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Short communication

Synthesis of a novel restricted access chiral stationary phase based on atom transfer radical polymerization and click chemistry for the analysis of chiral drugs in biological matrices

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ABSTRACT

A novel chiral restricted access material was synthesized via a combination of atom transfer radical polymerization (ATRP) and click chemistry. Poly(2-methyl-3-butyn-2-ol methacrylate) (pMBMA) was grafted onto porous silica gel by a surface-initiated ATRP in order to synthesize an inner layer for β-cyclodextrin (β-CD) immobilization. The azide-modified β-CD was bound to pMBMA by click chemistry. The results demonstrate that click chemistry provides an effective route for the immobilization of β-CD for chiral discrimination. A second ATRP reaction was then used to graft external poly(glycidyl methacrylate) (pGMA) layer onto the silica gel. The external hydrophilic layer was subsequently created by hydrolysis of the epoxy groups of the pGMA. This bi-layer grafted material exhibited both enantioseparation and protein exclusion. It can be used for the efficient separation of chiral compounds in biological samples with direct injection into an HPLC system.

1. Introduction

Restricted-access materials (RAMs) have been recognized as a suitable approach for direct HPLC injection of biological samples in pharmaceutical analysis to improve the analytical efficiency and accuracy of separations [1–6]. RAMs generally possess dual surface configurations. The inner layer is accessible only to small molecules and has the ability for their retention and separation. The outer surface is biocompatible but non-adsorptive to biomolecules. Proteins are excluded by a physical barrier due to the smaller pore diameters or by a chemical diffusion barrier created by the polymer network at the outer surface of the material. Different kinds of RAMs have been developed in recent years, but only a few articles have been published on RAMs with chiral selectors [7,8]. Very recently, we designed a RAM column that combined enantioselectivity and restricted-access properties [9]. In the synthesis of this material, atom transfer radical polymerization (ATRP) was employed to successively graft two layers of poly(glycidyl methacrylate) (pGMA) on the surface of porous silica gel. The internal layer of pGMA was used for immobilizing β-CD as a chiral selector. The external hydrophilic protein removal layer was created by hydrolysis of the outer layer of pGMA. The β-CD was immobilized by a reaction between the oxirane ring of GMA and the hydroxyl group of cyclodextrin. But this immobilization method has drawbacks like the uncertainty of the bonding position and the possible formation of multiple linkages of β-CD, since every hydroxyl group at the 2-, 3- and 6-positions of β-CD has the ability to react with the oxirane ring [10].

Click chemistry provides a way for selective chemical reactions with high yields. It can be used as a simple approach in the immobilization of highly functionalized molecules onto a solid-support. This method has been used to obtain well-defined structures and to avoid side-reactions in the preparation of chemically bonded β-CD stationary phases by Liang and his colleagues [11,12]. In this study, click chemistry was used as a more effective route to immobilize β-CD in the synthesis of a "comb-like" RAM (CD-click-RAM) by ATRP, in order to create a better CD bonding structure and improve the separation efficiency. A chiral RAM with good separation efficiency and the ability to exclude proteins was obtained.

2. Experimental

2.1. Preparation of CD-click-RAM

First, 3-(2-bromoisobutyryl)propyl triethoxysilane (BPE) was immobilized on silica gel as the ATRP initiator (Br-sil) [9]. A desirable initiator density (0.034 μmol m^{-2}) which determines the

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Table 1
The structures of the materials and protein recoveries from the stationary phases[a].

Material	Polymer grafted in ATRP	$n_{polymer}$[b]	Total pore area/$m^2\ g^{-1}$	Average pore diameter/Å	Recovery of BSA[c]
Silica gel	–	–	381.7	118.1	–
CD-click-sil	pMBMA	4.0	376.1	98.9	5.3%
CD-click-RAM	pGMA	49.2	280.7	80.7	98.2%

[a] Total pore area and pore diameter were measured by nitrogen sorption system.
[b] $n_{polymer}$ is the number of repeating unit of the grafted polymer chains, estimated by the carbon increment after the grafting reaction.
[c] The recovery of BSA was estimated by the peak area of BSA eluted from column by taking the area obtained without column as 100%. The concentration of BSA was 32 mg mL^{-1}.

