Talanta 152 (2016) 76–81

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Self-assembled and covalently linked capillary coating of diazoresin and cyclodextrin-derived dendrimer for analysis of proteins by capillary electrophoresis

Bing Yu [a,*], Ming Chi [a], Yuxing Han [b], Hailin Cong [a,*], Jianbin Tang [b,*], Qiaohong Peng [a]

[a] Laboratory for New Fiber Materials and Modern Textile, Growing Base for State Key Laboratory, College of Materials Science and Engineering, Qingdao University, Qingdao 266071, PR China

[b] Key Laboratory of Biomass Chemical Engineering of Ministry of Education and Center for Bionanoengineering, College of Chemical and Biological Engineering, Zhejiang University, Hangzhou, Zhejiang 310027, PR China

ARTICLE INFO

Article history:
Received 6 December 2015
Received in revised form
19 January 2016
Accepted 23 January 2016
Available online 26 January 2016

Keywords:
CE
Capillary coatings
Diazoresin
Dendrimer
Protein

ABSTRACT

Self-assembled and covalently linked capillary coatings of cyclodextrin-derived (CD) dendrimer were prepared using photosensitive diazoresin (DR) as a coupling agent. Layer by layer (LBL) self-assembled DR/CD-dendrimer coatings based on ionic bonding was fabricated first on the inner surface of capillary, and subsequently converted into covalent bonding after treatment with UV light through a unique photochemistry reaction of DR. Protein adsorption on the inner surface of capillary was suppressed by the DR/CD-dendrimer coating, and thus a baseline separation of lysozyme (Lys), myoglobin (Mb), bovine serum albumin (BSA) and ribonuclease A (RNase A) was achieved using capillary electrophoresis (CE). Compared with the bare capillary, the DR/CD-dendrimer covalently linked capillary coatings showed excellent protein separation performance with good stability and repeatability. Because of the replacement of highly toxic and moisture sensitive silane coupling agent by DR in the covalent coating preparation, this method may provide an environmentally friendly and simple way to prepare the covalently coated capillaries for CE.

1. Introduction

As a powerful separation tool for biomacromolecule analysis, capillary electrophoresis (CE) has the advantages of high efficiency, high sensitivity, high speed, and low cost [1,2]. However, one of the major impediments for CE analysis is severe protein adsorption onto fused-silica capillary walls when analyzing proteinaceous samples [3–5]. Consequently, sample loss, poor resolution, peak broadening, long migration times, and unstable electroosmotic flow (EOF) were generated by the protein fouling [6]. In order to suppress protein adsorption onto capillary surface, the most efficient and commonly used approach is surface modification with capillary coatings [7,8]. Many kinds of coated capillaries [9–11] had been prepared to obtain better separation effect and succeed.

Generally, capillary coatings are classified into non-covalently and covalently bonded ones. The non-covalent coatings can be produced simply by flushing the capillary with coating solutions, and the coating molecules absorb on capillary surface by weak interactions such as electrostatic, van der Waals, and hydrogen bonding, etc. [12–15]. Furthermore, the layer-by-layer (LBL) self-assembly technique can also be used to prepare the non-covalently bonded capillary coatings, which provides the coating with new structures and functions [16–19]. For example, Haselberg et al. [20] prepared polybrene-dextran sulfate-polybrene (PB-DS-PB) triple layer coatings by the LBL self-assembly technique, and the coatings were fully compatible with mass spectrometry (MS) detection, causing no background signals and ionization suppression. The coatings were used for the analysis of α-chymotrypsinogen, ribonuclease A (RNase A), cytochrome c (Cyt-c) and lysozyme (Lys) by CE-MS, and the detection limits for them were 16, 11, 14 and 19 nM, respectively. Compared with the non-covalently bonded coatings, the covalently bonded coatings are very stable and robust. For example, Xu et al. [21] prepared chemically bonded PVA coatings that were used for high-efficiency separation of Cyt-c, Lys, myoglobin (Mb) and trypsin inhibitor. Timperman et al. [22] prepared chemically bonded PEG coatings which were used for high efficiency separation of BSA, alcohol dehydrogenase, carbonic anhydrase and trypsin inhibitor. PVA and PEG covalently linked coatings not only showed very good anti-protein fouling properties, but also demonstrated excellent stabilities for

* Corresponding authors.
E-mail addresses: yubingqdu@yahoo.com (B. Yu), hailincong@yahoo.com (H. Cong).

http://dx.doi.org/10.1016/j.talanta.2016.01.043
0039-9140/

repeatable separations. However, the preparation process of covalently bonded capillary coatings is usually complicated which includes multi-steps such as capillary pretreatment, introducing coupling agents, and inserting target coating reagents, etc. [23–26]. Moreover, highly toxic and moisture sensitive silane coupling agents are traditionally used in the covalent coatings, which often cause environmental and quality problems during the manufacture and application [27,28]. In the fabrication process of capillary coatings with high quality and performance, how to combine the advantages of the non-covalently and covalently bonded coatings together, and avoid their disadvantages, is becoming one of the main development directions.

