ELSEVIER

Journal of Chromatography A, 898 (2000) 167–177

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

DNA adsorption onto polyethylenimine-attached poly(*p*-chloromethylstyrene) beads

Ender Unsal[a], Tahsin Bahar[a], Murvet Tuncel[b], Ali Tuncel[a,*]

[a]*Hacettepe University, Chemical Engineering Department, Beytepe, 06532 Ankara, Turkey*

[b]*Hacettepe University, Faculty of Medicine, Ankara, Turkey*

Received 27 March 2000; received in revised form 31 July 2000; accepted 1 August 2000

Abstract

In this study, DNA adsorption properties of polyethylenimine (PEI)-attached poly(*p*-chloromethylstyrene) (PCMS) beads were investigated. Spherical beads with an average size of 186 μm were obtained by the suspension polymerization of *p*-chloromethylstyrene conducted in an aqueous dispersion medium. Owing to the reasonably rough character of the bead surface, PCMS beads had a specific surface area of 14.1 m^2/g. PEI chains could be covalently attached onto the PCMS beads with equilibrium binding capacities up to 208 mg PEI/g beads, via a direct chemical reaction between the amine and chloromethyl groups. After PEI adsorption with 10% (w/w) initial PEI concentration, free amino content of PEI-attached PCMS beads was determined as 0.91 mequiv./g. PEI-attached PCMS beads were utilized as sorbents in DNA adsorption experiments conducted at +4°C in a phosphate buffer medium of pH 7.4. DNA immobilization capacities up to 290 mg DNA/g beads could be achieved with the tried sorbents. This value was approximately 50-times higher relative to the adsorption capacities of previously examined sorbents. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Adsorbents; DNA; Polyethylenimine; Poly(*p*-chloromethylstyrene)

1. Introduction

Immobilization of DNA onto the surface of polymeric materials allows the synthesis of DNA carrying specific sorbents. One of the most promising application of these materials is the blood detoxification by extracorporeal circulation devices. These systems have been utilized in the therapeutical applications of some autoimmune diseases, involving the removal of anti-DNA antibody from the plasma of the patient [1–4]. Antibody removal rate and equilibrium antibody adsorption capacity were shown to be proportional to the amount of immobilized DNA on the sorbent material [4–6]. The results of these studies also indicated that higher anti-DNA antibody removal rates could be achieved with the sorbents carrying higher amounts of immobilized DNA on their surfaces [4–6]. Different materials have been proposed as solid supports for DNA immobilization [3–15]. It was shown that more quantitative and stable immobilization of DNA – relative to the other possible interactions – could be achieved via polyion complexation between amine groups of support materials and phosphorus carrying segments of DNA molecules [5]. For this reason, aminated solid supports have been commonly employed in DNA immobilization studies [5,6,14,15].

*Corresponding author.

E-mail address: atuncel@hacettepe.edu.tr (A. Tuncel).

0021-9673/00/$ – see front matter © 2000 Elsevier Science B.V. All rights reserved.
PII: S0021-9673(00)00831-1

Amine functionality on the solid support can be generated by the chemical activation of surface-functional groups (hydroxyl, carboxyl, etc.) with the proper agents (i.e., cyanogen bromide, tosyl chloride or water-soluble carbodiimides) and then by treating the activated support with a diamine carrying ligand [14,15]. One of the recent methods used for generation of surface-amine groups was the graft copolymerization of amino containing acrylic monomers performed after the ozonization of support material [5,6]. Functionalized polystyrene, agarose, polyethylene-terephthalate (PET) or poly(hydroxyethylmethacrylate) (PHEMA) have been preferred as the base matrices in the preparation of DNA immobilized supports [4–6,13–15]. However, DNA binding capacities of the supports prepared from these polymers have been usually low. In these studies, the surface concentration of functional groups having a specific interaction ability with DNA (i.e., DNA specific ligands) was usually low on the inertial support materials. The surface concentration of activated groups on the inertial base matrix and the yield of activation process are the factors controlling the surface ligand concentration of these supports.

In our previous studies, functional beads for different biotechnological applications were obtained by different suspension polymerization procedures [16–20]. Recently, we produced spherical-swellable gel beads by suspension polymerization of an amino-carrying, acrylate-based monomer (i.e, *N*-3-dimethylaminopropylmethacrylamide) [20]. The use of these gel beads as a sorbent in batch DNA adsorption provided equilibrium DNA binding capacities up to 50 mg/g dry gel [20]. In the presented study, we selected a relatively hydrophobic material, poly(*p*-chloromethylstyrene) (PCMS) beads for the synthesis of a DNA immobilized sorbent. PCMS beads were obtained by a modified version of the suspension polymerization process described elsewhere [21]. An amino carrying polymer, polyethylenimine (PEI) – as a ligand having chemical interaction ability with DNA molecule – was covalently attached onto the bead surface via a single-step reaction between the chloromethyl and amino groups (i.e., without applying an additional activation process). The interaction of PEI-attached PCMS beads with DNA in an aqueous buffer medium provided equilibrium DNA immobilization capacities up to 290 mg DNA/g PCMS.

