Synthesis of Hyperbranched Polycarbosilane Modified with Cyclodextrin Derivatives and Its Application in Coated Capillary Electrophoresis Columns

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Received 9 March 2010; accepted 22 January 2011
DOI 10.1002/app.34273
Published online 15 June 2011 in Wiley Online Library (wileyonlinelibrary.com).

ABSTRACT: A series of hyperbranched polycarbosilanes were synthesized and coated on the inner surface of fused-silica capillaries by chemical bonding. The end groups of the hyperbranched polycarbosilanes were modified with β-cyclodextrin derivatives. The chiral isomers of ofloxacin and chlorpheniramine were separated by this new type of chiral capillary electrophoresis column and high-column efficiency was achieved. The results showed that ofloxacin was completely separated at pH 3.0; the separation resolution was 2.32, and theoretical plates were greater than 4.5×10^4 plates m^{-1}. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 122: 2167–2173, 2011

Key words: capillary coating; cyclodextrin derivatives; capillary electrophoresis column; chiral separation; hyperbranched polycarbosilane

INTRODUCTION

In recent years, the use of modified stationary phases in high-performance capillary electrophoresis (HPCE) has allowed for new and rapid column separation techniques,[1] especially in the analysis of chiral compounds.[2] Compared with HPLC technology, advantages possessed by HPCE are shown as followed: (1) the capillary column is easy to be cleaned and avoided being contaminated. (2) The higher resolutions are obtained with the simple pretreatment in short analysis times. (3) Complex samples, such as ribonucleic acid, which play important role in the process of life, can be separated effectively. (4) Provides a permanent surface requiring with much less material than the traditional methods that the chiral selection agent be simply added to the running buffer. Thus, HPCE has attracted significant attention in contemporary analytical science.[3,4]

The development of chiral separation environment is the key point to any successful capillary electrophoresis (CE) separation of enantiomers.[5] Currently, chiral separation has been accomplished through chiral additives to the running buffer, or the linear polymers are covalently bonded to the surface of capillary columns. The former method has low efficiency and a high cost of operation. By contrast, the polymers are covalently bonded on the surface of capillary columns exhibited higher column efficiency and lower cost of operation. More importantly, the capillary columns can be used repeatedly. The research of enantiomer separation is witnessing an explosion of interest in using the modified polymers.

Presently, traditional linear polymers, such as polyacrylamide and polysiloxane,[6–11] are used as coating materials for chiral capillary columns. However, some inevitable issues have appeared as below: first, the viscosity of linear polymers increases with the increasing concentration of the solution. This can result in the formation of a liquid droplet, which can block the capillary. Second, owing to the chain structure, functional groups are rarely available to bond with the silanol groups on the inner surface of the capillaries, so the separation efficiency is not optimized.[12–18] Therefore, developing coated materials with low viscosity and more available functional groups is a priority for the development of enantiomer separation technology in CE. Hyperbranched polymers are a new kind of functional polymer with a large amount of functional groups on their molecule periphery, which are available to bond to the inner surface of fused-silica capillaries and form a stable and reproducible coating. Subsequently, more chiral selectors can be grafted to the inner surface of capillary by chemical bonding. Additionally, owing to the highly branched structure, hyperbranched polymers possess a lower melting viscosity than the corresponding linear counterparts at the same molar mass.[19,20] This prevents the generation of chain

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entanglement and, consequently, it is easy to coat the capillary inner surface. Overall, hyperbranched polymers can significantly improve separation efficiency over linear polymers due to their unique chemical and physical properties.[19,20]

To our knowledge, it is not reported that hyperbranched polycarbosilanes are coated onto the inner surface of a fused silica capillary by chemical bonding to perform chiral separations. In this study, β-cyclodextrin (β-CD) derivatives were introduced to a fused silica capillary wall to generate a covalently bond stationary phase, in which a series of hyperbranched polycarbosilanes acted as a coupling agent. To demonstrate the effectiveness of this covalently bond coating, ofloxacin and chlorpheniramine were separated on a β-CD-modified fused silica capillary.