Dendrimers are highly branched macromolecules characterized by monodispersity, uniform and controlled sizes, copious surface functionalities [29,30], and low intrinsic viscosity in solution [30–32]. For example, Shou et al. [33] prepared a capillary coating based on 2,4,6,8-tetravinyl-2′,4′,6′,8′-tetramethyl cyclotetrasiloxane (D_4^{Vi}) for high efficiency CE separation of adenine. Kabir et al. [34] used sol–gel dendrimer coatings for capillary microextraction, and found the dendrimer coatings had excellent thermal and solvent stability. In this study, we developed a new method to fabricate the covalently linked cyclodextrin-derived (CD) dendrimer capillary coatings using the LBL self-assembly technique combined with photochemistry reactions. The fabrication, structure and property of the coatings were studied and discussed preliminarily.

2. Experimental

2.1. Reagents and solutions

Diazoresin (DR) (Mn=2500) was synthesized according to the method described elsewhere [35]. Lysozyme (Lys), cytochrome c (Cyt-c), bovine serum albumin (BSA), amyloglucosidase (AMG), myoglobin (Mb) and ribonuclease A (RNase A) were purchased from Sigma (St. Louis, USA). N,N-Dimethyl formamide (DMF) was purchased from Yongda Chemical Reagent Company (Tianjin, China). Phosphoric acid (H_3PO_4) was purchased from Fuyu Fine Chemical Company (Tianjin, China). Monosodium orthophosphate ($NaH_2PO_4 \cdot 2H_2O$) and dibastic sodium phosphate ($Na_2HPO_4 \cdot 2H_2O$) were bought from Shunqiang Chemical Reagent Company (Shanghai, China). Acetone was obtained from Sanhe Chemical Reagent Company (Tianjin, China). Sodium hydroxide (NaOH) and hydrochloric acid (HCl) were purchased from Hongyan Reagent Company (Tianjin, China). Phosphate buffer was used

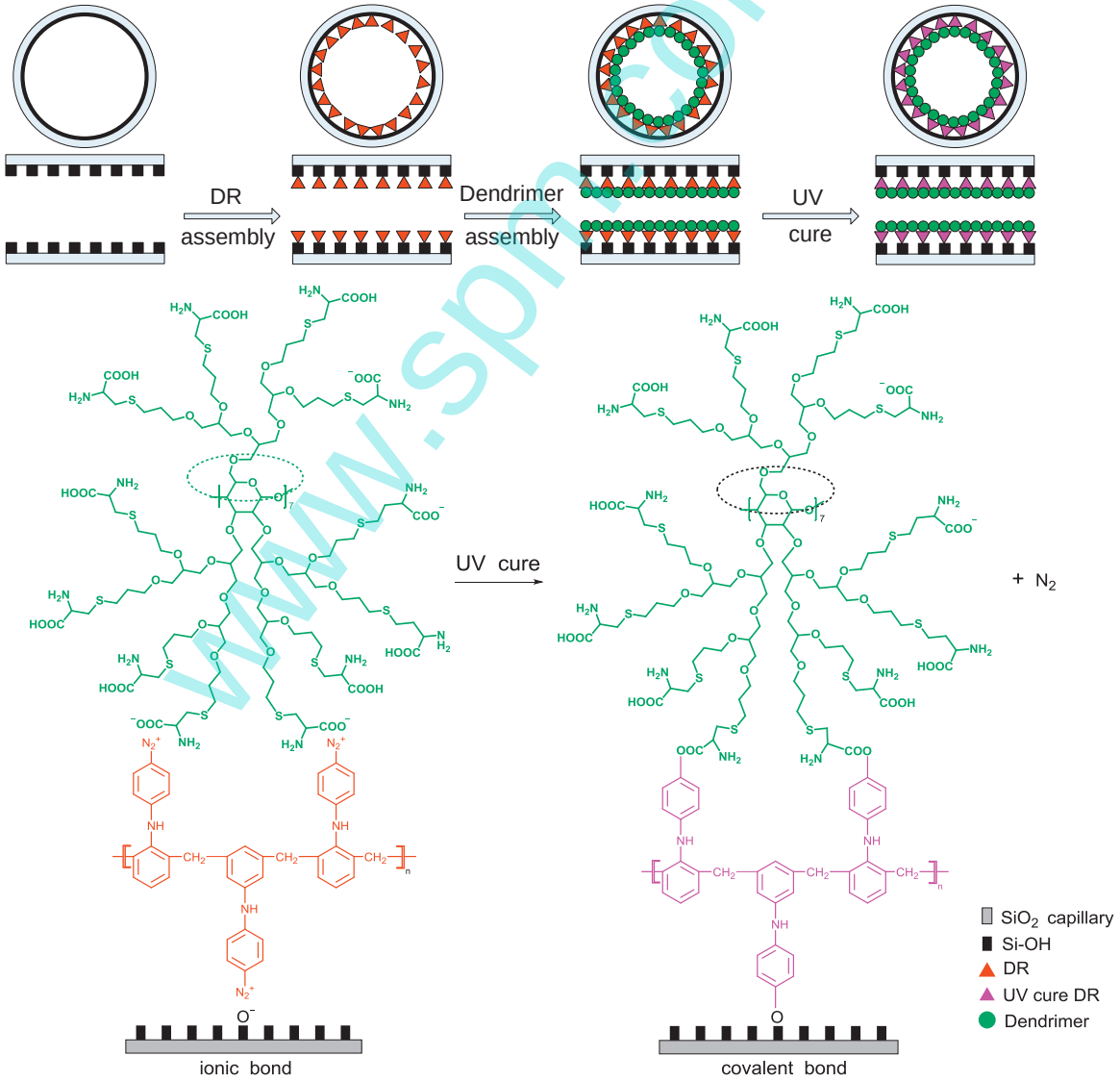

Fig. 1. Schematic illustration of preparation process of covalently bonded DR/CD-dendrimer coatings on capillary surface.

as separation medium, and the pH value was adjusted by NaOH (0.1 M) and H_3PO_4 (40 mM). The concentrations of Lys, Cyt-c, BSA, AMG, Mb, and RNase A in the testing samples were all 0.5 mg/mL. All solutions were filtered through 0.45 μm cellulose acetate membranes (Shanghai Xingya Cleaning Material Factory, China) before use.