2. Experimental

2.1. Materials

The monomer, 4-chloromethylstyrene (CMS; Aldrich, Milwaukee, WI, USA) and the crosslinker ethyleneglycol dimethacrylate (EGDMA; Aldrich) were used without further purification. Cyclohexane (CH; BDH, Poole, UK) was selected as the diluent. Azobisisobutyronitrile (AIBN; BDH) was crystallized from methanol and used as the initiator. Polyvinyl alcohol (PVA, M_r 85 000–146 000, 87–89% hydrolyzed; Aldrich) was the stabilizer. PEI samples with low and high molecular masses (M_n 1800, M_r 2000 and M_n 10 000, M_r 25 000; Aldrich) were used as received. Deoxyribonucleic acid (DNA, from salmon testes, catalog No. D1626) was supplied by Sigma (St. Louis, MO, USA). Distilled–deionized water was used in all experiments.

2.2. Preparation of PCMS beads

A modified version of suspension polymerization method proposed by Nonaka et al. was used for the preparation of spherical PCMS beads [21]. A typical preparation procedure was exemplified below. Continuous medium was prepared by dissolving PVA (100 mg) in the distilled–deionized water (50 ml). For the preparation of dispersed phase, CMS (2.5 ml), EGDMA (0.75 ml) and CH (3.6 ml) were mixed and AIBN (60 mg) was dissolved in the homogeneous organic phase. The organic phase was dispersed in the aqueous medium by stirring the mixture magnetically (300 rpm), in a sealed-cylindrical, pyrex polymerization reactor. The reactor content was heated to polymerization temperature (i.e., 78°C) within 40 min and the polymerization was conducted for 8 h with a 300 rpm stirring rate at 78°C. Final beads were extensively washed with ethanol and water to remove any unreacted monomer or diluent and then stored in distilled water at +4°C.

2.3. Characterization of PCMS beads

Surface morphology and internal structure of PCMS beads were observed in a scanning electron microscope (JEOL, JEM 1200EX, Tokyo, Japan). PCMS beads were dried at room temperature and coated with a thin layer of gold (about 100 Å) in vacuum and photographed in the electron microscope with ×800 magnification. The specific surface area of the PCMS beads was determined in BET apparatus. Total monomer conversion into the bead form (i.e., bead yield) was found by the determination of dry weight of gel beads. For this purpose, gel beads obtained from one batch were washed and then dried in a vacuum oven at 40°C for 48 h. Bead yield was calculated by taking the ratio of dry weight of beads to the initial monomer weight (including crosslinker). The average size and size distribution of the PCMS beads were determined by screen analysis performed by using Tyler Standard Sieves.

To determine the swelling ratio of plain PCMS beads, approximately 3 g of dry sample was placed in a cylindrical tube. First, the height of the bed formed by the dry beads (H_d) was measured. Then, 30 ml of swelling medium was added into the tube. In these experiments, toluene, hexane and water were utilized as the swelling medium. The dynamic swelling behavior of PCMS beads was followed by measuring the bed height at designated times (H_s). The volumetric swelling ratio of PCMS beads was calculated based on the following expression:

$$\text{Swelling ratio} = (H_s/H_d) \times 100 \quad (1)$$

2.4. PEI attachment onto PCMS beads

The following procedure was applied for the covalent attachment of PEI onto the PCMS beads. Dry PCMS beads (approximately 1.0 g) were put into an aqueous solution (30 ml) containing 10% (w/w) PEI (pH 10.6). The medium was shaken at 120 cpm for 6 h at 55°C. The preliminary experiments showed that PEI adsorption equilibrium was attained within this period. To find the equilibrium PEI adsorption capacity of PCMS beads, final PEI concentration (i.e., after completion of adsorption period) was determined by the potentiometric titration of a sample (5 ml) withdrawn from adsorption medium with 0.1 *M* standard HCl solution. Equilibrium PEI adsorption capacity (Q', mg PEI/g beads) was calculated based on Eq. (2) where, C_0 and C_f (mg/ml) are the initial and final PEI concentrations in the adsorption medium, respectively. V (ml) and M (g) are the volume of the adsorption medium and the amount of PCMS beads, respectively:

$$Q' = (C_0 - C_f) \cdot V/M \quad (2)$$

PEI-attached PCMS beads were extensively washed with distilled water to remove any physically adsorbed PEI from the beads. To determine the leakage of PEI, washing solutions were collected and analyzed by the same procedure. Then the amount of PEI released per g of particle was determined. This value was subtracted from the equilibrium adsorption capacity (Q) to calculate the covalently bound PEI onto the bead surface. Approximately 10–25% (w/w) of the PEI present on the bead surface was released by the washing. The reported PEI binding capacities were calculated based on the covalently attached amount of PEI onto per g of PCMS beads.

