ELSEVIER

International Journal of Pharmaceutics 205 (2000) 109–116

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Self-assembled hydrogel nanoparticles composed of dextran and poly(ethylene glycol) macromer

In-Sook Kim [a], Young-Il Jeong [b], Sung-Ho Kim [a,*]

[a] *College of Pharmacy, Chosun University, # 375 Seosuk-dong, Dong-gu, Kwangju 501-759, South Korea*
[b] *Department of Pharmacokinetics, Kyoto Pharmaceutical University, Yamashina-ku, Kyoto 607-8414, Japan*

Received 31 December 1999; received in revised form 12 June 2000; accepted 15 June 2000

Abstract

Biodegradable hydrogel nanoparticles were prepared from glycidyl methacrylate dextran (GMD) and dimethacrylate poly(ethylene glycol) (DMP). GMD was synthesized by coupling of glycidyl methacrylate to dextran in the presence of 4-(*N*,*N*-dimethylamino)pyridine (DMAP) using dimethylsulfoxide (DMSO) as an aprotic solvent. DMP was synthesized from poly(ethylene glycol) (PEG) and methacryloyl chloride. GMD/DMP (abbreviated as DP) hydrogel was prepared by radical polymerization of GMD and DMP using ammonium peroxydisulfate (APS) as an initiator and UV curing. DP hydrogel nanoparticles were obtained by diafiltration method using DMSO solution. The GMD and DMP were characterized by fourier transform infrared spectroscopy. Fluorescence probe technique was used to investigate the self-assembly of DP in water using pyrene as a hydrophobic probe. The critical association concentration (CAC) was determined to be 5.6×10^{-2} g/l. The shape of DP hydrogel nanoparticles was spherical when observed by transmission electron microscope (TEM). The size range of DP hydrogel nanoparticles was about $20 \sim 50$ nm. The hydrodynamic size of DP hydrogel nanoparticles was measured by photon correlation spectroscopy (PCS) and gradually increased with time in PBS (0.1 M, pH 7.4). Drug release study was performed using clonazepam (CNZ) as a hydrophobic model drug. In vitro release rate of CNZ from the DP hydrogel nanoparticles was dependent on the existence of dextranase and the pH of the release medium.

Keywords: Self-assembly; Hydrogel; Nanoparticle; Clonazepam; In vitro release

1. Introduction

Drug delivery systems can be used to achieve a sustained release of a drug, to alter the biodistribution, and to obtain triggered or pulsed release of a drug. A number of drug delivery systems are presently under investigation including liposomes (Crommelin and Schreier, 1994), polymeric nanoparticles and microspheres (Coevreur and Puisieux, 1993; Brannon-Peppas, 1995), implants made from biodegradable polymers, and hydrogels. Biodegradable polymeric systems for controlled release drug delivery have been extensively studied because an invasive technique such as

* Corresponding author. Tel.: +82-62-2306379; fax: +82-62-2225414.
E-mail address: shkim@mail.chosun.ac.kr (S.-H. Kim).

0378-5173/00/$ - see front matter
PII: S0378-5173(00)00486-5

surgery is not required after their use (Heller, 1993; Okano et al., 1994). Especially, biodegradable hydrogels have been represented as an attractive drug formulation because of their advantages such as biocompatibility, high responsibility for specific degradation, and a feasible approach to incorporate drugs into matrices.

Dextran is a polysaccharide consisting of glucose molecules coupled into long branched chains, mainly through a 1,6- and some through a 1,3-glucosidic linkages. Dextrans are colloidal, hydrophilic and water-soluble substances, inert in biological systems and do not affect cell viability. Because of these properties, dextrans have been used for many years as blood expanders to maintain or replace blood volume, and studied for use as a carrier system for a variety of therapeutic agents including antidiabetics, antibiotics, anticancer drugs, peptides and enzymes (Molteni, 1979; Poznansky and Cleland, 1980). Dextrans can be degraded by the dextranase which was found to be present in the colon (Sery and Hehre, 1956). Taking advantage of these enzymes, polymeric prodrugs for colonic drug delivery based on dextran were previously designed (Larsen et al., 1989). It was concluded that the dextran molecule was degraded by microbial dextranases making the ester bond accessible to hydrolysis, releasing drugs.