2.2. Synthesis of cyclodextrin-derived (CD) dendrimer

CD-dendrimer (Generation 3, Mn = 19,300) was divergently synthesized with β-CD as the core and allyl bromide as the monomer through alternate allylation of the –OH groups and dihydroxylation of the double bond, and its zwitterionic surface (Gn-Cys) was constructed by conjugating cysteine onto the surface through thiol-ene reaction under UV radiation according to our previously reported method [36].

2.3. Preparation of dendrimer-coated capillary

As shown in Fig. 1, the CD-dendrimer coated capillary was prepared as follows: a new bare fused silica capillary was rinsed with 0.1 M NaOH for 30 min and deionized (DI) water for 10 min. Then coating was performed by flushing the capillary with aqueous solution of DR (2 mg/ml) for 5 min, and then flushed with DI water for 1 min and air dried for 5 min. Subsequently, the capillary was flushed with aqueous solution of CD-dendrimer (4 mg/mL) for 5 min, and then flushed with DI water for 1 min and air dried for 5 min. A self-assembled DR/CD-dendrimer bilayer coating was completed. The coating cycle was repeated for several times to obtain multilayer DR/CD-dendrimer coated capillary. Afterwards, the coated capillary was exposed to 365 nm UV light with an intensity of 350 μW/cm^2 for 15 min in order to form the covalently linked dendrimer capillary coatings.

2.4. Instrumentation and characterization

UV–vis spectrometer (TU-1810, China) was used for monitoring the LBL self-assembly coating process. The photo-crosslinking of the DR/CD-dendrimer coating on the capillary was carried out using a 365 nm UV curing system (EXFO Omnicure S1000) with a lamp power of 100 W. Atomic force microscope (AFM, CSPM 5500, China) was used for surface characterization of the coatings. The CE experiments were performed on a CL1020 high performance capillary electrophoresis instrument (Huayang liming instrument Co., China). Fused-silica capillaries of 75 μm ID and 375 μm OD were provided by Yongnian Optic Fiber (Hebei, China). The EOF measurements were carried out using a method reported elsewhere [19]. Phosphate buffers (40 mM) with pH values in the range of 3.0–9.0 were applied for determination of EOF. DMF with a concentration of 0.5 vol% was used as the EOF marker.

3. Results and discussion

3.1. Formation of DR/CD-dendrimer coatings on the inner wall of capillary

3.1.1. LBL self-assembly

UV–visible spectroscopy was used to monitor the assembly process of DR and CD-dendrimer. The UV–visible absorbance of DR/CD-dendrimer film at 380 nm, which derives from the characteristic π–π* transition absorption of the diazo group of DR, increases linearly with the number of assembly cycles (Fig. 2). This indicates that the LBL assembly is carried out successfully and uniformly. The driving force of the assembly mainly comes from the electrostatic interaction between the positive charged diazonium group (–N_2^+) of DR and negative charged carboxyl group of dendrimer (Fig. 1).

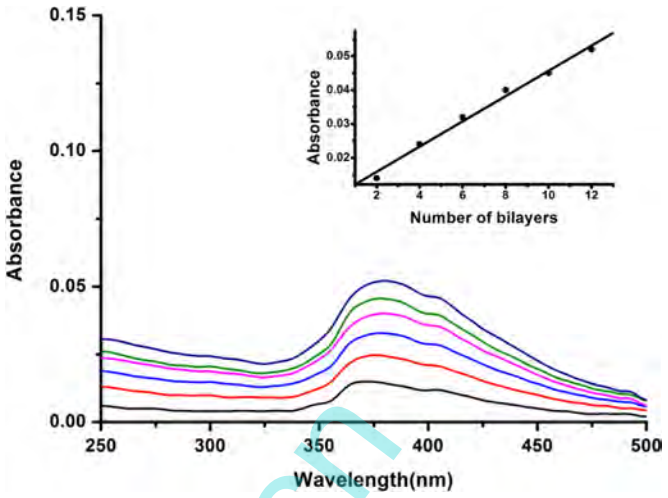

Fig. 2. The UV–vis spectra of the assembly from the DR and CD-dendrimer. Number of assembly cycles (bottom to top): 1, 2, 3, 4, 5 and 6. The inset plot shows that the absorbance of the films at 380 nm changes linearly with the number of assembly cycles.

3.1.2. UV crosslinking

DR is a non-toxic photoactive component often used as cell culture supports [37,38], and the diazonium groups involved in the DR/dendrimer multilayer films will be decomposed under UV irradiation, which results in a gradual decrease in the absorbance of the film at 380 nm (Fig. 3). The photoreaction that takes place in the multilayer films, which originates from the diazonium decomposition, is a first-order reaction: $\ln[(A_0-A_e)/(A_t-A_e)]$ changes linearly with irradiation time (Fig. 2, inset), where A_0, A_t and A_e represent the absorbance of the film before irradiation, after irradiating for time t, and at the end of irradiation (35 s), respectively. As illustrated in Fig. 1, following the decomposition of the diazonium group in the film under UV irradiation, the ionic bonds were converted into covalent bonds [39]. The unique photo-crosslinking reaction of DR has been applied to the fabrication of covalently attached self-assembly films [40], hollow microcapsules [41], and bio chips [42].