Free amino contents of PEI-attached PCMS beads were determined by potentiometric titration. For this purpose, PEI-attached PCMS beads (approximately 1.0 g) were treated with 0.1 *M* standard HCl solution (100 ml) for 6 h at a 120 cpm shaking rate. After consumption of HCl by the free amine groups of beads, the final HCl concentration in the aqueous medium was determined by a potentiometric titration with a 0.048 *M* NaOH solution.

2.5. DNA adsorption experiments

DNA adsorption isotherms were obtained by using both plain and PEI-attached PCMS beads as sorbents. For this purpose DNA initial concentration in the adsorption medium was varied between 0.4 and 4.0 mg/ml. A typical DNA adsorption procedure may be described as follows. To prepare the adsorption medium, a certain amount of DNA was dissolved in a phosphate buffer solution (50 ml, pH 7.4, ionic strength: 0.1) by stirring the medium at 200 rpm for 24 h at +4°C. PCMS beads (approximate dry weight: 0.25 g) equilibrated in phosphate buffer solution were transferred to the adsorption medium. The adsorption process was conducted at

+4°C, with 200 rpm stirring rate for 8 h. To determine the equilibrium DNA adsorption capacity, the sample withdrawn from the adsorption medium after completion of the equilibrium adsorption period was assayed according to the method of Spirin for the determination of DNA concentration [22]. Details of DNA assay were given elsewhere [20].

3. Results and discussion

3.1. Characterization of PCMS beads

The suspension polymerization procedure provided crosslinked PCMS beads in the spherical form. The chemical structure of these beads is given in Fig. 1. An optical micrograph of the PCMS beads is given in Fig. 2. As seen here, the selected suspension polymerization method provided PCMS particles in an excellent spherical form. Under a light microscope, the particles were observed as dark spheres. This view should be evaluated as an indication for the macroporous character of the bead interior [16,17]. Surface morphology and internal structure of PCMS beads were exemplified by the electron micrographs in Fig. 3. As seen in Fig. 3A, PCMS beads had a reasonably rough surface. However, the surface of the PCMS beads contained no macropores. The micrograph in Fig. 3B was taken with the broken beads to observe the internal part. The presence of macropores within the bead interior was clearly seen in this photograph. It could be concluded that the PCMS beads had a macroporous interior surrounded by a reasonably rough surface, in the dry state. The roughness of the bead surface should be considered as a factor providing an increase in the specific surface area. This conclusion was supported by the BET measurement. Specific surface area of the PCMS beads was found to be 14.1 m^2/g. However, no significant pore volume was obtained in the same measurement. The determined cumulative pore volume (i.e., 0.03 cm^3/g) was too low for representing the porosity of the bead interior observed in Fig. 3B. In the BET measurement, such a low value should only be obtained with a particle sample having an impermeable surface against nitrogen. However, this conclusion is valid for dry beads. To test the permeability of the bead surface against different solvents, the swelling behavior of PCMS

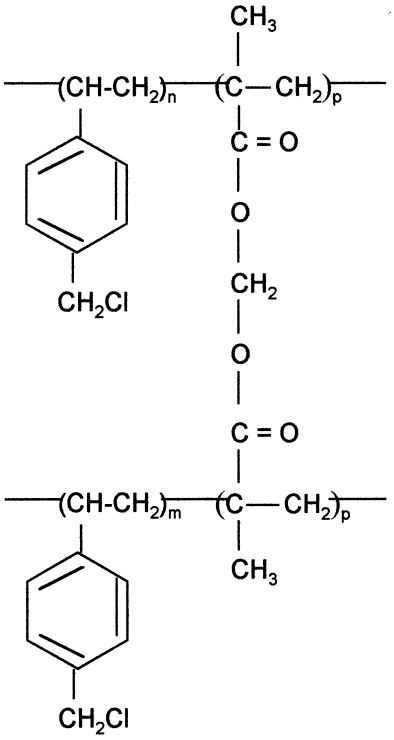

Fig. 1. Chemical structure of PCMS beads.

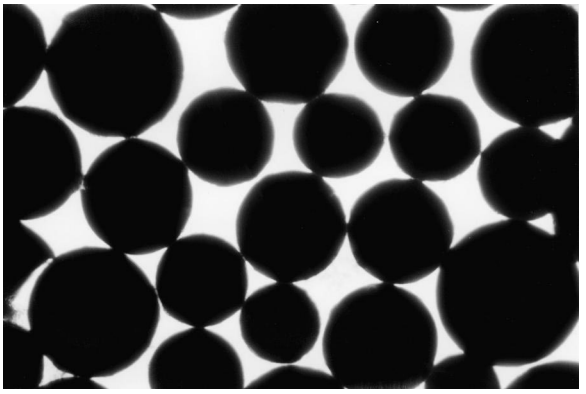

Fig. 2. The optical micrograph of plain PCMS beads in distilled water (magnification: ×150).

