

Retention Behavior of β -Cyclodextrin-bonded Stationary Phase Synthesized via Atom-transfer Radical Polymerization and Click Chemistry

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A new chiral stationary phase was designed and synthesized via surface-initiated atom-transfer radical polymerization (ATRP) and click chemistry. Poly(2-methyl-3-butyn-2-yl methacrylate-co-divinylbenzene), poly(MBMA-co-DVB), was grafted on silica surface via ATRP ("grafting-from" technique) first, and then azide-modified β -CD was immobilized on the alkyne of the polymer layer as chiral separation material. Different crosslinking degree of poly(MBMA-co-DVB) was selected to improve the hydrophobicity of silica surface. The materials were packed into a stainless-steel column (150 mm \times 4.6 mm i.d.) for high-performance liquid chromatography (HPLC), and evaluated by separation of aromatic and chiral compounds. The result demonstrated that the crosslinked benzene ring in poly(MBMA-co-DVB) can well improve the selectivity and retention time.

The specific structure of β -cyclodextrin (β -CD) demonstrates multiple retention mechanisms as chromatographic stationary phase, especially to recognize chiral compounds.^{1,2} The hydrophilic exterior-surface of β -CD is suitable for hydrophilic interaction liquid chromatography.³ The inner-surface also possesses hydrophobic properties, which may be beneficial for reversed-phase liquid chromatography (RPLC). The RPLC is the most commonly used chromatographic mode for separation of chiral compounds, disubstituted benzenes, alkylbenzene, etc.⁴⁻⁶ Under RPLC mode, the β -CD-bonded stationary phase shows great separation efficiency for positional and stereo optical isomers. However, the separation ability for aromatic compounds is undesirable.⁵ It might be affected by the properties of the stationary substrate. For example, even with the same method to immobilize β -CD, different substrates can have a great impact on the chromatographic behavior of flavanone isomers.^{7,8}

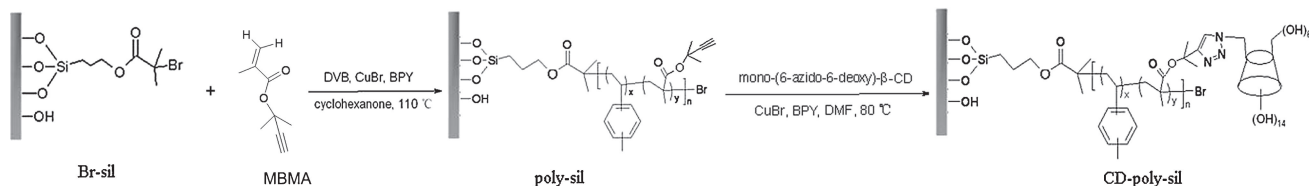
In this study, we designed a series of new stationary substrate for grafting β -CD to improve the separation properties of aromatic compounds as well as chiral compounds. Polymer-modified silica gel was prepared as the stationary substrate. For grafting polymer layer on the silica surface, atom-transfer radical polymerization (ATRP) was employed. ATRP can be used for

the synthesis of (co)polymers with predetermined molecular weight and narrow molecular-weight distributions, which make this method very attractive for the multifunctional material preparation.⁹

Herein surface-initiated ATRP on silica gel was used for the polymerization. The surface-attachable initiator 3-(2-bromoisobutyryloxy)propyltriethoxysilane (BPE) was prepared according to the literature methods.¹⁰ This initiator involves triethoxysilane reactive group which is able to bind to silica surfaces and a bromoisobutyryl group that can initiate the ATRP. The reaction between the triethoxysilane anchoring group and the surface OH-groups of silica resulted in the formation of SiI-initiator (Br-sil). High bromoisobutyryl group density on the surface of silica (0.9 $\mu\text{mol m}^{-2}$) was selected for grafting thin polymer membranes via ATRP.

For preparing the β -cyclodextrin-bonded stationary phase (CD-poly-sil), 2-methyl-3-butyn-2-yl methacrylate (MBMA)¹¹ as monomer and divinylbenzene (DVB) as crosslinker were used to graft polymer layer on the silica surface. The polymer layer was prepared by different crosslinking degree. The alkyne groups on the polymer layer were then reacted with β -CD bearing an azide group¹² via click chemistry (Scheme 1¹³ and Table 1). The click method is suitable for immobilization of CDs on silica supports to afford a well-defined structure due to its high reaction selectivity. In the click immobilization process of the β -CD, only the azide group of β -CD could react with the alkyne group on the silica supports. Therefore, the side reaction generated by competition of hydroxy groups at other position of the β -CD can be avoided, and the resulting β -CD bonded on the support surface was envisioned to keep its internal structure and performances.

The CD-poly-sils were slurry-packed into stainless-steel columns (150 mm \times 4.6 mm). To understand the chromatographic properties of the synthesized stationary phase with different hydrophobicity, the separation of aromatic compounds on CD-poly-sils was investigated under RPLC mode in the same mobile phase condition. We observed that with higher hydrophobicity, CD-poly-sils could yield much higher retention and better separation efficiency for aromatic compounds (Figure 1). We suppose the interactions (carbonyl π -benzene π and benzene



Scheme 1. Synthesis of CD-poly-sil.