Table 2
Enantioseparation of chiral compounds on CD-click-sil and CD-click-RAM stationary phases[a].

Analyte	Retention factor (k_1)		Enantioselectivity (α)		Resolution (R_s)	
	CD-click-sil	CD-click-RAM	CD-click-sil	CD-click-RAM	CD-click-sil	CD-click-RAM
1. Chlorthalidone	9.60	8.13	1.44	1.29	1.58	1.34
2. Ibuprofen	4.07	3.51	1.13	1.07	0.50	0.43
3. Mandelic acid	4.03	3.41	1.43	1.15	1.60	1.41
4. Benzoin	12.42	11.17	1.18	1.09	0.88	0.69
5. Aminoglutethimide	9.04	8.16	1.12	1.05	0.92	0.76
6. Chlorpheniramine	3.16	2.90	1.81	1.54	1.87	1.51

[a] HPLC column size was 150 mm × 4.6 mm. The flow rate was 0.5 mL min^{-1}. Detection wavelength was 254 nm. The mobile phases were: MeOH/(0.3% TEAA, pH 4.9) (1/9, v/v) for analytes 1 and 3, MeOH/(0.3% TEAA, pH 4.9) (5/95, v/v) for 4, MeCN/(0.3% TEAA, pH 6.8) (1/9, v/v) for 6, MeOH/(0.3% TEAA, pH 5.4) (5/95, v/v) for 5, MeOH/(0.3% TEAA, pH 4.9) (8/2, v/v) for 2, respectively.

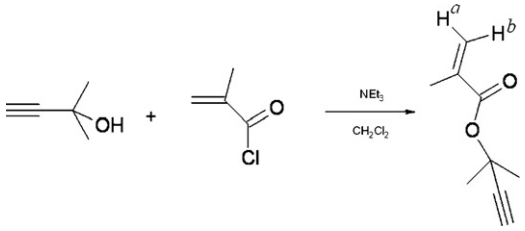

Fig. 1. Synthesis of 2-methyl-3-butyn-2-ol methacrylate (MBMA).

distance between the polymer chains, was selected to obtain a brush-like chain structure. Next, 2-methyl-3-butyn-2-ol methacrylate (MBMA)[1] was synthesized (Fig. 1) and grafted onto silica gel by a surface-initiated ATRP reaction with CuBr/[2,2′-bipyridine (BPY)] as the catalyst/ligand composite. The molar ratio for initiator/CuBr/BPY/MBMA was 1:4:8:200. Mono-6-deoxy-6-azido-β-CD[2] was synthesized according to the method in the literature [13] and reacted with the alkyne groups of poly-MBMA (pMBMA) via click chemistry (Fig. 2). The external pGMA layer was then grafted on CD-click-sil via a second ATRP for the preparation of CD-click-RAM. Hydrophilic poly(glycerol monomethacrylate), (pGMMA) was created on the surface of the material by hydrolysis of pGMA which forms a barrier for protein exclusion (Fig. 2).

Elemental analysis was performed with an elemental analyzer (Elementar Vario EL, Elementar, Germany). The pore structures of the materials were analyzed by a nitrogen sorption system (Quantachrome instrument, model Nova 2000, USA). The IR spectra were obtained with an AVATAR-360 FTIR instrument (Nicolet, USA).

2.2. Chromatographic evaluation

The CD-click-sil and CD-click-RAM were slurry-packed into stainless steel columns (150 mm × 4.6 mm). A Shimadzu 20A HPLC instrument equipped with a LC-20 AD pump and a SPD-20A UV detector was employed for the experiment.