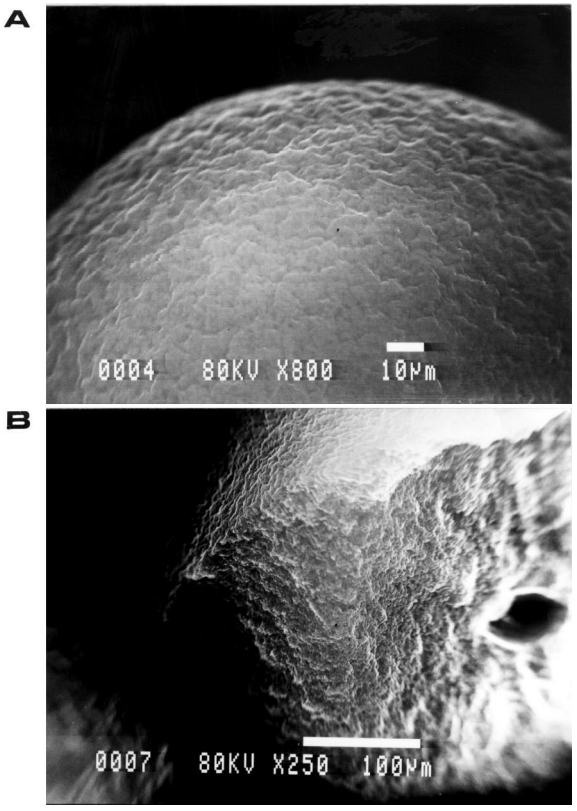

Fig. 3. The electron micrographs showing (A) surface morphology (magnification: ×800) and (B) internal structure (magnification: ×250) of plain PCMS beads.

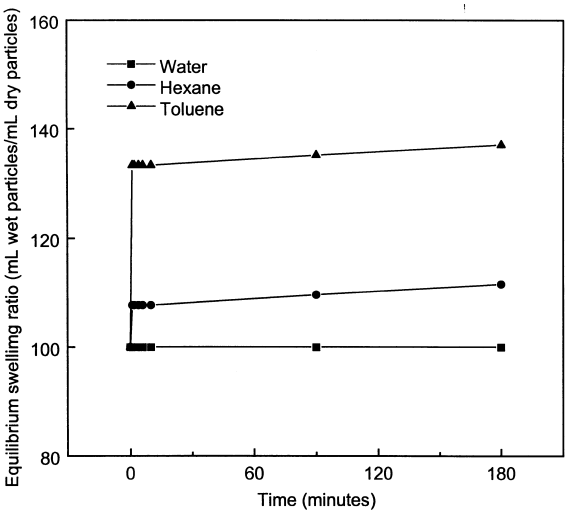

Fig. 4. Swelling behavior of plain PCMS beads.

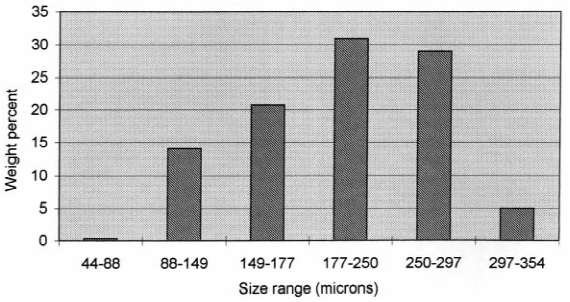

Fig. 5. The histogram showing the size distribution of PCMS beads.

beads was examined in different media (i.e., water, hexane and toluene). The swelling behavior of PCMS beads is given in Fig. 4. As seen here, the PCMS beads reached equilibrium swelling within approximately 2–3 min. Although the PCMS beads exhibited no significant swelling in water, appreciable equilibrium swelling ratios were observed in the organic solvents (i.e., hexane and toluene). This behavior may be attributed to the fact that the surface of the PCMS beads was permeable against hexane and toluene since the bead surface probably possessed gel-type microporosity in the swollen form (i.e., swelling porosity) [23–25]. In some of the studies relating to the synthesis of macroporous spherical beads, a less porous surface relative to the bead interior or a bead surface containing no macropores in the dry state were also observed [23–25]. The histogram showing the size distribution of PCMS beads is given in Fig. 5. Note the given histogram was obtained by the screen analysis. As seen here, approximately 60% (w/w) of the PCMS beads was collected in the size range of 177–297 μm. Based on the given distribution, the average bead size was calculated as 186 μm.

3.2. PEI attachment onto PCMS beads

PEI is known as one of the most effective agents having a complexation ability with DNA [26–30]. In our work, PEI was directly attached onto the surface of the PCMS beads via a chemical reaction between

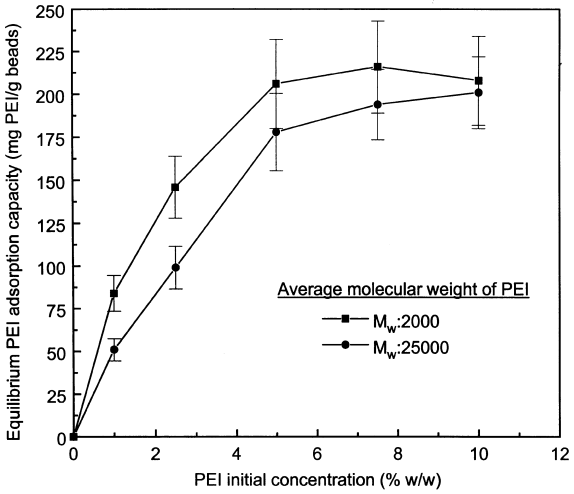

Fig. 6. The variation of equilibrium PEI adsorption capacity with initial PEI concentration (conditions: pH: 10.6, temperature: 55°C, time: 6 h, shaking rate: 120 cpm, PCMS beads: 1 g in 30 ml aqueous medium).