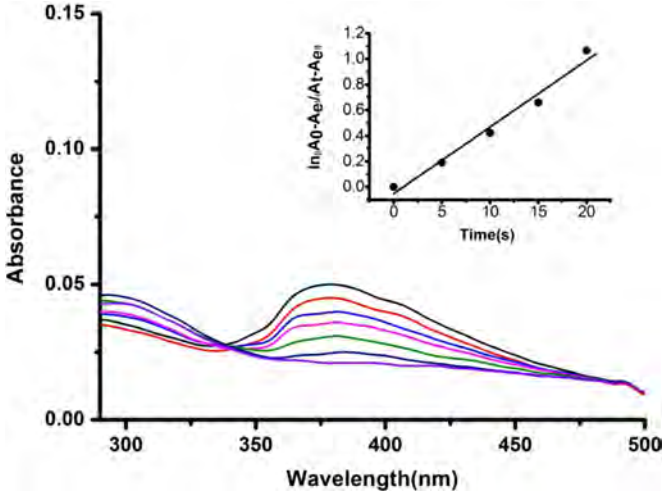

Fig. 3. UV–vis spectra of DR/CD-dendrimer multilayer coatings at different irradiation times. Irradiation time (s) (top to bottom): 0, 5, 10, 15, 25 and 35 s; Irradiation intensity (at 365 nm): 350 μW/cm^2. Inset: relationship between $\ln[(A_0-A_e)/(A_t-A_e)]$ and irradiation time.

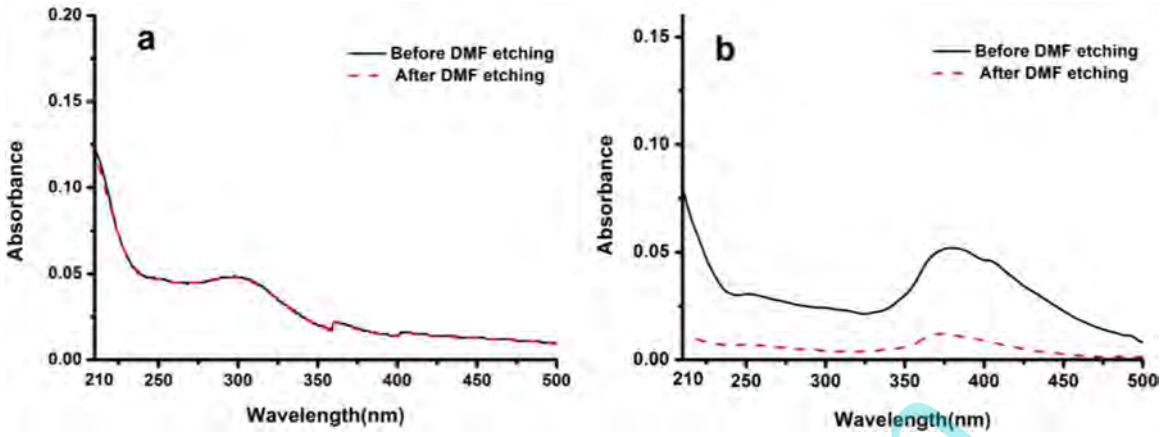

Fig. 4. UV–vis spectra of irradiated (A) and nonirradiated (B) DR/dendrimer multilayer coatings before (solid lines) and after (dash lines) etching with DMF at 25 °C for 30 min.

3.1.3. Stability

As can be seen in Fig. 4, the spectrum of the UV irradiated coating does not change after immersion in DMF for 30 min (Fig. 4a), due to its covalently crosslinked structure. However, the spectrum of the non-irradiated film (Fig. 4b) changes dramatically because of the etching by the DMF.

3.1.4. Morphology

AFM images (Fig. 5) shows the surface morphology of the bare and DR/CD-dendrimer covalently coated capillaries. The inner surface bare capillary is very smooth which has an average surface roughness (Ra) of 0.225 nm, and after surface modification with 2 and 12 layers of DR/CD-dendrimer covalent coatings, the Ra increases to 0.582 and 4.305 nm, respectively. The average thickness for the 2 and 12 layers of DR/CD-dendrimer covalent coatings is about 4.1 and 25.6 nm, respectively. The increased Ra and thickness with layer numbers indicates that the LBL coating process is carried out successfully. Since the 2-layer capillary coating is smoother than the 12-layer coating and facile to fabrication, it is adopted for the following studies.

3.2. Performance of the DR/CD-dendrimer coatings for CE analysis of proteins

3.2.1. EOF

Fig. 6 compares the EOF at different buffer pH values in bare, DR/CD-dendrimer non-covalently and covalently coated capillary columns. The magnitude of EOF in a capillary is dependent on the net surface charge density of chargeable groups. For bare capillaries, the dissociation of silanol groups is responsible for the generation of EOF, which increases with the increase of buffer pH, due to the strong dissociation of silanol groups in a high pH environment. By contrast, when the capillary is coated with DR/CD-dendrimer, especially the covalently coated DR/CD-dendrimer, the EOF decreases. For example, the EOF of the DR/CD-dendrimer covalently coated column at pH 4.0 is 5.40×10^{-9} m^2 V^{-1} S^{-1} that is much less than that of the uncoated one (1.96×10^{-8} m^2 V^{-1} S^{-1}), because the silanol groups on capillary surfaces which are responsible for the generation of EOF are mainly reacted and shielded by the DR/CD-dendrimer coating.