MATERIALS AND METHODS

Instrument and reagent

The chiral separation was performed on a QL-1000 CE instrument, which was purchased from Shandong Normal University, (Shandong, China). Data collection and processing were accomplished on an N2000 workstation (Zhejiang University, Zhejiang, China). Fused silica capillaries with a 75 μm i.d. were used for the preparation of the capillary column, which can be acquired from Hebei Yongnian Optical Fiber Factory, (Hebei, China).

2,4,6,8-Tetramethyl-2′,4′,6′,8′-tetra-vinyl-cycle-tetrasiloxane (D_4^{vi}) was purchased from Shanghai Jiancheng Industry & Trade (Shanghai, China). Methyldichlorosilane was obtained from Jiangxi Lanxing Chemical Industry (Jiangxi, China). Methyl hydrogen diallyl silane and Karstedt catalyst were all prepared in house. Ofloxacin, which was the chemical reference, was provided by the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Chlorpheniramine was bought from Shenyang Xindi Pharmaceutical (Liaoning, China). All the reagents above were chemically pure. 2-*O*-(2-Hydroxypropyl)-β-cyclodextrin (HP-β-CD) with analytical purity was supplied by Tianjin Kermel Chemical Reagent (Tianjin, China). Phosphoric acid, sodium hydroxide, dichloromethane, dimethyl sulfoxide, and methanol were all analytically pure reagents. In this study, secondary deionized water was used in the preparation of the coated capillaries and buffer. The phosphate buffers were prepared by dissolving a weighed amount of sodium dihydrogenphosphate in water and the addition of sodium hydroxide to adjust pH values.

The synthesis of hyperbranched polycarbosilane

In this experiment, hyperbranched polycarbosilanes were synthesized by hydrosilylation in which we used methyl hydrogen diallyl silane as the growth monomer, D_4^{vi} as the core molecule, and Karstedt catalyst as the catalyst for hydrosilylation. Hyperbranched polycarbosilanes of different generations were prepared by mixing various ratios of monomer to centronucleus.

The purified D_4^{vi} (1.03 g), 0.20 g of Karstedt-tetrahydrofuran (THF) solution and 50 mL of refined THF were added to the flask equipped with an agitator under a constant stream of nitrogen, respectively. The 3.05 g of refined methyl hydrogen diallyl silane and 50 mL of refined THF were added to the reaction system drop by drop within 5 ~ 6 h by controlling velocity with dropping funnel. The reaction lasted 12 h at room temperature, and then the second-generation hyperbranched polycarbosilane (G2) was obtained after removal of solvents and residual monomer through vacuum distillation. With the same synthesis method, the third-generation hyperbranched polycarbosilane (G3) was obtained by changing the molar ratio of reactants. The FTIR and NMR spectra of G2 and G3 were quite similar, because their chemical structures were similar.

G2: ^{1}H NMR ($CDCl_3$, 400 MHz): 0. 04 (s, 12H, Si Me), 0.08 (s, 12H, SiMe), 0. 68 (m, 8H, CH_2), 1.22 (m, 16H, CH_2), 5.59 (m, 48H, —CH=CH_2). FTIR (KBr pellet, cm^{-1}): 1632 cm^{-1} (—CH=CH_2), 2930 cm^{-1}, 1450 cm^{-1} (—CH_2—), 2970 cm^{-1}, 2875 cm^{-1}, 1385 cm^{-1}(—CH_3). The synthesis route is shown in Figure 1.

The modification of end groups of hyperbranched polycarbosilane

The second-generation hyperbranched polycarbosilane and Karstedt-THF solution were mixed under nitrogen. Then, methyl hydrogen diallyl silane (According to the theory, the ratio of molar percentage of double bonds to methyl hydrogen diallyl silane equals 4 : 1) and refined THF were added to the reaction system by drops within 2 h. After 12 h at room temperature, refined THF was added by dropping over 3 h, in which β-CD derivatives had been dissolved. Keep the reaction 3 h at room temperature, and the second-generation hyperbranched polycarbosilane modified with HP-β-CD (G2-HP-β-CD) was obtained. Similarly, the third-generation hyperbranched polycarbosilane modified with HP-β-CD (G3-HP-β-CD) was prepared by changing the molar ration of reactants.