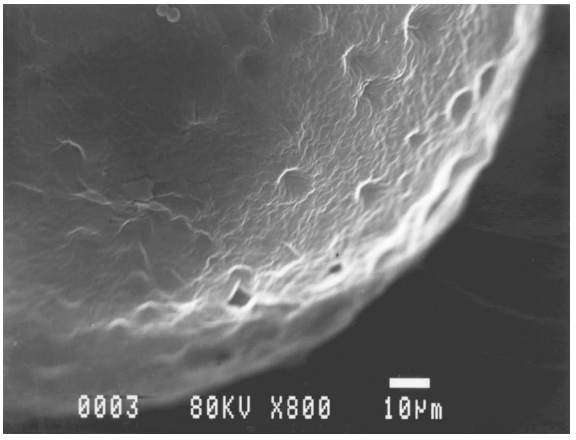

Fig. 7. A typical electron micrograph of PEI-attached PCMS beads prepared with the initial PEI concentration of 10% (magnification: ×800).

the amino and chloromethyl groups [31]. Owing to the selection of PCMS as the base material, no additional activation procedure was employed for the attachment of the ligand having interaction ability with DNA. The variation of the equilibrium PEI adsorption capacity (i.e., the amount of covalently bound PEI onto per g of PCMS) with the initial PEI concentration is given in Fig. 6. Here, two different PEIs with the average molecular masses of 2000 and 25 000 were utilized. As seen in Fig. 6, the equilibrium amount of PEI attached onto the PCMS beads markedly increased until 5% (w/w) initial PEI concentration. No significant difference was observed between the attached amounts of PEI with different molecular masses. The maximum amount of PEI attached onto the PCMS beads was approximately 200 mg/g for both PEI types. Such an unusual amount of PEI could be attached covalently onto the bead surface probably due to highly reactive character of *p*-chloromethylstyrene. Then a hairy type-structure comprised of a PCMS sphere and PEI tails on the bead surface was probably obtained. In the case of reasonably high PEI attachment, it was possible to show the presence of a PEI layer on the surface of the PCMS beads by scanning electron microscopy. A typical electron micrograph of PEI-attached PCMS beads prepared with the initial PEI concentration of 10% is given in Fig. 7. It should be noted that surface morphology of PEI-attached PCMS beads was reasonably different than that of plain PCMS (Fig. 3A). The roughness of bead surface observed in Fig. 3A disappeared with the attachment of PEI. Free amino ($-NH_2$) contents of PEI-attached PCMS beads obtained with different PEI initial concentrations were also determined after the PEI adsorption and are given in Table 1. As seen here, the amino content increased as the initial concentration of PEI increased. The amino content was approximately proportional to the amount of PEI present on the bead surface (i.e., Fig. 6). The possible chemical interactions between PEI and

Table 1
Free amino contents of PEI-attached PCMS beads prepared with different PEI initial concentrations

PEI initial concentration (%, w/w)	Amino content (mequiv./g)	
	M_r PEI: 2000	M_r PEI: 25 000
1.0	0.396±0.091	0.348±0.044
2.5	0.567±0.125	0.436±0.106
5.0	0.787±0.132	0.743±0.155
7.5	0.869±0.124	0.796±0.126
10.0	0.913±0.182	0.875±0.148