3.2.2. Effect of coating types

Fig. 7a–c shows CE separation results of four proteins by using bare capillary, DR/CD-dendrimer non-covalent, and DR/CD-dendrimer covalent capillary coatings in the optimized conditions, respectively. The bare capillary shows a strong adsorption to the

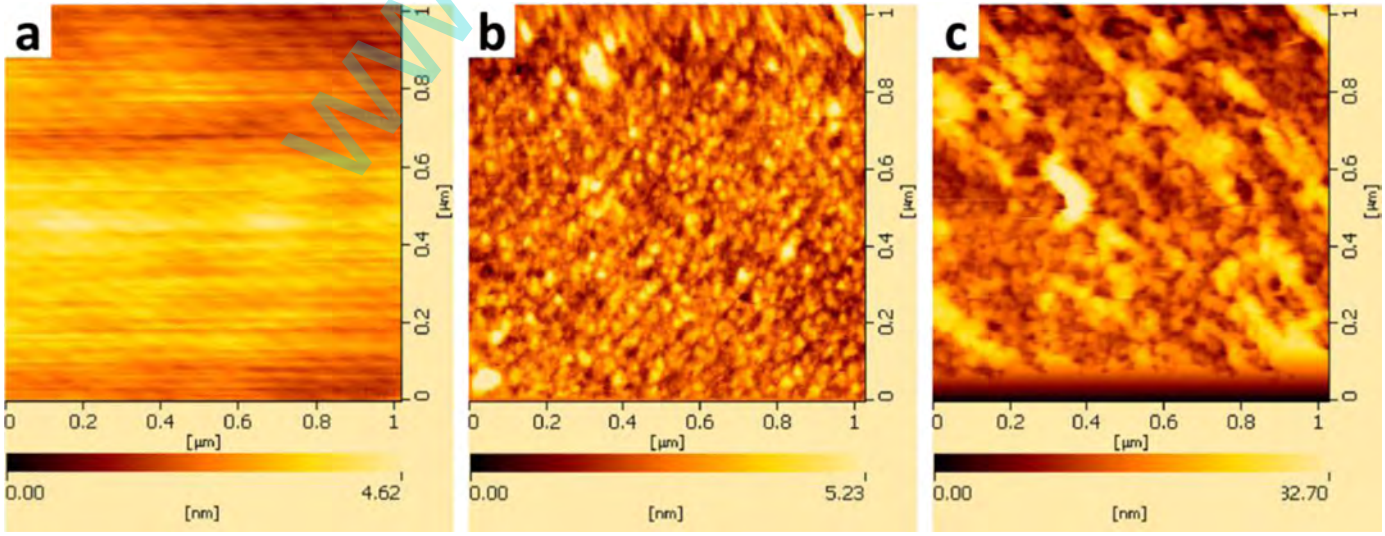

Fig. 5. AFM images of inner surface of (a) bare capillary, (b) two layers of DR/dendrimer covalently coated capillary, and (c) 12 layers of DR/dendrimer covalently coated capillary.

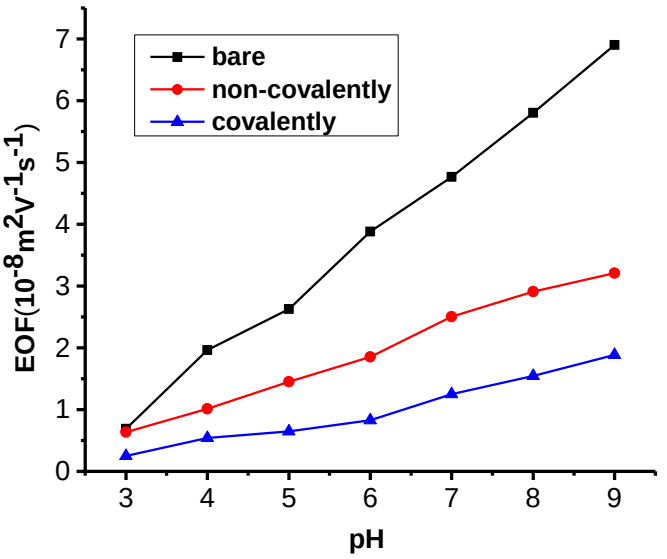

Fig. 6. Influence of pH on EOF of bare, DR/CD-dendrimer non-covalently and covalently coated capillary columns. Test conditions: buffer, 40 mM phosphate; injection, 20 s with a height difference of 20 cm; applied voltage, +15 kV; UV detection, 214 nm; capillary, 75 μm ID × 50 cm (41 cm effective); capillary temperature, 25 °C.