Table 1. Synthesis of CD-poly-sils with different conditions

CD-poly-sils	Initiator/CuBr/BPY /MBMA/DVB /molar ratio	ΔC^a /g	β -CD density ^b / $\mu\text{mol m}^{-2}$
CD-poly-sil-1	1:2:4:10:0	0.010	0.21
CD-poly-sil-2	1:2:4:10:10	0.023	0.17
CD-poly-sil-3	1:2:4:10:20	0.027	0.05

^a ΔC is the carbon content increment in one gram of the substrate after ATRP obtained by elemental analysis. ^bThe β -CD density on the CD-poly-sils was determined according to reference.¹⁰

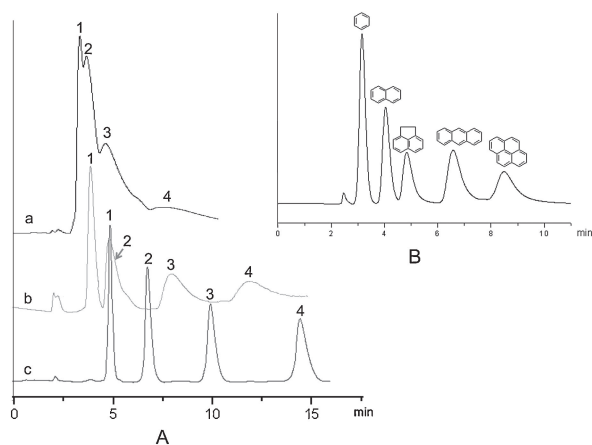


Figure 1. Chromatogram for aromatic compounds with CD-poly-sils. Flow rate: 1.0 mL min^{-1} . Detection wavelength: 254 nm. The mobile phase was MeCN/H₂O (38/62, v/v). A: the separation of alkylbenzene (1: methylbenzene; 2: ethylbenzene; 3: propylbenzene; 4: butylbenzene) on CD-poly-sils (a: CD-poly-sil-1; b: CD-poly-sil-2; c: CD-poly-sil-3); B: the separation of polycyclic aromatic hydrocarbons on CD-poly-sil-2.

π -benzene π) mainly contribute to the retention of the analyte. Higher retention factors were observed with CD-poly-sil-2 and CD-poly-sil-3, owing to the presence of crosslinked benzene ring in the substrate surface providing higher hydrophobicity. However, the CD-poly-sil-1 contains no crosslinking agent, and the separation efficiency of alkylbenzene is obviously inferior (seriously tailing) compared with CD-poly-sil-2 and CD-poly-sil-3.

The chiral recognition ability of the CD-poly-sil stationary phases toward structurally diverse chiral compounds was investigated. Table 2 summarized capacity factors (k) for first eluted enantiomers, separation factors (α), resolution (R_s) in the chiral separation. To have a better comparison for CD-poly-sils, the same mobile phase was used for each compound on both materials. Even the CD-poly-sil-3 provided better separation for alkylbenzene, there was no obvious resolution for chiral compounds. According to Table 1, the β -CD density on CD-poly-sil-3 stationary phase is very low, which might be the reason for lack of chiral recognition ability. As we can see, from CD-poly-sil-1 to CD-poly-sil-3, the β -CD density gradually decreases with the increase of crosslinking degree. We attribute the low β -CD density on CD-poly-sil-3 to the high crosslinked

Table 2. Enantioseparation of chiral compounds on CD-poly-sil stationary phases^a

Analyte	CD-poly-sil-1			CD-poly-sil-2 ¹³			CD-poly-sil-3		
	k_1	α	R_s	k_1	α	R_s	k_1	α	R_s
A	3.13	1.29	1.23	5.83	1.55	1.61	7.58	1.09	0.43
B	2.06	1.14	1.16	4.62	1.36	1.50	6.04	1.16	0.65
C	8.92	1.06	0.69	13.74	1.14	1.09	17.33	—	—
D	1.81	1.13	1.07	3.46	1.72	1.49	5.18	1.15	0.55

^aHPLC column size was $150 \text{ mm} \times 4.6 \text{ mm}$. Flow rate: 0.5 mL min^{-1} . Detection wavelength: 254 nm. The mobile phases: A (chlorthalidone) and C (benzoin) were MeOH/(1.0% TEAA, pH 4.9) (15/85, v/v); B (mandelic acid) was MeOH/(1.0% TEAA, pH 4.9) (5/95, v/v); D (chlorpheniramine) was MeCN/(0.3% TEAA, pH 6.8) (25/75, v/v). TEAA (triethylammonium acetate buffer) was prepared by addition of HOAc into 1.0% or 0.3% triethylamine aqueous solution to obtain desired pH.

polymer layer, which can embed the alkynyl groups and restrict the reaction of click chemistry for immobilizing β -CD.

Compared with CD-poly-sil-1, CD-poly-sil-2 shows better enantioseparation with appropriate retention time. It demonstrates that the crosslinked benzene ring in the substrate surface can interact with aromatic groups of chiral compounds through π - π interaction, which increases the retention time. This retention process can provide more chance for the chiral compounds to form inclusion complexes with β -CDs during the separation, and then better chiral selectivity can be achieved.

In conclusion, the use of surface-initiated ATRP ("grafting-from" technique) and click chemistry can prepare the chiral stationary phase with controlled molecular architecture. The chromatographic behavior of aromatic compounds demonstrates poly(MBMA-co-DVB) can increase the hydrophobicity of the silica surface and improve the separation efficiency. Appropriate retention time for the chiral compounds can be achieved by changing the hydrophobicity of silica surface, and also the chiral selectivity will be optimized.

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