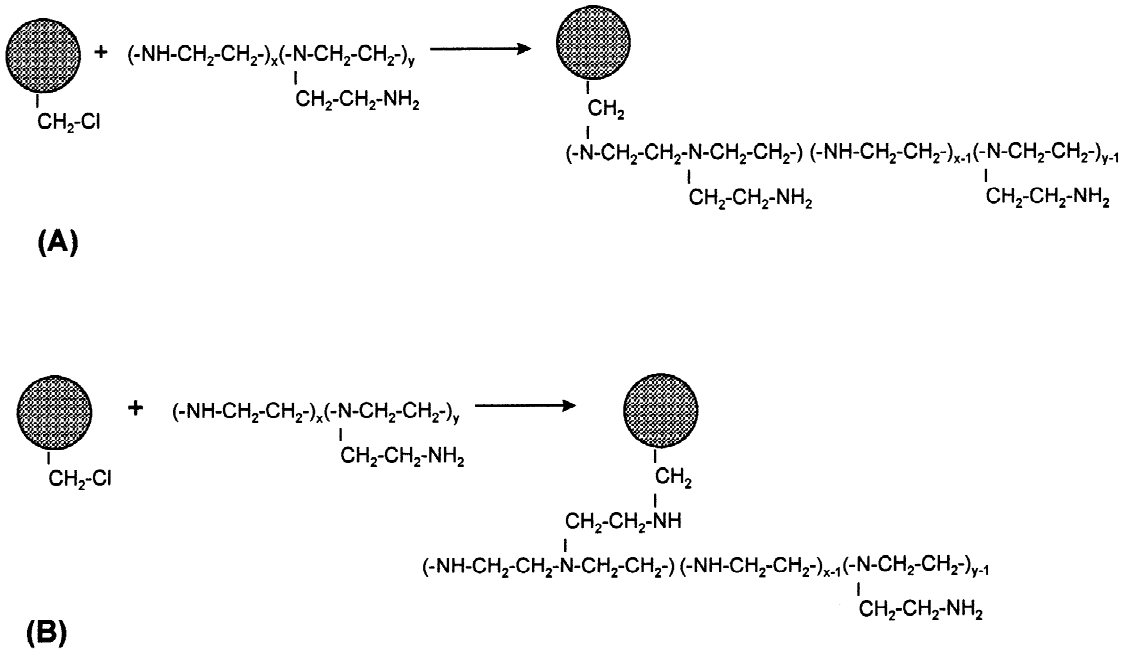

Fig. 8. Possible chemical interactions between PEI and the chloromethyl groups of PCMS beads.

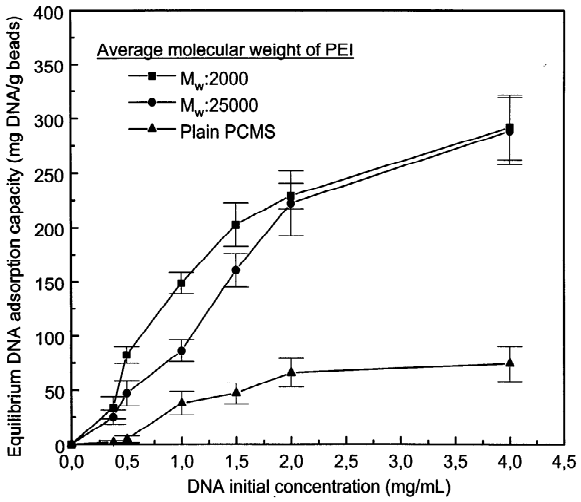

Fig. 9. The variation of equilibrium DNA adsorption capacity of plain and PEI-attached PCMS beads with initial DNA concentration (conditions: pH: 7.4, temperature: +4°C, time: 8 h, shaking rate: 120 cpm, PCMS beads: 0.25 g in 50 ml aqueous buffer medium).

chloromethyl groups of the PCMS beads are shown in Fig. 8. As seen here, PEI can react with the chloromethyl groups via either primary or secondary amine groups. The possibility of first reaction (i.e., the reaction between the chloromethyl groups of the PCMS beads and secondary amine groups of PEI) is higher because of the reactivity of secondary amines against chloromethyl is higher relative to that of primary amines. Therefore, the first reaction is probably more effective for binding of PEI onto the PCMS beads.

3.3. DNA adsorption experiments

In the DNA adsorption experiments, the temperature and pH were fixed at +4°C and 7.4, respectively. DNA initial concentration was varied between 0.4 and 4.0 mg/ml by using plain and PEI-attached PCMS beads as sorbents. Here, two types of PEI-carrying PCMS beads prepared by the attachment of

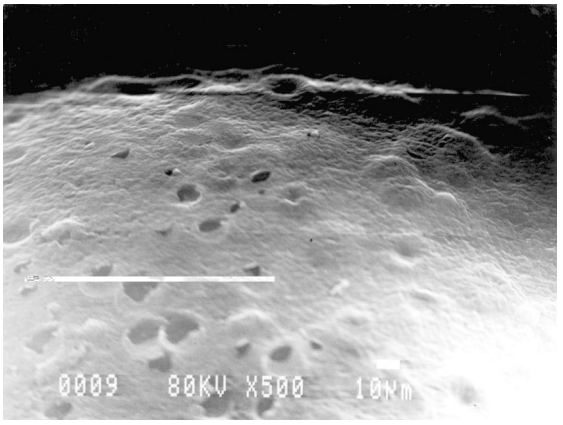

Fig. 10. The electron micrograph showing the surface morphology of DNA-adsorbed PEI-attached PCMS beads (magnification: ×500).

low- and high-molecular-mass PEIs were used. These beads were obtained with the initial PEI concentration of 10% (w/w) in the adsorption medium. Final PEI contents of the beads prepared with low- and high-M_r PEIs were 208 and 201 mg/g, respectively. The variation of equilibrium DNA adsorption capacity with the initial DNA concentration is given in Fig. 9 for plain and PEI-attached PCMS beads. In these experiments, plain PCMS beads were included as reference material. For a certain DNA initial concentration, the equilibrium DNA adsorption capacity of PEI-attached PCMS beads was reasonably higher than those observed with plain PCMS. This result indicated that PEI attachment onto the PCMS surface provided a drastic increase in the DNA immobilization capacity. For PEI-attached beads, the equilibrium adsorption