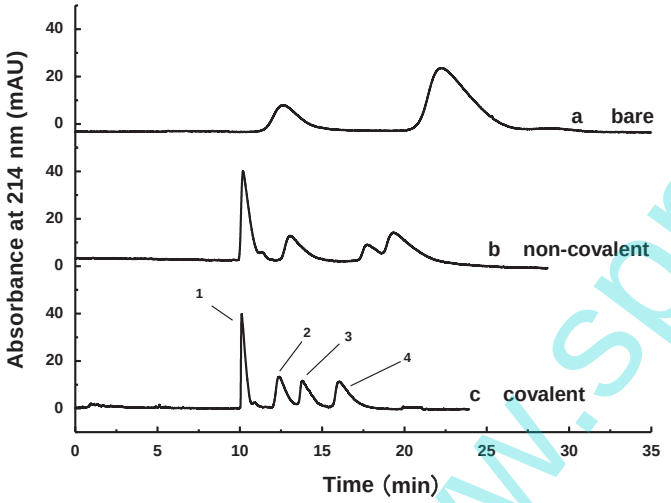

Fig. 7. Separation of four proteins using the bare capillary (a), two-layer DR/CD-dendrimer non-covalently coated capillary (b) and two-layer DR/CD-dendrimer covalently coated capillary (c). Separation conditions: buffer, 40 mM phosphate (pH=4.0); injection, 20 s with a height difference of 20 cm; applied voltage, +15 kV; UV detection, 214 nm; sample, 0.5 mg/mL for each protein; capillary, 75 μm ID × 50 cm (41 cm effective); capillary temperature, 25 °C. Peak identification: 1, Lys; 2, BSA; 3, Mb; 4, RNase A.

proteins, and thus a poor separation occurs with only two characteristic peaks obtained after a long elution time. Although the separation performance of DR/CD-dendrimer non-covalent capillary coating is better than that of bare capillary, the analysis time is also long and the characteristic peaks are broad. Consequently, effective separation of the proteins cannot be achieved, and the stability of the coating is very poor due to lack of strong bonding to the capillary. Compared with the other two, the DR/CD-dendrimer covalent capillary coating has the best separation performance, and a stable and baseline separation of the Cyt-c, Lys, BSA and RNase A is achieved within 18 min.

3.2.3. Separation performance

The 2-layer DR/dendrimer covalent coatings prepared by this method have very good stability and repeatability. Table 1

Table 1
Chemical stability of two-layer DR/CD-dendrimer covalent capillary coatings.

Rinse solvent	EOF before rinsing ($\times 10^{-8}$ m^2 V^{-1} S^{-1})	EOF after rinsing 15 min ($\times 10^{-8}$ m^2 V^{-1} S^{-1})	Degradation ratio[a] (%)
0.1 M NaOH	0.2441	0.2410	−1.27
0.1 M HCl	0.2401	0.2429	1.17
Acetone	0.2431	0.2476	1.85
DMF	0.2489	0.2441	−1.81

[a] EOF test conditions: the same as Fig. 6, and buffer pH=3.0.

Table 2
Separation performance of the two-layer DR/CD-dendrimer covalent capillary coatings.

Protein	Migration time RSD (%)[a]			
	Run to run (n=5)	Day to day (n=3)	Capillary to capillary (n=3)	Continuous 60 times running
Lys	0.48	1.10	2.11	1.07
BSA	0.53	2.08	3.30	2.19
Mb	0.86	2.31	3.63	2.89
RNase A	0.81	2.03	3.25	2.01

[a] Separation conditions: the same as Fig. 7.

indicates that the coatings can resist the long time flush of 0.1 M NaOH, 0.1 M HCl, and DMF, and the degradation ratio of EOF is less than 2%, which demonstrates that the DR/CD-dendrimer covalent coatings has excellent stability and good tolerance to the strong alkaline, acid and organic solvent.

As shown in Table 2, the run-to-run (n=5) RSD of migration time for the proteins is less than 1%, day-to-day (n=3) RSD is less than 2.5%, and capillary-to-capillary (n=3) RSD is less than 3%. After a continuous 60 times running in a coating column, the RSD of migration time for the proteins are all less than 3%, and the separation performance of the DR/dendrimer covalent coatings do not degraded. Therefore, the DR/dendrimer covalently coated capillaries are robust and may be used in heavy duty analysis.

4. Conclusions

In this work, a new type of covalently linked CD-dendrimer capillary coating is prepared successfully using photosensitive DR as coupling agents combined with the LBL self-assembly technique. The ionic bonding between the DR and dendrimer is converted into covalent bonding after treatment with UV light through the unique photochemistry of DR. Compared with the bare capillary and non-covalently bonded DR/dendrimer coatings, the covalently linked DR/CD-dendrimer capillary coatings improved the CE separation performance for proteins and exhibited good stability and repeatability. The covalently bonded coatings suppressed the protein adsorption on the inner surface of silica capillary, and thus a baseline separation of Lys, Mb, BSA and RNase A was achieved within 18 min under optimized conditions. Furthermore, this method is greener and simpler than traditional method for the use of DR instead of highly toxic and moisture sensitive silane coupling agent.

Acknowledgments

This work is financially supported by the National Natural Science Foundation of China (NSFC) (21375069, 21404065,

21574072), the Natural Science Foundation for Distinguished Young Scientists of Shandong Province (JQ201403), the Project of Shandong Province Higher Educational Science and Technology Program (J15LC20), the Graduate Education Innovation Project of Shandong Province (SDYY14028), the Scientific Research Foundation for the Returned Overseas Chinese Scholars of State Education Ministry (20111568), and the Postdoctoral Scientific Research Foundation of Qingdao.