2.3. Sample preparation for the direct HPLC injection of plasma

Racemic chlorthalidone or mandelic acid was dissolved in 100 μL HPLC eluent and mixed with 100 μL human plasma collected from healthy volunteers. A 20 μL aliquot of the solution was injected onto the CD-click-RAM column. The mobile phase flow rate was 0.5 mL min^{-1}. The analytes were detected at 254 nm. The solution for protein recovery evaluation was prepared by dissolving bovine serum albumin (BSA) in the HPLC eluent. The concentration of BSA was 32 mg mL^{-1}, similar to the protein concentration in human plasma samples. The recovery was calculated based on the peak area of BSA eluted from the column by taking the area obtained without a column as 100%.

3. Results and discussion

The structures of CD-click-sil and CD-click-RAM were analyzed by FTIR (Fig. 3). The diminution of the peak at 2010 cm^{-1} (C≡C stretching vibration) indicates a decrease in the number of alkynyl groups after the click reaction [14]. The peaks at 1724 cm^{-1} (ester carbonyl) and 2957 cm^{-1} (C–H stretching vibration) appear in the spectrum of the CD-click-RAM, which demonstrates that pGMMA has been grafted into the material.

The CD-click-sil with short pMBMA chains and the CD-click-RAM with longer pGMA chains were synthesized using the conditions that had been optimized for good enantioseparation and protein exclusion. The material pore structures and the grafted polymer lengths, represented by the number of repeating polymer units on the chains after each ATRP, are shown in Table 1. The β-CD density on the surface of the silica gel was 0.13 μmol m^{-2} determined by the same method in literature [9]. A solution prepared

[1] Procedure for the synthesis of 2-methyl-3-butyn-2-ol methacrylate (MBMA) 2-Methyl-2-propenoyl chloride (20.0 mL, 207 mmol) was added dropwise into a solution containing 2-methyl-3-butyn-2-ol (21.0 mL, 217 mmol) and triethylamine (29.8 mL, 207 mmol) in dry dichloromethane (120 mL). The mixture was stirred for 0.5 h at 0 °C and then for another 20 h at room temperature. After filtration, the filtrate was washed with 1% HCl (2× 100 mL), saturated $NaHCO_3$ aqueous solution (2× 100 mL) and distilled water consecutively. The organic phase was separated and dried over $MgSO_4$. The MBMA was obtained as a colorless liquid by vacuum distillation (63–65 °C/12 mmHg) with a yield of about 74%. NMR: δ_H (300 MHz; $CDCl_3$; Me_4Si), 6.06 (s, 1H, H^b), 5.54 (s, 1H, H^a), 2.56 (s, 1H, C≡C*H*), 1.93 (s, 3H, C=CCH_3), 1.53 (s, 6H, OC(CH_3)$_2$). IR: 3280, 2124, 1715, 1638 cm^{-1}.

[2] Caution: sodium azide is acutely toxic and presents a severe explosion risk when shocked or heated. It must be handled with care inside a hood using appropriate personal protective equipment (gloves, lab coat, and safety glasses).

