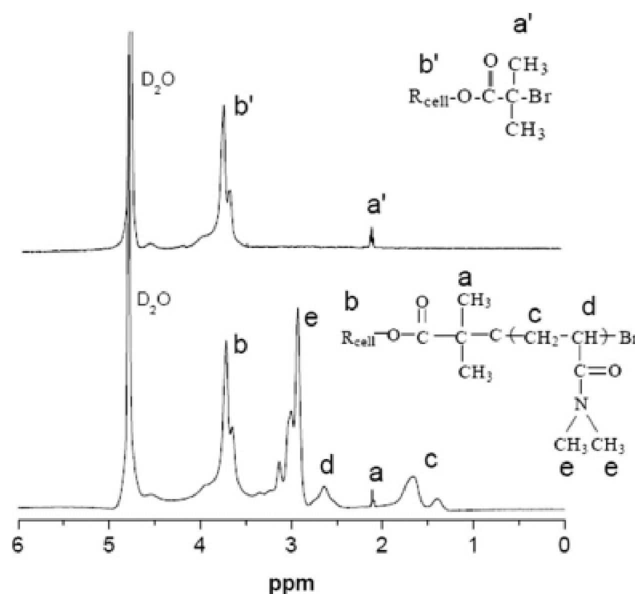

Figure 3 1H NMR spectra of HEC-Br and HEC-*g*-PDMA.

TABLE I
Graft Copolymerization of Hydroxyethylcellulose and *N, N*-dimethylacrylamide[a]

No.	Time (h)	Conversion (%)	$\bar{M}_n$ of PDMA[b] ($\times 10^{-4}$)	$\bar{M}_n$ of graft copolymer[c] ($\times 10^{-5}$)	PDI (M_w/M_n)[d]
HEC	0	0	0	2.5	2.90
HEC-*g*-PDMA-1	1	14.2	7	3.2	3.42
HEC-*g*-PDMA-2	2	23.4	12	3.7	3.86
HEC-*g*-PDMA-3	4	48.4	25	5.0	3.81
HEC-*g*-PDMA-4	6	61.5	31	5.6	4.17

[a] DMA: HEC-Br(C-Br): CuBr: $Me_6[14]aneN_4$ = 100 : 1 : 1 : 3 (molar ratio), Temperature: 30°C.
[b] Molecular weight of PDMA calculated from ^{1}H NMR.
[c] Molecular weight of HEC is about 2.5×10^{-5} g/mol (data supply from Dow Chemical Company).
[d] Molecular weight distributions were obtained using GPC.

HEC. The HEC-*g*-PDMA copolymers have been successfully synthesized. The results are listed in Table I, which indicate control over the polymerization.

Separation of basic proteins with HEC-*g*-PDMA coating

Our previous work[3] has proved that HEC-*g*-PDMA is an efficient noncovalent coating for basic protein separation. Thus, the separation performance of three different pHs was investigated here by using HEC-*g*-PDMA-3 coating capillary. Figure 4 showed protein separation results in pH 2.2, 4.6, and 6.0. As shown, highly efficient protein separation was obtained at this pH range. At these conditions, proteins were separated with sufficient efficiencies above 200,000 plates/m. Previous works[17] proved that neutral polymer adsorption on silica may involve two types of interactions: (1) hydrogen bonding between hydrogen bond-forming groups on the polymer and the silanol groups on the silica surface, and (2) hydrophobic interactions between polymer segments and the hydrophobic siloxane groups of the skeleton structure of the silica surface. The copolymer will have a greater tendency to adsorb on the wall because of the rather hydrophobic property of PDMA.[18] We found that the HEC-*g*-PDMA coating of the capillary give a stable surface film with a broad working range of pH 2–7. The polymer efficiently coated the capillary inner surface by a dynamic process, thereby leading to high efficiency, reproducibility, and recovery of basic proteins analyzed by CE. The separations of basic proteins can't be achieved under identical conditions using a bare fused-silica capillary at pH 4.6 and pH 6.0. The results shown that the adsorption phenomenon between the proteins (positively charged) and the capillary wall (negatively charged) is so strong that leads to the analysis of protein mixtures using the bare silica capillary is impossible.[13]