Because of the branching nature of hyperbranched polycarbosilane, the more reactive groups with exponential are available than traditional linear polymers. Consequently, the target groups are obtained by modification of double bonds in the outer end of hyperbranched polycarbosilanes and then reacted with hydroxyl located on C-6 of HP-β-CD. The mechanism of modification is shown in Figure 2.

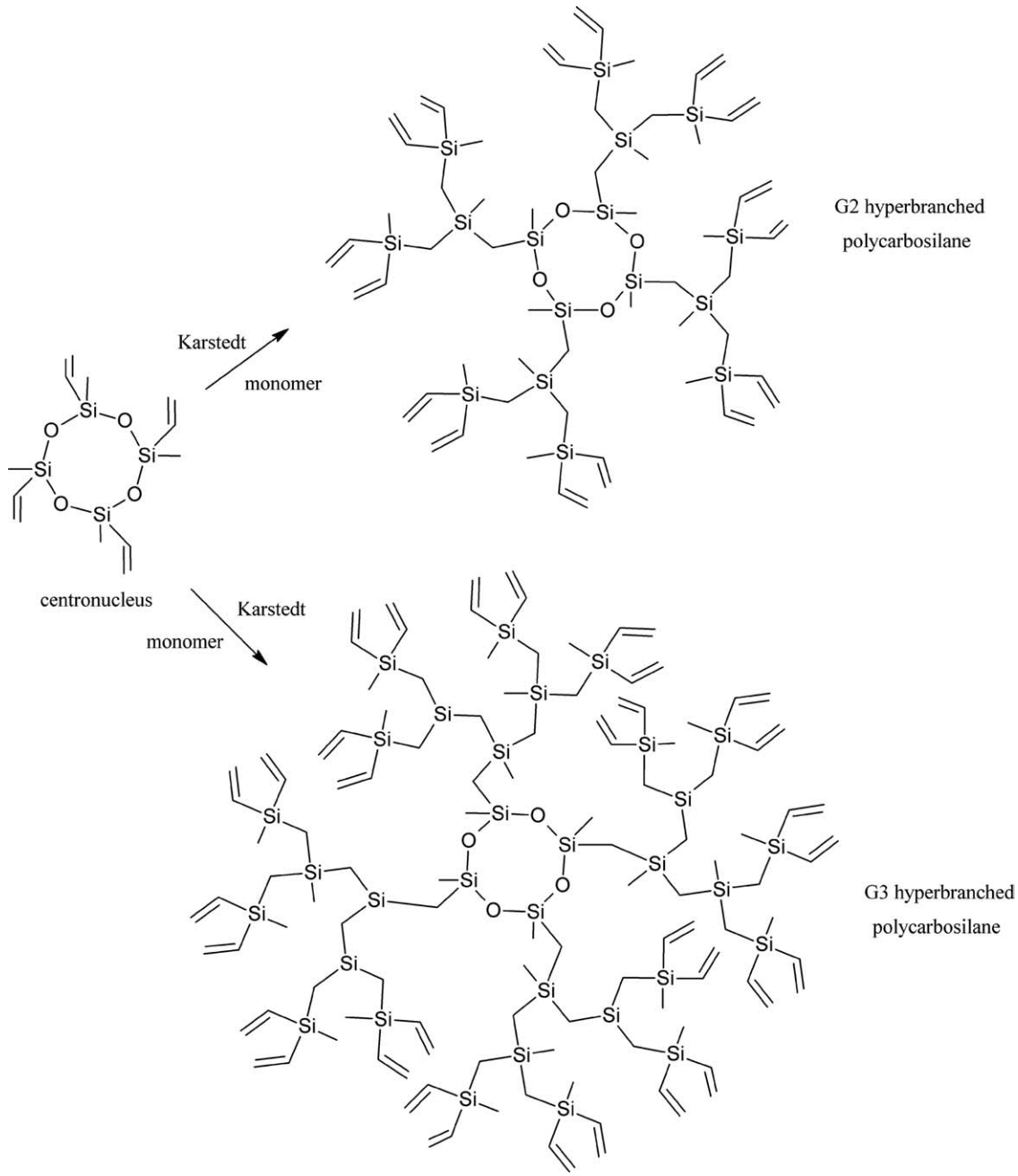

Figure 1 Synthesis of the hyperbranched polycarbosilane with D_4^{vi} as the core molecule and methyl hydrogen diallyl silane as the growth monomer.

Preparation of chemically bonded coating for capillary column

First, the Si—OH groups on the inner surface of the capillary are modified by ethylene linkages with silane coupling agent. Then, with azodiisobutyronitrile (AIBN) as a thermal initiator, the carbon–carbon double bonds on the inner surface of the capillary are thermally cured with the carbon–carbon double bonds in the outer end of hyperbranched polycarbosilanes; then, the hyperbranched polycarbosilanes are bonded on the inner surface of the capillary, and this process is shown in Figure 3.