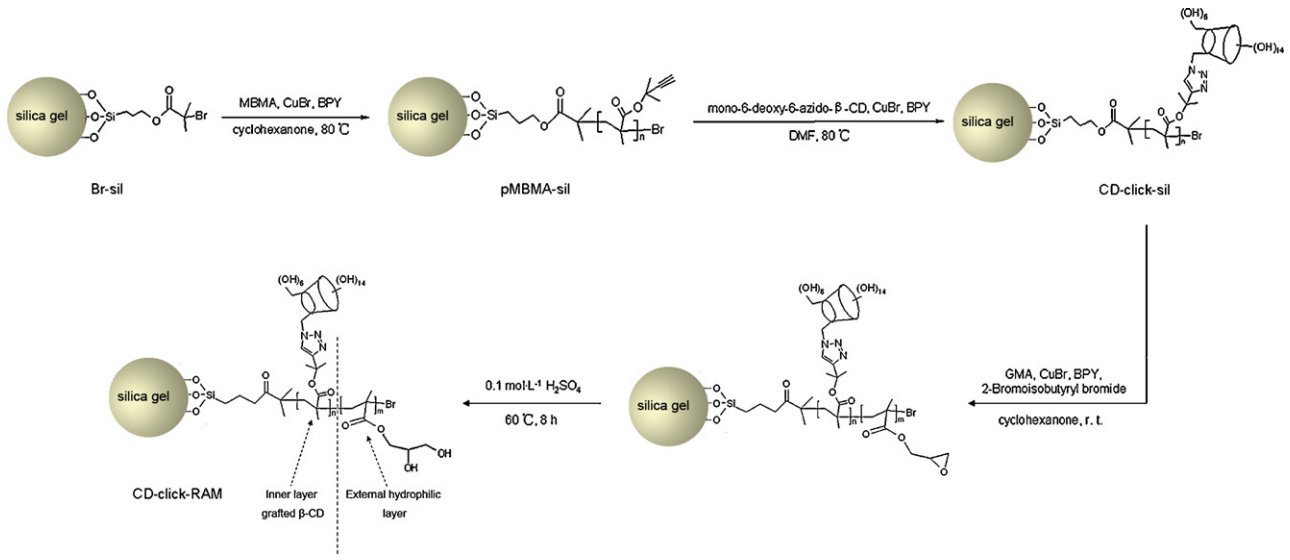

Fig. 2. Synthesis of CD-click-RAM.

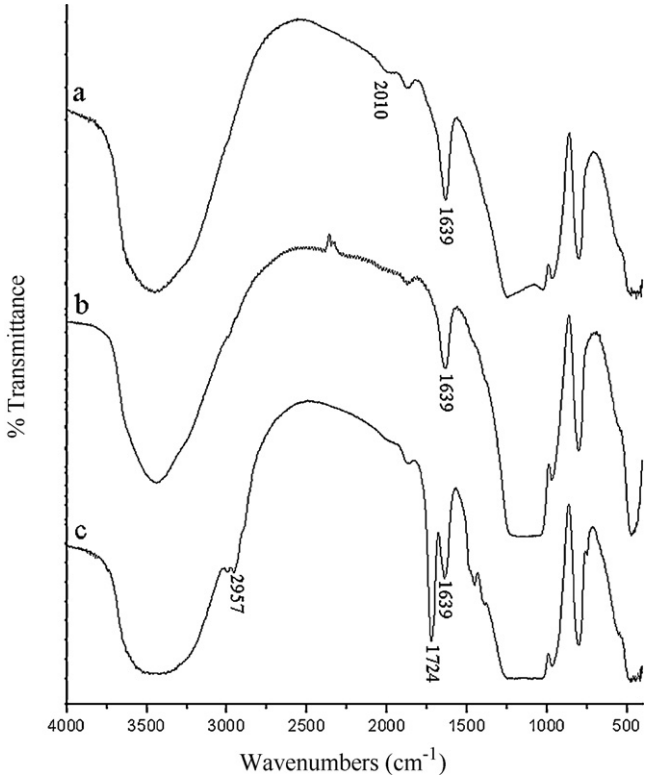

Fig. 3. FTIR spectra (KBr) of pMBMA-sil (a), CD-click-sil (b) and CD-click-RAM (c).

by dissolving bovine serum albumin (BSA) in the eluent was used to evaluate the protein exclusion ability of the CD-click-RAM. The BSA recovery was 98.2% for the CD-click-RAM, which demonstrates that proteins can be almost completely excluded from the column (Table 1).