In this study, we synthesized GMD and DMP, and prepared hydrogel nanoparticles with DP. The hydrogel nanoparticles can complex various hydrophobic substances, including soluble proteins or enzymes (Nishikawa et al., 1994; Akiyoshi et al., 1998). We have investigated the physicochemical properties of DP hydrogel nanoparticles and the feasibility as drug carrier was studied with hydrophobic model drug in vitro.

2. Materials and methods

2.1. Materials

Dextran from *Leuconostoc mesenteroides* with average molecular weights of 70 000 PEG with average molecular weight of 8000, dextranase from *Penicillium sp*. (EC 3.2.1.11, 3.6 U/mg solid) were purchased from Sigma (St. Louis, MO). APS and glycidyl methacrylate were purchased from Fluka AG (Buchs, Switzerland). Methacryloyl chloride and DMAP were purchased from Aldrich Chemical Co. Inc. Clonazepam (CNZ) was obtained from Roche (Switzerland). All other chemicals were reagent grade or above, and used without further purification.

2.2. Synthesis of GMP and DMP

GMD was synthesized by the method reported previously (Van Dijk-Wolthuis et al., 1995). Briefly, dextran (50.0 g) was dissolved in 450 ml of dimethylsulfoxide (DMSO) under nitrogen stream. After dissolving 10 g of DMAP, a calculated amount of glycidyl methacrylate was added. The solution was stirred at room temperature for 48 h, and the reaction was stopped by adding an equimolar amount of concentrated HCl to neutralize the DMAP. The reaction mixture was transferred to a dialysis tube (molecular weight cutoff (MWCO) 12 000 g/mol) and extensively dialyzed for 2 weeks against distilled water at 4°C. GMD was lyophilized to obtain white fluffy product and was stored at -20°C until use.

Purified PEG (2×10^{-3} mol) was dissolved in 150 ml of benzene in a 500 ml round bottom flask and cooled to 0°C. DMP was synthesized by the method we reported previously (Kim et al., 1996). Methacryloyl chloride (90.57 ml) and triethylamine (0.49 ml) were added to the flask and stirred for 3 h at 80°C. The reaction mixture was filtered to remove triethylamine hydrochloride, and the macromer DMP was obtained by pouring the filtrate into an excess of *n*-hexane. Finally, it was dried at 40°C under reduced pressure for a day.

2.3. Synthesis of DP hydrogels and preparation of DP hydrogel nanoparticles

DP hydrogels were prepared by radical polymerization of GMD and DMP in DMSO solution using ammonium peroxydisulfate (APS) as an

initiator and UV curing. The DP hydrogel nanoparticles was prepared by a diafiltration method (Yokoyama et al., 1994; Kwon et al., 1995; Cho et al., 1997; Jeong et al., 1998; Kim et al., 2000). Briefly, 20 mg of DP was dissolved in 10 ml of DMSO. To form hydrogel nanoparticles, the solution was dialyzed using MWCO 12 000 g/mol dialysis tube against distilled water. The medium was replaced every 1 h for the first 3 h and every 3 h for 21 h. Then, the solution was freeze-dried.

2.4. Drug loading

To prepare CNZ-loaded DP hydrogel nanoparticles, 20 mg of DP was dissolved in 10 ml of DMSO followed by the addition of 20 mg of CNZ. After complete dissolution of the drug at room temperature, the solution was dialyzed using MWCO 12 000 g/mol dialysis tube against distilled water. The CNZ-loaded nanoparticles were obtained by freeze-drying the solution and they were kept in a refrigerator at 4°C until use.