The preparation process is as follows: the pretreated capillary was cleaned with secondary deionized water and dried with a high-purity nitrogen flow, flushed for 15 min with the toluene solution and nitrogen dried, and then the toluene solution containing 10% of vinyl triethoxy silane was introduced into the capillary with negative pressure, sealed with the soluble glass before being placed in a drying oven at 110°C and reacted for 40 min. In the end, the capillary was flushed with toluene and methanol for 10 min, respectively, and the silanization capillary column was prepared.

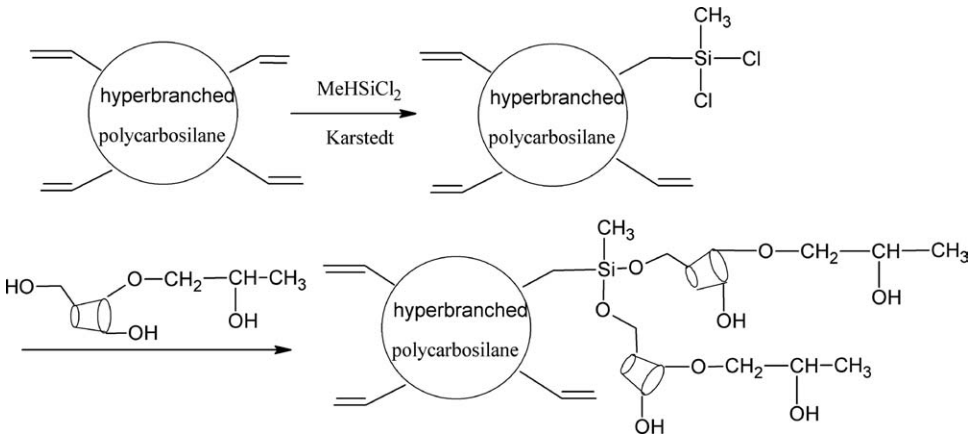

Figure 2 Modification of the end groups of hyperbranched polycarbosilane with β-cyclodextrin derivatives through reacting with hydroxyl located on C-6.

The second-generation hyperbranched polycarbosilane with modification (4.13 g) was dissolved by 200 g of *N*,*N*-dimethylformamide, and then 3.5 g of AIBN was added, and mixed for 20 min. The extracted solutions of 10 mL were added to the capillary modified with silane using coating column equipment. The reaction lasted 2 h in an incubator at 60–65°C. Then, the column was taken out and washed with methanol and secondary deionized water twice for 15 min, respectively.

The prepared column was placed in a gas chromatography oven and held at a constant temperature of 50°C for 30 min. The column was then heated from 50 to 150°C at the rate of 1°C min^{-1} and held at the final temperature for 3 h under a gentle nitrogen flow.