References

[1] R. Haselberg, G.J. de Jong, G.M. Sonmsen, Capillary electrophoresis–mass spectrometry for the analysis of intact protein, J. Chromatogr. A 1159 (2007) 81–109.
[2] W.J. Chen, K. Mou, B. Xu, X.M. Ling, J.G. Cui, P. Xu, Capillary electrophoresis for screening of 20S proteasome inhibitors, Anal. Biochem. 394 (2009) 62–67.
[3] B. Yu, P. Liu, H.L. Cong, J.G. Tang, L.X. Zhang, Novel covalently coated diazoresin/polyvinyl alcohol capillary column for the analysis of proteins by capillary electrophoresis, Electrophoresis 33 (2012) 3066–3072.
[4] G. Zhu, L. Sun, N.J. Dovichi, Thermally-initiated free radical polymerization for reproducible production of stable linear polyacrylamide coated capillaries, and their application to proteomic analysis using capillary zone electrophoresis–mass spectrometry, Talanta 146 (2016) 839–843.
[5] S. de Jong, S.N. Krylov, Pressure-based approach for the analysis of protein adsorption in capillary electrophoresis, Anal. Chem. 84 (2012) 453–458.
[6] B. Yu, M.M. Jiao, H.L. Cong, X. Shu, S.J. Yang, A novel diazoresin/poly(N-vinyl aminobutyric acid) covalent capillary coating for the analysis of proteins by capillary electrophoresis, J. Sep. Sci. 37 (2014) 725–730.
[7] A. Yone, R.R. Carballo, D.A. Grela, I.N. Rezzano, N.M. Vizioli, Study of peptide-ligand interactions in open-tubular capillary columns covalently modified with porphyrins, Electrophoresis 32 (2011) 2840–2847.
[8] S. Bachmann, R. Vallant, R. Bakry, C.W. Huck, D. Corradini, G.K. Bonn, CE coupled to MALDI with novel covalently coated capillaries, Electrophoresis 31 (2010) 618–629.
[9] X.F. Guo, X.M. Guo, H. Wang, H.S. Zhang, One step physically adsorbed coating of silica capillary with excellent stability for the separation of basic proteins by capillary zone electrophoresis, Talanta 144 (2015) 110–114.
[10] B. Yu, W. Cui, H.L. Cong, M.M. Jiao, P. Liu, S.J. Yang, A novel diazoresin/polyethylene glycol covalent capillary coating for analysis of proteins by capillary electrophoresis, RSC Adv. 3 (2013) 20010–20015.
[11] L. Vitali, F.D. Betta, A.C.O. Costa, F.A.S. Vaz, M.A.L. Oliveira, J.P. Vistuba, G. A. Micke, New multilayer coating using quaternary ammonium chitosan and κ-carrageenan in capillary electrophoresis: application in fast analysis of betaine and methionine, Talanta 123 (2014) 45–53.
[12] F.H. Cao, Z.F. Luo, D. Zhou, R.J. Zeng, Y.M. Wang, Hydroxyethylcellulose-graft-poly(2-(dimethylamino)ethyl methacrylate) as physically adsorbed coating for protein separation by CE, Electrophoresis 32 (2011) 1148–1155.
[13] M.D. Gulcev, C.A. Lucy, Factors affecting the behavior and effectiveness of phospholipid bilayer coatings for capillary electrophoretic separation of basic proteins, Anal. Chem. 80 (2008) 1806–1812.
[14] Y. Liu, X.F. Fu, Y. Bai, M.L. Zhai, Y.P. Liao, J. Liao, H.W. Liu, Improvement of reproducibility and sensitivity of CE by using the capillary coated dynamically with carboxymethyl chitosan, Anal. Bioanal. Chem. 399 (2011) 2821–2829.
[15] C. Zheng, Y.P. Liu, Q.H. Zhou, X. Di, Capillary electrophoresis with non-covalently bilayer-coated capillaries for stability study of allergenic proteins in simulated gastrointestinal fluids, J. Chromatogr. B 878 (2010) 2933–2936.
[16] B. Yu, H.L. Cong, H.W. Liu, Y.Z. Li, F. Liu, Ionene-dynamically coated capillary for analysis of urinary and recombinant human erythropoietin by capillary electrophoresis and online electrospray ionization mass spectrometry, J. Sep. Sci. 28 (2005) 2390–2400.
[17] C. Nilsson, K. Becker, I. Harwigsson, L. Bulow, S. Birnbaum, S. Nilsson, Hydrophobic interaction capillary electrochromatography of protein mutants. Use of lipid-based liquid crystalline nanoparticles as pseudostationary phase, Anal. Chem. 81 (2009) 315–321.
[18] J.R. Catai, J.S. Torano, P.M.J.M. Jongen, G.J. de Jong, G.W. Somsen, Analysis of recombinant human growth hormone by capillary electrophoresis with bilayer-coated capillaries using UV and MS detection, J. Chromatogr. B 852 (2007) 160–166.
[19] Y. Sultan, R. Walsh, C. Monreal, M.C. Derosa, Preparation of functional aptamer films using layer-by-layer self-assembly, Biomacromolecules 10 (2009) 1149–1154.
[20] R. Haselberg, G.J. de Jong, G.W. Somsen, Capillary electrophoresis-mass spectrometry of intact basic proteins using polybrene-dextran sulfate-polybrene-coated capillaries: system optimization and performance, Anal. Chim. Acta 678 (2010) 128–134.