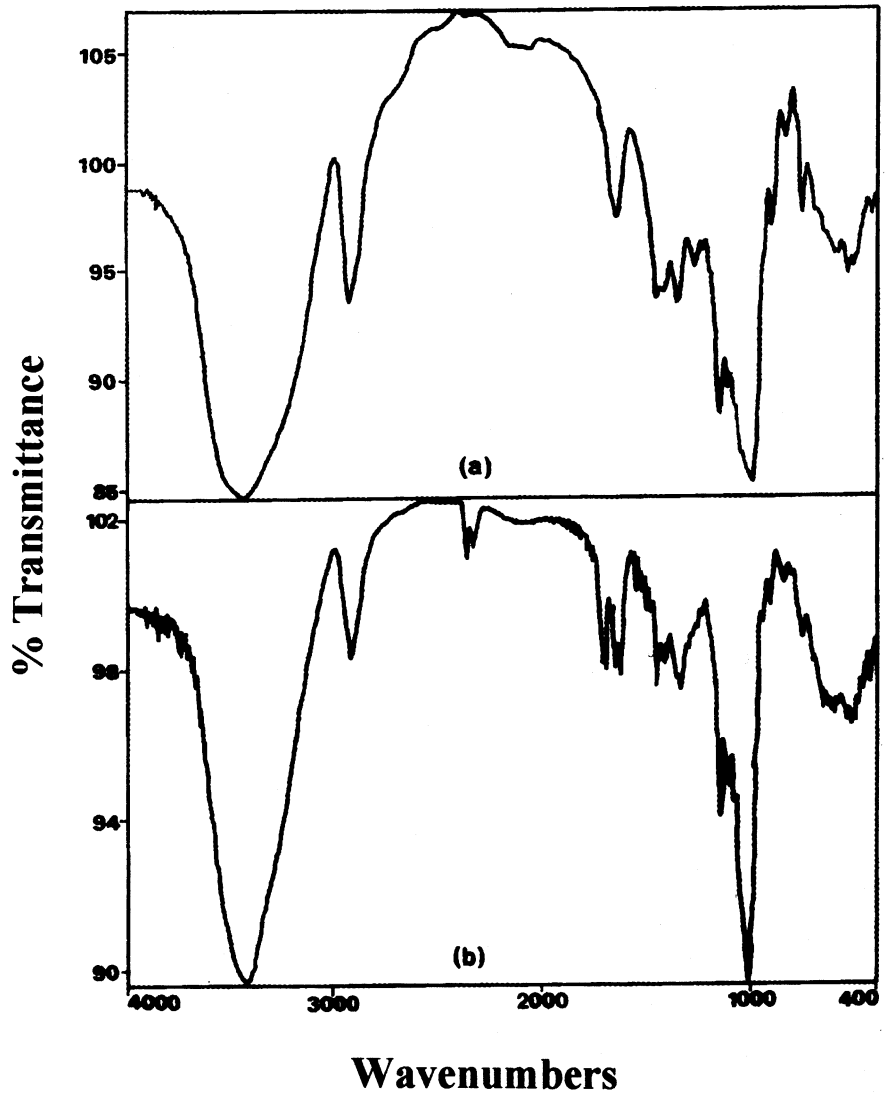

Fig. 1. FT-IR spectra of dextran (a) and GMD (b).