The electrophoresis running conditions

A 1% (V/V) dimethyl sulfoxide solution in secondary deionized water was selected to detect electro-osmotic flow, and the solution used in separating samples of 1 $\times$ 10^{-4} g mL^{-1} were introduced into the capillary by hydrodynamic injection by 10 cm high difference for 7 s at a constant voltage(16 kV); the 40 $\times$ 10^{-3} mol L^{-1} phosphate solution was selected as the running phosphate buffer; the UV detection wavelength was 214 nm; the length of capillary was 55 cm; and the inner diameter was 75 μm, all the solvent and solution were filtered with a 0.45-μm filter membrane and got rid of air in ultrasonic cleaner for 10 min.

RESULTS AND DISCUSSION

The enantiomers of ofloxacin and chlorpheniramine were separated with a chemically bonded chiral coating, and the results are shown in Figure 4 and Table I. As can be seen from Figure 4 and Table I, a chemically bonded, coated capillary column modified with HP-β-CD is able to achieve baseline separation of ofloxacin isomers. The resolution and theoretical plates reached 2.32 and 4.50 $\times$ 10^4, respectively. Whereas the resolution was 0.91 and fell short of baseline separation when chlorpheniramine was separated.

The influence of pH value on chiral separation

The influence of different pH values of phosphate buffer on separation of ofloxacin and chlorpheniramine isomers were examined, and the coated materials were G2-HP-β-CD. The results are shown in Figure 5.

It was evident from Figure 5 that the separation efficiency of chiral isomers was different at various pH values. Before pH 3.0, the column efficiency and resolution increased with higher pH values of the running buffer. Ofloxacin was separated completely with a higher resolution of 2.32 when pH values was 3.0, and then decreased quickly with an increase in the pH of the running buffer. The column efficiency reached the upper value at pH 3.0. However, the column efficiency and resolution reached a maximum at pH 3.5 for chlorpheniramine though the resolution fell short of baseline separation. The

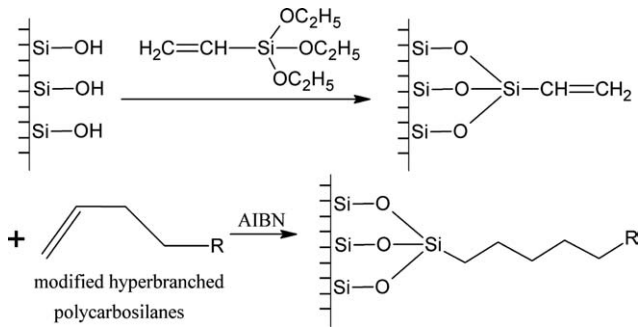

Figure 3 Scheme of the modified hyperbranched polycarbosilanes chemically bonded to the fused-silica capillary.