[21] L. Xu, X.Y. Dong, Y. Sun, Novel poly(vinyl alcohol)-based column coating for capillary electrophoresis of proteins, Biochem. Eng. J. 53 (2010) 137–142.
[22] T.T. Razunguzwa, M. Warrier, A.T. Timperman, ESI-MS compatible permanent coating of glass surfaces using poly(ethylene glycol)-terminated alkoxysilanes for capillary zone electrophoretic protein separations, Anal. Chem. 78 (2006) 4326–4333.
[23] P. Tuma, E. Samcova, K. Stulik, Determination of the spectrum of low molecular mass organic acids in urine by capillary electrophoresis with contactless conductivity and ultraviolet photometric detection – an efficient tool for monitoring of inborn metabolic disorders, Anal. Chim. Acta 685 (2011) 84–90.
[24] J. Mei, Y.P. Tian, W. He, Y.X. Xiao, J. Wei, Y.Q. Feng, Preparation approaches of the coated capillaries with liposomes in capillary electrophoresis, J. Chromatogr. A 1217 (2010) 6979–6986.
[25] S.Y. Zhou, J.J. Tan, Q.H. Chen, X.C. Lin, H.X. Lu, Z.H. Xie, Carboxymethylchitosan covalently modified capillary column for open tubular capillary electrochromatography of basic proteins and opium alkaloids, J. Chromatogr. A 1217 (2010) 8346–8351.
[26] J. Lokajova, H. Tiala, T. Viitala, M.L. Riekkola, S.K. Wiedmer, Covalent binding of phospholipid vesicles on fused silica capillaries for electrochromatography, Soft Matter 7 (2011) 6041–6050.
[27] K. Omae, T. Sakai, H. Sakurai, K. Yamazaki, T. Shibata, K. Mori, M. Kudo, H. Kanoh, M. Tati, Acute and subacute inhalation toxicity of silane 1000 ppm in mice, Arch. Toxicol. 66 (1992) 750–753.
[28] G.D. Clayton, F.E. Clayton (Eds.), Patty's Industrial Hygiene and Toxicology, Wiley, New York, 1981.
[29] X.P. Wang, J.B. Tang, M.H. Sui, X.P. Wang, J.X. Xu, Y.Q. Shen, Degradable water soluble hyperbranched polymers for drug delivery, J. Control. Release 152 (2011) e76–e78.
[30] R.K. Tekade, P.V. Kumar, N.K. Jain, Dendrimers in oncology: an expanding horizon, Chem. Rev. 109 (2009) 49–87.
[31] T.H. Mourey, S.R. Turner, M. Rubinstein, J.M.J. Frechet, C.J. Hawker, K.L. Wooley, Unique behavior of dendritic macromolecules: intrinsic viscosity of polyether dendrimers, Macromolecules 25 (1992) 2401–2406.
[32] X.P. Ma, Z.X. Zhou, E.L. Jin, Q.H. Sun, B. Zhang, J.B. Tang, Y.Q. Shen, Facile synthesis of polyester dendrimers as drug delivery carriers, Macromolecules 46 (2012) 37–42.
[33] C.Q. Shou, X.X. Xing, J.F. Kang, Z.L. Zhang, N.J. Song, P. Shang, Carbosilane dendrimer for the coated capillary electrophoresis column, Chin. J. Anal. Chem. 36 (2008) 167–171.
[34] A. Kabir, C. Hamlet, K.S. Yoo, G.R. Newkome, A. Malik, Capillary microextraction on sol–gel dendrimer coatings, J. Chromatogr. A 1034 (2004) 1–11.
[35] C. Zhao, J.Y. Chen, W.X. Cao, Synthesis and characterization of diphenylamine diazonium salts and diazoresins, Angew. Makromol. Chem. 259 (1998) 77–82.
[36] Y.X. Han, M.Z. Ye, J.B. Tang, M.H. Sui, X.R. Liu, Y.Q. Shen, Synthesis and properties of zwitterionic dendrimer as drug and imaging probe carrier, J. Control. Release 213 (2015) e144–e145.
[37] A. Plewa, W. Niemiec, J. Filipowska, A.M. Osyczka, R. Lach, K. Szczubialka, M. Nowakowska, Photocrosslinkable diazoresin/pectin films–synthesis and application as cell culture supports, Eur. Polym. J. 47 (2011) 1503–1513.
[38] Y. Fu, G.J. Yao, Y.P. Dong, Y.J. Cao, E.K. Duan, Covalent-attached self-assembly film based on diazor esin and bovine serum albumin and the cell culture on its surface, Chem. J. Chin. Univ. 24 (2003) 182–183.
[39] J.Y. Chen, W.X. Cao, Fabrication of a covalently attached self-assembly multilayer film *via* H-bonding attraction and subsequent UV-irradiation, Chem. Commun. 17 (1999) 1711–1712.
[40] Z.H. Yang, J.Y. Chen, W.X. Cao, Self-assembled ultra-thin films fabricated from diazoresin and phenol–formaldehyde resin via H-bonding interaction, Polym. Int. 53 (2004) 815–819.
[41] H. Zhu, M.J. Mcshane, Macromolecule encapsulation in diazoresin-based hollow polyelectrolyte microcapsules, Langmuir 21 (2005) 424–430.
[42] B. Yu, H.L. Cong, H.W. Liu., C.H. Lu, F. Wei, W.X. Cao, Fabrication and characterization of stable ultrathin film micropatterns containing DNA and photosensitive polymer diazoresin, Anal. Bioanal. Chem. 384 (2006) 385–390.