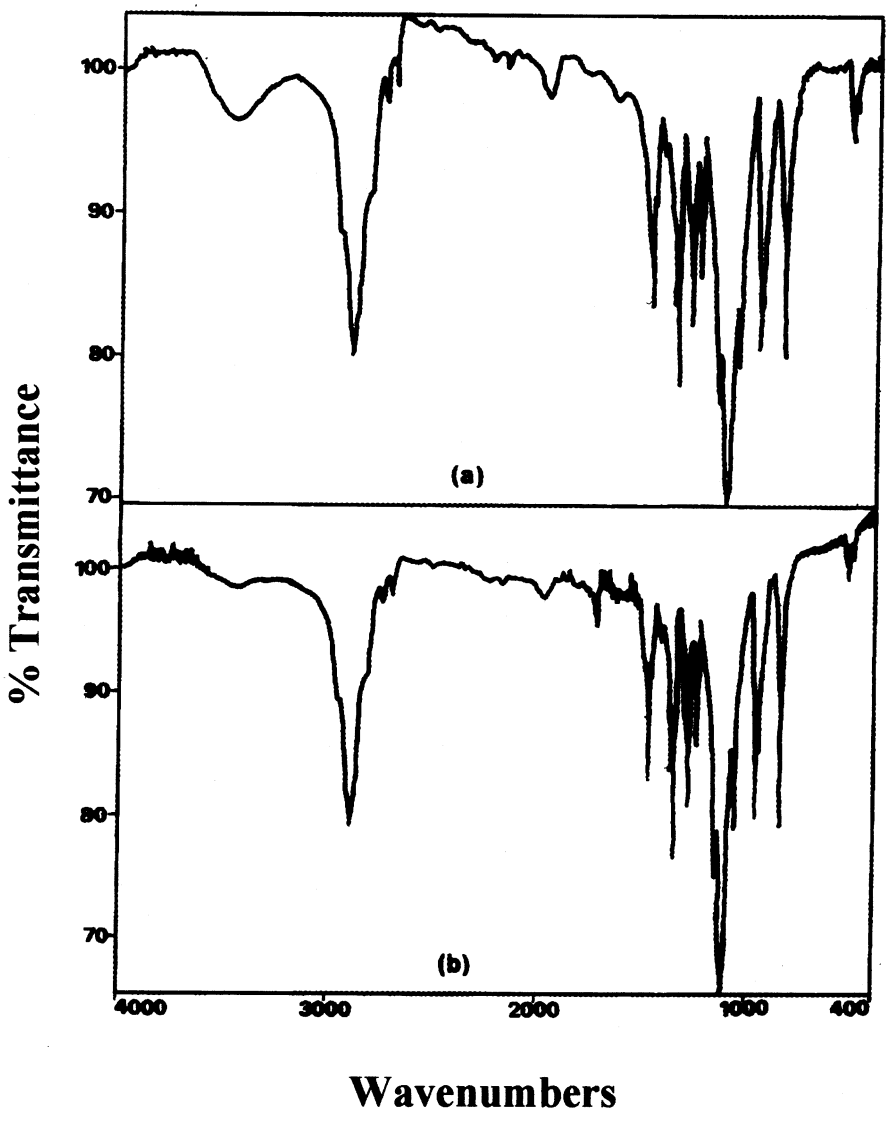

Fig. 2. FT-IR spectra of PEG (a) and DMP (b).

2.5. FT-infrared (IR) measurement

Infrared spectrum of GMD and DMP were obtained by Fourier Transform Infrared Spectroscopy (FT-IR, Nicolet, Magna IR 550).

2.6. Measurement of fluorescence spectroscopy

Critical association concentration (CAC) of the DP was studied by fluorescence spectroscopy (Shimadzu RF-5301 PC spectrofluorophotometer, Shimadzu Co. Ltd., Japan) using pyrene as a hydrophobic probe (Kalyanasundaram and Thomas, 1977; Wilhelm et al., 1991; Kim et al., 2000). Various concentrations of DP solutions were added to each vial containing 6.0×10^{-7} M of pyrene and heated for 3 h at 65°C to equilibrate the pyrene and the DP nanoparticles. The samples were left to cool overnight at room temperature. Fluorescence emission spectra were mea-

sured at excitation wavelength of 339 nm, and the excitation spectra were measured at emission wavelength of 390 nm. Excitation and emission bandwidths were 1.5 and 1.5 nm, respectively.

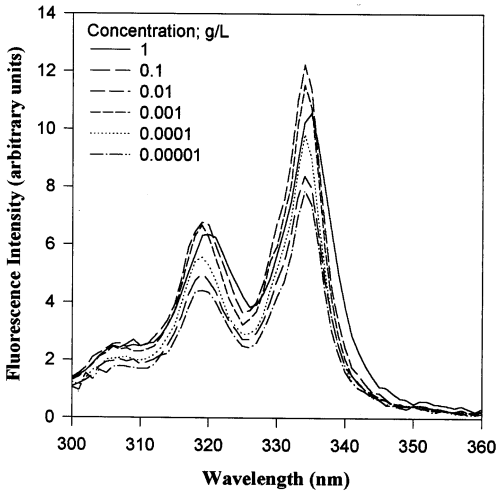

Fig. 3. Fluorescence excitation spectra of pyrene against concentration of DP hydrogel in distilled water ($\lambda_{em} = 390$ nm). [pyrene] $= 6.0 \times 10^{-7}$ M.