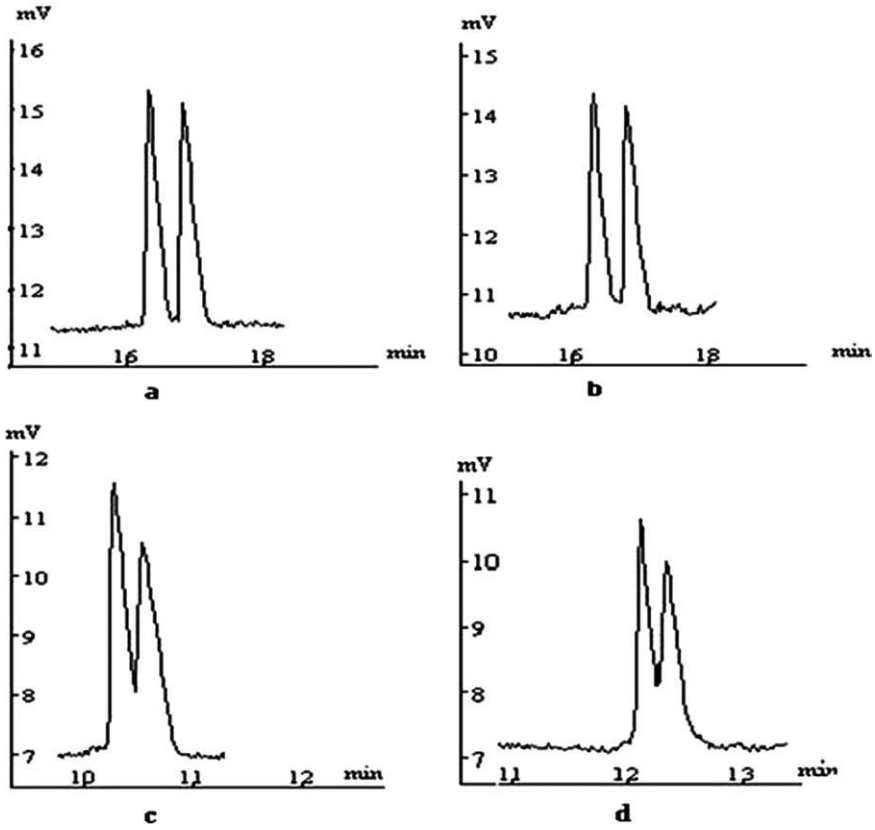

Figure 4 Separation of ofloxacin(a, b) and chlorpheniramine(c, d) by capillary modified by covalently bonding of hyperbranched polycarbosilane with HP-β-CD: (a) and (c) were G2-HP-β-CD-coated columns, (b) and (d) were G3-HP-β-CD-coated columns. (conditions: capillary, 55 cm × 75 μm i.d.; buffer, 0.04 mol L^{-1} phosphate; operation voltage, 16 kV; hydrodynamic injection, 7 s at 10 cm).

separation theoretical plate number reached a maximum of 3.91×10^4. From data above, pH 3.0 and pH 3.5 were chosen as the optimal conditions to separate ofloxacin and chlorpheniramine, respectively.

The influence of generations of hyperbranched polycarbosilane on separation of chiral isomers

In this study, the coating materials of the capillary were different generations of hyperbranched polycarbosilane after modification. The relationship between column efficiency and generation of hyperbranched polycarbosilane was examined, in which the end group modification reagent was HP-β-CD.

It was shown from Table I that the separation theoretical plate number of the hyperbranched polycarbosilane-coated capillary column modified with G3 was close to G2, and the difference was less than 4.5%. However, the result of separation resolution was reversed, the difference was higher than 30% when ofloxacin was separated. The more modified end groups in the outer end of G3, which can bond more cyclodextrin molecules, improved the separation efficiency of the coated capillary column.

From the discussion in a paragraph above, the conclusion was demonstrated that G2-coated column modified with HP-β-CD was an ideal coated capillary

TABLE I
The Resolution and Theoretical Plates of Enantiomers in Chiral Separation

	Ofloxacin		Chlorpheniramine	
Columns	Resolution (Rs)	N ($\times 10^4$) (plates m^{-1})	Resolution (Rs)	N ($\times 10^4$) (plates m^{-1})
Blank column	–	–	–	–
G2-HP-β-CD	2.32	4.51	0.91	3.91
G3-HP-β-CD	1.59	4.67	0.87	4.08
Difference	31%	3.5%	4.4%	4.3%