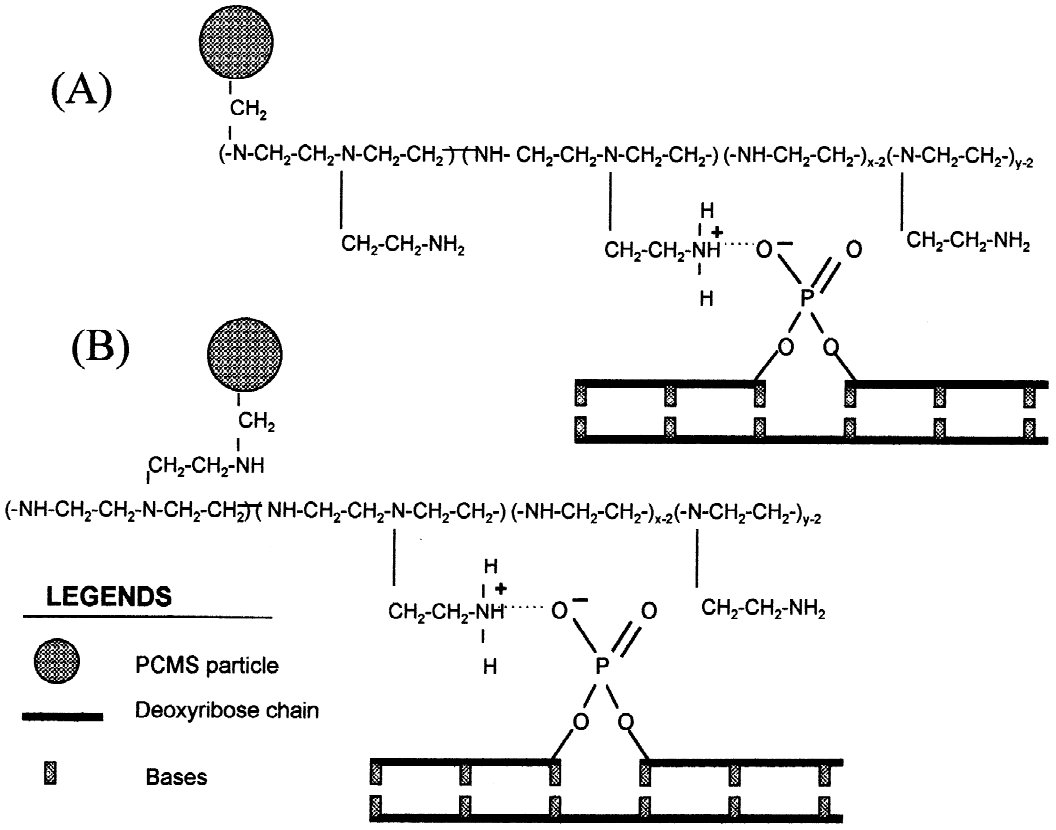

Fig. 11. Two possible mechanisms for the DNA adsorption onto PEI-carrying PCMS particles.

binding of DNA to the PEI chains bound onto the PCMS particles via primary amine groups is given in Fig. 11B. After adsorption, DNA-carrying plain PCMS and PEI-attached PCMS beads (approximate dry weight: 0.25 g) were transferred to the desorption medium including 1 *M* NaCl, 1 m*M* EDTA and 10 m*M* Tris–HCl buffer (pH 8.0) [15]. The desorption was conducted at 23°C for 2 h. The desorption ratios obtained with the PEI-attached PCMS beads are given in Table 2. As seen here, only a small fraction of adsorbed DNA could be desorbed from the PEI-attached PCMS beads. This behavior probably originated from the multipoint-attachment of DNA onto the PEI chains on the bead surface. It should be noted that desorption yields with the DNA-adsorbed plain PCMS beads were lower than 1%. This result probably indicated a covalent interaction between DNA and the chloromethyl groups on the bead surface. Possible covalent interactions are shown in Fig. 12. As seen here, chloromethyl groups can react with the primary amine groups of adenine (i.e., located in the adenine–thymine pairs of DNA, Fig. 12A) and guanine bases (i.e., located in cytosine–guanine pairs, Fig. 12B). Kato and Ikada investigated DNA adsorption onto the poly(ethylene) terephthalate microfibers with a high specific surface area [5]. After ozonization, the microfibers were exposed to graft copolymerization of monomers including acrylic acid, methacroyloxyethyl phosphate, *N*,*N*-dimethylaminoethyl-methacrylate, *N*-vinylformamide and glycidyl methacrylate. In the referred study, the highest DNA adsorption capacity was obtained as approximately 5 mg DNA/g with the poly(methacroyloxyethyl phosphate) grafted PET microfibers [5]. On the other hand, the maximum equilibrium DNA adsorption capacity achieved with the CNBr activated poly(2-hydroxyethyl-methacrylate) microbeads was 2.75 mg DNA/g [4]. The high DNA adsorption capacity observed in our study may be explained by the high PEI content of PCMS beads. The hairy structure of examined sorbent (i.e., the presence of long and flexible PEI chains attached to the solid PCMS sphere) was probably responsible for reasonably high DNA binding. The high DNA content achieved in our study can be an advantage both for the applications involving the use of DNA immobilized sorbents in the removal of different biological agents like anti-DNA antibodies from the plasma and the applications aiming to prevent DNA contamination.