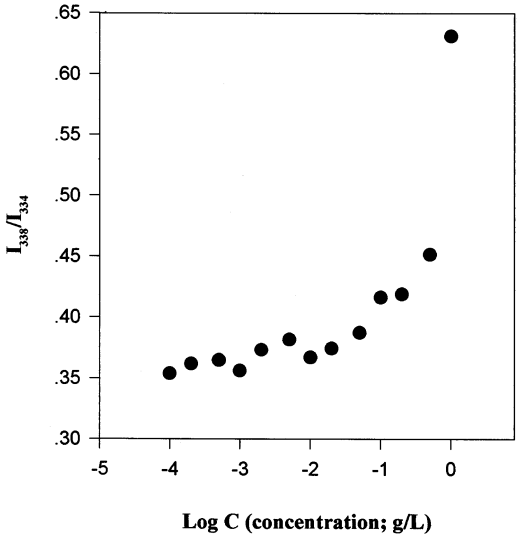

Fig. 4. Plot of intensity ratio (I_{338}/I_{334}) of pyrene excitation spectra versus log C of the DP hydrogel in distilled water ($\lambda_{em} = 390$ nm). [Pyrene] $= 6.0 \times 10^{-7}$ M.

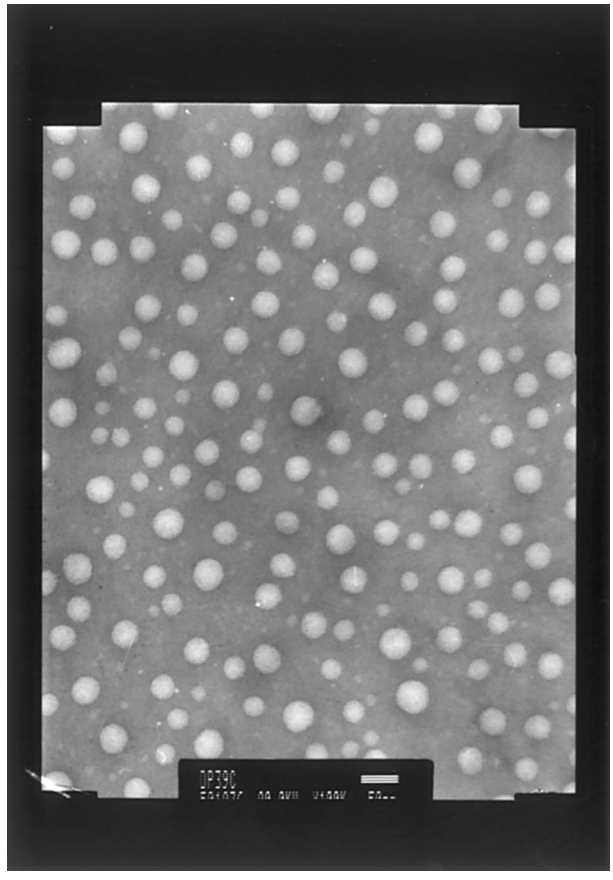

Fig. 5. Morphology of DP hydrogel nanoparticles observed by TEM.

2.7. Transmission electron microscope (TEM) observation

A drop of DP hydrogel nanoparticle suspension containing 0.01% phosphotungstic acid was placed on a copper grid coated with carbon film, and dried at 25°C. The observation was done at 80 kV with JEM-2000 FX II (Jeol, Japan).

2.8. Photon correlation spectroscopy (PCS) measurement

PCS was measured with a Zetasizer 3000 (Malvern Instruments, UK) with He–Ne laser beam at a wavelength of 633 nm at 37°C. The size changes were measured in PBS (0.1 M, pH 7.4) against swelling time at 37°C. The content of

nanoparticle in the sample suspension was 1 g/l and measured without filtering.

2.9. Drug release studies in vitro

The release experiment in vitro was carried out using a dialysis tube. The 5 mg of CNZ-loaded DP hydrogel nanoparticles and 1 ml of PBS (0.1 M, pH 7.4) were put into a dialysis tube (MWCO 12 000 g/mol). Then the dialysis tube was introduced into a vial with 10 ml PBS. The media was stirred at 100 rpm at 37°C. At predetermined time intervals, the entire medium was removed and replaced with the same amount of fresh PBS. The amount of CNZ released from DP hydrogel nanoparticles was measured with an UV spectrophotometer (UV-1201, Shimadzu, Japan) at 306 nm. CNZ release from the DP hydrogel nanoparticles was evaluated by the existence of dextranase and at different values of pH.