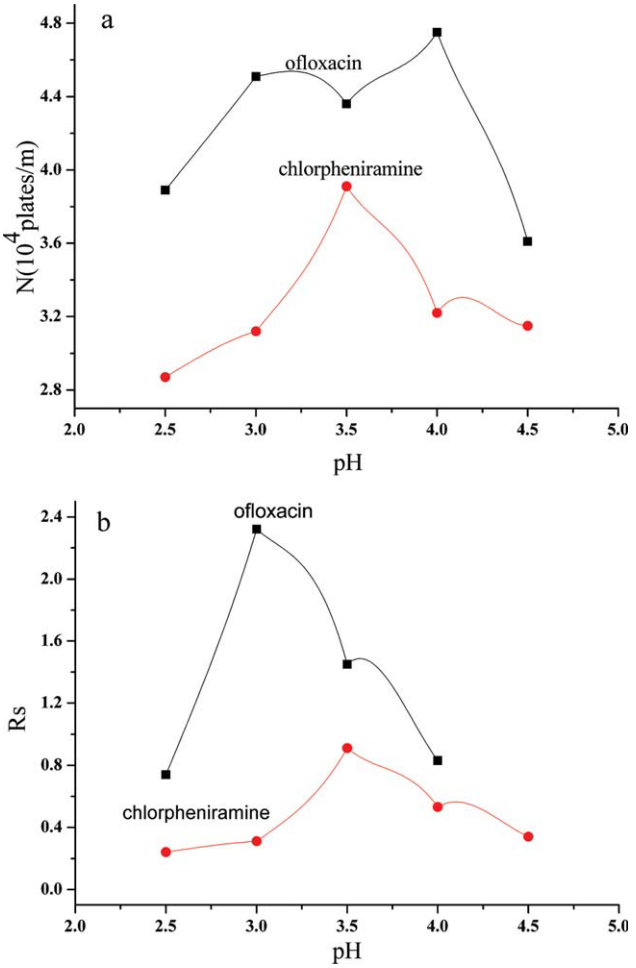

Figure 5 Effect of the buffer pH on the column efficiency (a) and resolution (b). (conditions: capillary, 55cm × 75μm i.d. G2-HP-β-CD coated fused-silica capillary column; buffer, 0.04 mol L^{-1} phosphate; operation voltage, 16 kV; hydrodynamic injection, 7 s at 10 cm). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

with the characteristics of stable operation, simple preparation, and excellent separation efficiency.

The influence of running number on coated column performance

The chiral isomers of ofloxacin and chlorpheniramine were separated at pH3.0 and pH3.5 with a G2-HP-β-CD modified capillary column, respectively. The stability of the hyperbranched polycarbosilane capillary column modified with G2 and G3 was measured.

TABLE II
Influence of Theoretical Plates of Running Number of Ofloxacin

Running numbers	N ($\times 10^4$) (plates m^{-1})	Resolution (Rs)	N Decline rate (%)
1	4.5	2.32	–
50	4.32	1.52	4.00
100	4.18	1.53	7.11
150	4.27	1.56	5.11

TABLE III
Influence of Theoretical Plates of Running Number of Chlorpheniramine

Running numbers	N ($\times 10^4$) (plates m^{-1})	Resolution (Rs)	N Decline rate (%)
1	3.91	0.91	–
50	3.67	0.71	6.10
100	3.52	0.82	9.97
150	3.55	0.78	9.21

From Tables II and III, we can see that there was little decrease in efficiency with the hyperbranched polycarbosilane coated capillary column after continuous running of 150. Meanwhile, the decline rate was less than 10%, and there was little change of resolution. The reason was possibly that the silica–carbon bonds consisted in main chain of hyperbranched polycarbosilane macromolecule could resist acid–base, in addition, the silica–oxygen bonds, which generated between HP-β-CD and modified hyperbranched polycarbosilane, were steady under the condition of acid–base. Thus, the stability of coated columns could keep well within long time.

CONCLUSIONS

In this study, a new style of chiral coating column for CE was developed with a series of hyperbranched polycarbosilanes functionalized with β-CD derivatives, in which the modified hyperbranched polycarbosilanes are coated on the inner surface of the capillary by chemical bonding and a covalently bonded stationary phase is formed. A higher separation efficiency for the two chiral enantiomers ofloxacin and chlorpheniramine was achieved by the prepared columns. Efficiencies greater than 4.6×10^4 plates for ofloxacin and 4.0×10^4 plates for chlorpheniramine were achieved. The application of hyperbranched polymers in analytical science has become broader and new routes in separation of chiral enantiomers are presented by hyperbranched polycarbosilane-modified capillaries.

The author gratefully acknowledges the Scott for overall help and discussion.

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