The enantioselectivity of the CD-click-sil and the CD-click-RAM were evaluated by the HPLC enantioseparation of six chiral pharmaceuticals. For comparison purposes, the same mobile phase was used for both CD-click-sil and CD-click-RAM stationary phases when the same compound was analyzed. The results (Table 2) demonstrate that the CD-click-sil has good enantioselectivity for the chiral compounds used in the study. The hydrophilic external chains of the CD-click-RAM resulted in shorter retention times for the analytes in this reverse-phase separation. Lower resolutions were obtained on the CD-click-RAM stationary phase.

In our previous work, a ring opening reaction between pGMA and the sodium salt of β-CD was used for immobilizing the CD in the "CD-sil-2" material [9]. Although the CD-click-sil in this study and the "CD-sil-2" in our previous work have the same ATRP initiator density (0.034 μmol m^{-2}), similar polymer chain lengths for CD immobilization and similar β-CD grafting densities (0.13 μmol m^{-2} and 0.14 μmol m^{-2}, respectively), better enantioseparation efficiency has been achieved on the CD-click-sil stationary phase. For example, the enantiomers of chlorthalidone can be separated on CD-click-sil with $\alpha = 1.44$ and $k_1 = 9.60$ (Table 2), whereas a lower selectivity ($\alpha = 1.04$) and a similar retention factor ($k_1 = 8.57$) were obtained on the CD-sil-2 using the same column size and mobile phase. A longer column (250 mm × 4.6 mm) has to be used to obtain better selectivity on the CD-sil-2 [9]. We attribute the better enantioseparation of the CD-click-sil to the more homogenous bonding of CD that is achieved with click chemistry. Furthermore, possible hydrogen bonding between

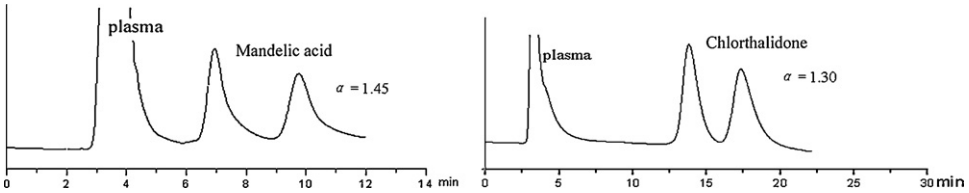

Fig. 4. Liquid chromatograms of the enantioseparation of human plasma samples spiked with mandelic acid or chlorthalidone on the CD-click-RAM stationary phase. HPLC column was 150 mm × 4.6 mm. Mobile phases: MeOH/(0.3% TEAA, pH 4.9) (97/3, v/v) for chlorthalidone and 0.3% TEAA (pH 4.9) for mandelic acid. The flow rate was 0.5 mL min^{-1}. Detection was at 254 nm.

the –NH or –OH groups of the analytes and the triazole groups of the CD-click-sil may contribute to the better separation. In contrast, all the –OH groups (2, 3, and 6-hydroxy groups) on β-CD can react with pGMA in the "CD-sil-2" synthesis. The bonding with the 2- and 3-OH groups of β-CD may result in poorer chiral separation.

The potential applicability of the material was evaluated by direct injection of spiked human plasma into an HPLC using the CD-click-RAM as the stationary phase. Two racemic compounds were used in the evaluation. The chromatographic results showed that proteins were excluded on the column in 6 minutes and the enantiomers were well separated (α = 1.45 for mandelic acid and α = 1.30 for chlorthalidone) (Fig. 4).

4. Conclusion

In summary, a novel chiral restricted access material was synthesized using surface-initiated ATRP and click chemistry. The chiral stationary phase showed better enantioseparation ability than the material synthesized in our previous work. This demonstrates that click chemistry provides a more effective route for the immobilization of β-CD compared with the general ring-opening reaction. An external hydrophilic layer was created on the material and has shown the ability to exclude proteins. This multifunctional material will facilitate the efficient separation and determination of chiral compounds in biological samples with direct injection into an HPLC system.

Acknowledgments

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