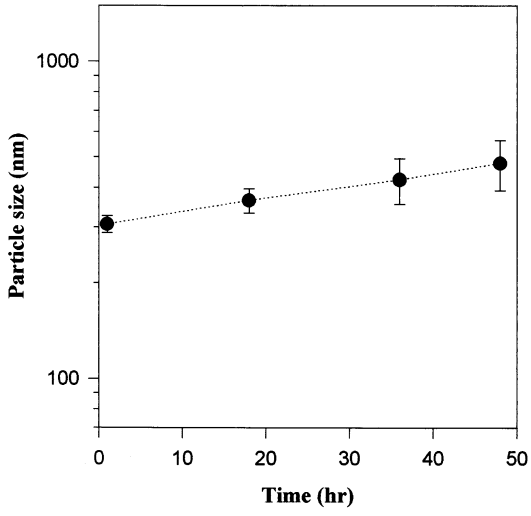

Fig. 6. Time dependence of the hydrodynamic size of DP hydrogel nanoparticles in PBS (0.1 M, pH 7.4).

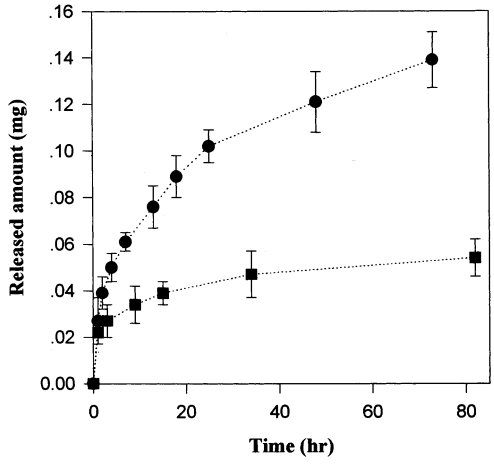

Fig. 7. CNZ release from DP hydrogel nanoparticles with (●) and without (■) dextranase in PBS (0.1 M, pH 7.4) in vitro. [Dextranase] = 0.1 U/ml.

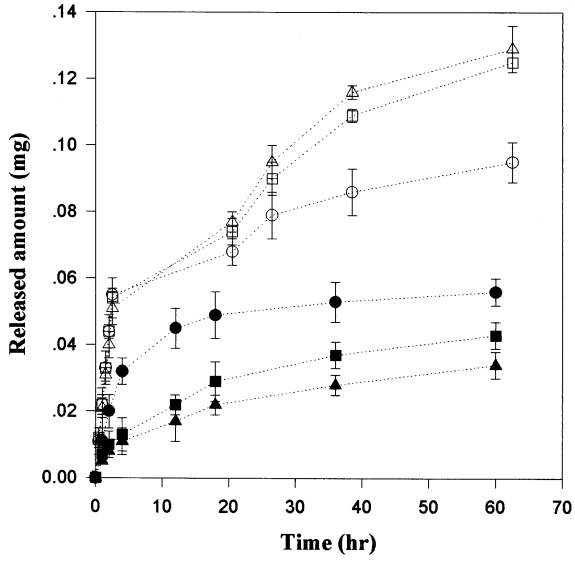

Fig. 8. CNZ release from DP hydrogel nanoparticles against various pH solutions with (○ pH 2; □ pH 4; △ pH 6) and without (●, pH 2; ■, pH 4; ▲, pH 6) dextranase. [Dextranase] = 0.1 U/ml.

3. Results and discussion

3.1. Synthesis of DP hydrogel nanoparticles

Fig. 1 shows the FT-IR spectra of dextran (a) and GMD (b) broad absorption band around 3427 cm^{-1} was observed at all spectra because of many hydroxyl groups of dextran. At 1701 cm^{-1}, a new absorption band was detected in the GMD due to the ester carbonyl group of glycidyl methacrylate. Fig. 2 shows the synthetic evidence of DMP. The FT-IR spectrum of the PEG

showed an absorption band at 3459 cm^{-1} due to the terminal hydroxyl group as shown in Fig. 2 (a) (Kalyanasundaram and Thomas, 1977; Deng et al., 1990). This band became weak in the PEG macromer due to methacrylation as shown in Fig. 2 (b). A new absorption band was seen at 1725 cm^{-1} in the DMP due to the methacryloyl group (Pavia et al., 1979). The band at 2889 cm^{-1} was attributed to the C–H stretching (Andini et al., 1988) and was present in both polymers.