CONCLUSIONS

The ATRP of DMA with HEC-Br has been successfully carried out. The nature of the polymerization is evidenced by evolution of molecular weight with conversion. NMR, IR and GPC data are supportive of the grafting reaction between DMA and HEC. The HEC-*g*-PDMA copolymers have been successfully synthesized. The separation of proteins with pH ranging from 2.2 to 6.0 was successful by using HEC-*g*-PDMA copolymer, with the advantages of high efficiency. The experimental results indicate that HEC-*g*-PDMA coating capillary has great

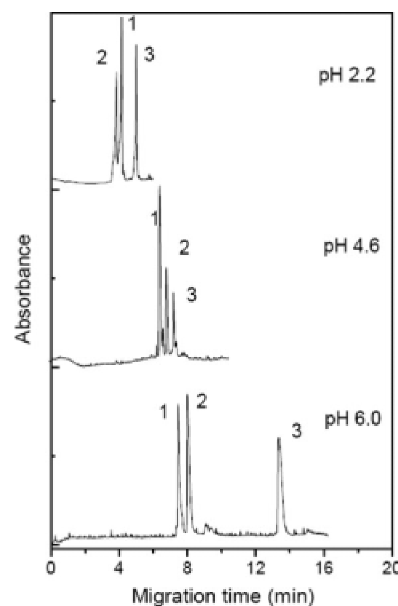

Figure 4 Effect of buffer pH on the separation of basic proteins. Separation buffer: phosphate-citrate of pH 2.2, pH 4.6 and pH 6.0, separations were taken in HEC-g-PDMA coated capillary. Conditions: separation voltage, 20 kV; temperature, 25°C; detection, UV 214 nm; injection, 0.5 psi for 5 s; polymer concentration, 0.2% w/v. Sample: 0.5 mg/mL proteins mixture, peak identification: 1, lysozyme; 2, cytochrome *c*; 3, ribonuclease A. Capillary, bare fused-silica of 40 cm total length (30 cm to the detector) ×75 μm id.

potential for use in proteomics applications. Ongoing research in our laboratory is investigating the influence of the length of group chain and the potential use of HEC-*g*-PDMA for other types of biomolecular separations.

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References

1. Gilges, M.; Kleemiss, M. H.; Schomburg, G. Anal Chem 1994, 66, 2038.
2. Fischer, A.; Brembilla, A.; Lochon, P. Eur Polym J 2001, 37, 33.
3. Peng, S. H.; Shi, R. H.; Yang, R. M.; Zhou, D.; Wang, Y. M. Electrophoresis 2008, 29, 4351.
4. Wang, J. S.; Matyjaszewski, K. Macromolecules 1995, 28, 7901.
5. Carlmark, A.; Malmstrom, E. Biomacromolecules 2003, 4, 1740.
6. Carlmark, A.; Malmstrom, E. J Am Chem Soc 2002, 124, 900.
7. Yang, R. M.; Wang, Y. M.; Zhou, D. Electrophoresis 2007, 28, 3223.
8. Senoo, M.; Kotani, Y.; Kamigaito, M.; Sawamoto, M. Macromolecules 1999, 32, 8005.
9. Teodorescu, M.; Matyjaszewski, K. Macromol Rapid Commun 2000, 21, 190.
10. Neugebauer, D.; Matyjaszewski, K. Macromolecules 2003, 36, 2598.
11. Ding, S. J.; Radosz, M.; Shen, Y. Q. Macromol Rapid Commun 2004, 25, 632.
12. Yang, R. M.; Wang, Y. M.; Wang, X. G.; He, W. D.; Pan, C. Y. Eur Polym J 2003, 39, 2029.
13. Yang, R. M.; Shi, R. H.; Peng, S. H.; Zhou, D.; Liu, H.; Wang, Y. M. Electrophoresis 2008, 29, 1460.
14. Cordova, E.; Gao, J.; Whitesides, G. M. Anal Chem 1997, 69, 1370.
15. Erny, G. L.; González, N.; Elvira, C.; San, J.; Cifuentes, A. Electrophoresis 2006, 27, 1041.
16. Kato, M.; Imamura, E.; Sakai-Kato, K.; Kato, M.; Nakajima, T.; Toyo'oka, T. Electrophoresis 2006, 27, 1895.
17. Tanahashi, T.; Kawaguchi, M.; Honda, T.; Takahashi, A. Macromolecules 1994, 27, 606.
18. Doherty, E. A. S.; Meagher, R. J.; Albarghouthi, M. N.; Barron, A. E. Electrophoresis 2003, 24, 34.