References

[1] D.S. Terman, R. Harbeck, A. Hoffman, I. Steward, J. Robinetto, R. Carr, Fed. Proc. 34 (1975) 976.
[2] D.S. Terman, I. Steward, J. Robinetto, R. Carr, R. Harbeck, Clin. Exp. Immunol. 24 (1976) 231.
[3] V.G. Nicolaev, V.V. Sarnatskaya, E.V. Eretskaya, E.A. Snezhkova, N.V. Belitser, Clin. Mater. 11 (1992) 125.
[4] A. Denizli, E. Piskin, J. Chromatogr. B 666 (1995) 215.
[5] K. Kato, Y. Ikada, Biotechnol. Bioeng. 51 (1996) 581.
[6] B. Zhu, H. Iwata, D. Kong, Y. Yu, K. Kato, Y. Ikada, J. Biomater. Sci. Polymer Edition 10 (1999) 341.
[7] A. Weissbach, M. Poonian, Methods Enzymol. 34 (1974) 463.
[8] T.Y. Shih, M.A. Martin, Biochemistry 13 (1974) 3411.
[9] D.S. Terman, D. Petty, R. Harbeck, R.I. Carr, G. Buffaloe, Clin. Immunol. Immunopathol. 8 (1977) 90.
[10] Y. Yan, Y. Yu, J. Song, S. Qian, W. Quan, X. Shao, R. Cui, J. Wang, Artif. Organs 12 (1988) 444.
[11] F. Hiepe, K. Wolbart, W. Schossler, A. Speer, T. Montag, F. Mielke, E. Apostoloff, Biomater. Artif. Cells Artif. Organs 18 (1990) 683.
[12] K. Suzuki, M. Hara, M. Harigai, T. Ishizuka, T. Hirose, Y. Matsuki, Y. Kawaguchi, A. Kitani, M. Kawagoe, H. Nakamura, Arthritis Rheum. 34 (1991) 1546.
[13] D. Letourneur, M. Josefowicz, Biomaterials 13 (1992) 59.
[14] I.J. Bruce, M.J. Davies, K. Howard, D.E. Smethurst, M.J. Todd, Pharm. Pharmacol. 48 (1996) 147.
[15] M.J. Davies, D.E. Smethurst, K.M. Howard, M. Todd, L.M. Higgins, I.J. Bruce, Appl. Biochem. Biotechnol. 68 (1997) 95.
[16] S. Senel, H. Cicek, A. Tuncel, J. Appl. Polymer Sci. 61 (1997) 1319.
[17] A. Tuncel, K. Ecevit, K. Kesenci, E. Piskin, J. Polymer Sci. Polymer Chem. Edition 31 (1996) 1275.
[18] M. Bayhan, A. Tuncel, J. Appl. Polymer Sci. 67 (1998) 1127.
[19] A. Tuncel, J. Appl. Polymer Sci. 74 (1999) 1025.
[20] A. Tuncel, E. Unsal, H. Cicek, J. Appl. Polymer Sci. 77 (2000) 3154.
[21] T. Nonaka, Y. Uemura, K. Ohse, K. Jyono, S. Kurihara, J. Appl. Polymer Sci. 66 (1997) 1621.
[22] A.S. Spirin, Biochemistry 23 (1958) 617.
[23] A. Jayakrishnan, B.C. Thanoo, J. Biomed. Mater. Res. 24 (1990) 913.
[24] D. Horak, F. Lednicky, V. Rehak, F. Svec, J. Appl. Polym. Sci. 49 (1993) 2041.
[25] D. Horak, F. Lednicky, M. Bleha, Polymer 37 (1996) 4243.
[26] W.T. Godbey, K.K. Wu, A.G. Mikos, J. Control. Release 60 (1999) 149.
[27] W.T. Godbey, K.K. Wu, G.J. Hirasaki, A.G. Mikos, Gene Ther. 6 (1999) 1880.

[28] W.T. Godbey, K.K. Wu, A.G. Mikos, J. Biomed. Mater. Res. 45 (1999) 268.
[29] D. Goula, J.S. Remy, P. Erbacher, M. Wasowicz, G. Levi, B. Abdallah, B.A. Demeneix, Gene Ther. 5 (1998) 712.
[30] S.S. Diebold, H. Lehrmann, M. Kursa, E. Wagner, M. Cotton, M. Zenke, Hum. Gene Ther. 10 (1999) 775.
[31] S. Margel, E. Nov, I. Fisher, J. Polymer Sci.: Polymer Chem. Edition 34 (1996) 175.