3.2. Analysis of DP hydrogel nanoparticles

To investigate the self-association of DP hydrogel nanoparticles, fluorescence probe technique was used and the critical association concentration (CAC) was determined using pyrene as a hydrophobic probe as for block copolymer micelles (Kalyanasundaram and Thomas, 1977; Wilhelm et al., 1991; Marctic and Nair, 1994; Kim et al., 2000). Fig. 3 shows the fluorescence excitation spectra of pyrene at a fixed emission wavelength of 390 nm in the presence of various concentrations of DP hydrogel. A red shift was observed with increasing concentration of DP hydrogel. A plot of I_{338}/I_{334} versus log C is shown in Fig. 4. A flat region in the low concentration and sigmoidal region were noted. This result indicates that signal change in the region of 5.6×10^{-2} g/l can be evaluated to the CAC values of DP hydrogel.

Fig. 5 shows TEM photograph of the DP hydrogel nanoparticles dried at 25°C. The shapes of the DP hydrogel nanoparticles were observed as mostly spherical shape, and the range of diameter of these nanoparticles was about $20 \sim 50$ nm when dehydrated. The size of hydrated state was measured by PCS. Fig. 6 represents the time dependent hydrodynamic size of DP hydrogel nanoparticles in PBS (0.1 M, pH 7.4). The size was gradually increased with time, indicating that the DP nanoparticles possess the characteristics of hydrogel.

3.3. Drug release study

Fig. 7 shows the CNZ release from the DP hydrogel nanoparticles with and without dextranase in PBS (0.1 M, pH 7.4). The concentration of dextranase was 0.1 U/ml. CNZ was released at a higher rate with dextranase than without dextranase. This result may be attributable to the degradation of dextran portion in DP hydrogel nanoparticles by dextranase.

The influence of pH on the CNZ release from the DP hydrogel nanoparticles was examined at three different pH values (Fig. 8). Without dextranase, CNZ tends to be released more quickly at lower pH than at higher pH. It is thought that the hydrogen bonds are formed between water and DP polymer chains at low pH, and the swelling is increased by water absorption. However, the effect of pH values on CNZ release rate was reversed with dextranase. This result can be explained by the optimum pH of the enzyme. The optimum pH of dextranase is between pH 5.0 and 7.0. Accordingly, the CNZ release rate was increased more by the enzymatic degradation of dextran chain at pH 6.0 than at 2.0 and 4.0. In conclusions, it can be expected that the DP hydrogel nanoparticles have the potential of colon-specific drug delivery system.

Acknowledgements

This work was supported by the Brain Korea 21 Project.

References

Akiyoshi, K., Kobayashi, S., Shichibe, S., Mix, D., Baudys, M., Kim, S.W., Sunamoto, J., 1998. Self-assembled hydrogel nanoparticles of cholesterol-bearing pullulan as a carrier of protein drugs: complexation and stabilization of insulin. J. Controll. Rel. 54, 313–320.

Andini, S., Ferrara, L., Maglio, G., Palumbo, R., 1988. Synthesis of block polyesteramides containing biodegradable poly(L,L-lactide) segments. Macromol. Chem. Rapid Commun. 9, 119–124.

Brannon-Peppas, L., 1995. Recent advances in the use of biodegradable microparticles and nanoparticles in controlled drug delivery. Int. J. Pharm. 116, 1–9.

Cho, C.S., Cheon, J.B., Jeong, Y.I., Kim, I.S., Kim, S.H., Akaike, T., 1997. Novel core-shell type thermo-sensitive nanoparticles composed of poly(γ-benzyl L-glutamate) as the core and poly(*N*-isopropylacrylamide) as the shell. Macromol. Rapid Commun. 18, 361–369.

Coevreur, P., Puisieux, F., 1993. Nano- and microparticles for the delivery of polypeptides and proteins. Adv. Drug Deliv. Rev. 10, 141–162.

Crommelin, D.J.A., Schreier, H., 1994. Liposomes. In: Kreuter, J. (Ed.), Colloidal Drug Delivery Systems. Marcel Dekker, Dorcecht, pp. 73–190.

Deng, X.M., Xiong, C.D., Cheng, L.M., Xu, R.P., 1990. Synthesis and characterization of block copolymers from D,L-lactide and poly(ethylene glycol) with stannous chloride. J. Polym. Sci., Polym. Lett. Ed. 28, 411–416.

Heller, J., 1993. Polymers for controlled parenteral delivery of peptides and proteins. Adv. Drug Deliv. Rev. 10, 163–204.

Jeong, Y.I., Cheon, J.B., Kim, S.H., et al., 1998. Clonazepam release from core-shell type nanoparticles in vitro. J. Controll. Rel. 51, 169–178.

Kalyanasundaram, K., Thomas, J.K., 1977. Environmental effects on vibronic band intensities in pyrene monomer fluorescence and their application in studies in micellar systems. J. Am. Chem. Soc. 99, 2039–2044.

Kim, I.S., Kim, S.H., Cho, C.S., 1996. Drug release from pH-sensitive interpenetrating polymer networks hydrogel based on poly(ethylene glycol) macromer and poly(acrylic acid) prepared by UV cured method. Arch. Pharm. Res. 1, 18–22.

Kim, I.S., Jeong, Y.I., Kim, S.H., 2000. Thermo-sensitive self-assembled micelles prepared by cholic acid and poly(*N*-isopropylacrylamide). Arch. Pharm. Res. 23 (1), 87–89.

Kwon, G.S., Naito, M., Yokoyama, M., Okano, T., Sakurai, Y., Kataoka, K., 1995. Physical entrapment of adriamycin in AB block copolymer micelles. Pharm. Res. 12, 192–195.

Larsen, C., Harboe, E., Johansen, M., Olesen, H.P., 1989. Macromolecular prodrugs. XVI. Colon-targeted delivery-comparison of the rate of release of naproxen from dextran ester prodrugs in homogenates of various segments of the pig gastrointestinal (GI) tract. Pharm. Res. 5, 995–999.

Marctic, P.A., Nair, M., 1994. Microenvironment sensing of block copolymer micelles by fluorescence spectroscopy. J. Colloids Interface Sci. 163, 517–519.

Molteni, L., 1979. In: Gregoriadis, G. (Ed.), Drug Carriers in Biology and Medicine, Academic Press, London, pp. 107–125.

Nishikawa, T., Akiyoshi, K., Sunamoto, J., 1994. Supramolecular assembly between nanoparticles of hydrophobized polysaccharide and soluble protein complexation between the self-aggregate of cholesterol-bearing pullulan and α-chymotrypsin. Macromolecules 27, 7654–7659.

Okano, T., Yui, N., Yokoyama, M., Yoshida, R., 1994. Advances in Polymeric Systems for Drug Delivery. Gordon and Breach Science, Yverdon.

Pavia, D.L., Lampmam, G.M., Kriz, G.S., 1979. Introduction to Spectroscopy. W.B. Saunders, Philadelphia.

Poznansky, M.J., Cleland, L.G., 1980. In: Juliano, R.L. (Ed.), Drug Delivery Systems, Oxford University Press, New York, pp. 253–315.

Sery, T.W., Hehre, E.J., 1956. Degradation of dextrans by enzymes of intestinal bacteria. J. Bacteriol. 71, 373.

Van Dijk-Wolthuis, W.N.E., Franssen, O., Talsma, H., Van Steenbergen, M.J., Kettenes-van den Bosch, J.J., Hennink, W.E., 1995. Synthesis, characterization and polymerization of glycidyl methacrylate derivatized dextran. Macromolecules 28, 6317–6322.

Wilhelm, M., Zhao, C.L., Wang, Y., Xu, R., Winnik, M.A., Mura, J.L., Riess, G., Croucher, M.D., 1991. Poly(styrene–ethylene oxide) block copolymer micelle formation in water: a fluorescence probe study. Macromolecules 24, 1033–1040.

Yokoyama, M., Okano, T., Sakurai, Y., Kataoka, K., 1994. Improved synthesis of adriamycin-conjugated poly(ethylene oxide)-poly(aspartic acid) block copolymer and formation of unimodal micellar structure with controlled amount of physically entrapped adriamycin. J. Controll. Rel. 32, 269–277.