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Fabrication of Chiral Stationary Phases via Continuous Assembly of Polymers for Resolution of Enantiomers by Liquid Chromatography

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Abstract

Precise stereochemical determination of chiral molecules is highly important, especially in the pharmaceutical industry where one enantiomer may have a therapeutic effect while the other has detrimental effects. Herein, the continuous assembly of polymers (CAP) mediated via ring-opening metathesis polymerization (ROMP) is used to fabricate immobilized-type chiral stationary phases (CSPs) – as cross-linked thin films on solid supports – for enantiomeric separation. Optically-active polysaccharides (chitosan and amylose) with aromatic substituents were pre-functionalized with pendent norbornene groups and subsequently employed as macrocross-linkers in the CAP_{ROMP} process, building cross-linked

films from initiator-functionalized mesoporous silica particles. The immobilized cross-linked films on mesoporous silica particles can act as CSPs. Therefore, the chiral recognition abilities of the CSPs were explored by liquid chromatography (LC). It was found that CSPs with higher amount of polysaccharide cross-linked films – made from multiple CAP reactions – have better chiral separation capabilities. This work demonstrates the versatility of the CAP approach to fabricate CSPs to tailor specific separation needs.

1. Introduction

Chiral centers are found in various compounds designed for biological and environmental applications, including drugs, agrochemicals, food additives and other natural products. The study of chirality has attracted significant interest in many scientific areas as a result of the unique physiological properties of specific enantiomers or stereoisomers.[1-3] Protein binding, pharmacodynamics, pharmacokinetics and even toxicity can vary significantly between enantiomers.[1,4,5] Biological enantioselectivity was first reported by Louis Pasteur in 1848 who realized that the (+)- isomer of ammonium tartrate was consumed more rapidly by microorganisms compared to the corresponding (-)- isomer.[6,7] In 1957, precise stereochemical determination became a major focus, especially in the pharmaceutical industry after it was found that one enantiomer of chiral drugs may have a therapeutic effect while the other is toxic.[8,9] For instance, the R-(+)- isomer of thalidomide (*N*-phthalylglutamic acid imide) induces sedative and hypnotic effects while the S-(-)- isomer is tetratogenic and can cause fetal malformations.[8,9]

There are a number of approaches that have been developed to obtain optically pure compounds. They are divided into two categories; the first approach involves the asymmetric

synthesis of a specific enantiomer,[10-13] whereas the second method involves the resolution of a racemate into individual enantiomers.[14-16] The second approach has grown in popularity because both enantiomers can be obtained at the same time, which is difficult to achieve using the asymmetric synthesis approach. Amongst the established methods for the separation of enantiomers including crystallization, and kinetic and chromatographic resolution, direct separation using chiral stationary phases (CSPs) with high performance liquid chromatography (HPLC) has emerged as the most versatile approach.[5,16-18] It is well recognized as the most reliable method for both analysis of enantiomer compositions and preparation of enantiomers of high optical purity. The main criteria for CSPs include high chiral recognition abilities, wide mobile phase tolerance, and high throughput capacity for large-scale separations.[18-19] To date, the most powerful CSPs for both analytical and preparative separations are polysaccharide-based CSPs.[1,3-5,19-21]

Polysaccharides, such as cellulose and amylose, are abundant in nature and they are optically active. Although the native form of these polysaccharides show low chromatographic and enantioselective properties, their modified derivatives, which include triacetate, tribenzoate and trisphenylcarbamates, are significantly more effective and have been commercialized to separate a broad range of racemates.[1,3-5,19,20] These modified polysaccharides possess highly-ordered helical structures that play a key role in providing chiral recognition. It has been suggested that the 3,5-dimethylphenylcarbamates derivative of cellulose and amylose have left-handed 3/2 and 4/3 helices, respectively.[22,23] As a result, the polysaccharide backbone is arranged in regular repeat patterns along the helical axis, and a chiral helical groove with polar carbamate and aromatic groups is located along the main chains.[3] Chiral molecules may possibly interact with the polar carbamate groups via hydrogen bonding on the amino and carbonyl groups or dipole-dipole interaction on the carbonyl groups. In addition, π-π

interactions between the aromatic groups of a racemate and the phenyl groups of the phenylcarbamates on the polysaccharides may also play a role in chiral recognition and separation.[3,24]

In comparison to conventional coating methods that rely on physical adsorption, the immobilization of polysaccharide derivatives onto silica substrates by covalent bonding has proven to be more versatile as it allows a wider range of mobile phases to be used in conjunction with HPLC.[1,3,4,20] There are several immobilization methods that have been examined over the past few decades, which include: (i) the cross-linking of the polysaccharide derivatives bearing hydroxyl groups or vinyl groups with diisocyanates or vinyl monomers, respectively; (ii) chemical bonding of amylose derivatives at a chain-end, and; (iii) photochemical cross-linking methods.[25-29] Despite their excellent ability to resolve chiral compounds, precise control over film thickness and composition, and surface confinement cannot be achieved using current cross-linking methods.

Herein, the viability of the continuous assembly of polymers (CAP) approach to produce CSPs with engineered features is investigated. The CAP approach utilizes controlled polymerization methodologies to mediate the continuous growth of macrocross-linkers – (bio)macromolecules functionalised with pendent polymerizable moieties – from initiator-functionalized surfaces to form surface-confined, cross-linked films in a single-step.[30-36] As the CAP films are cross-linked, the resulting CSPs are expected to be robust towards a wide range of HPLC eluents. Furthermore, the film composition and thickness can be tailored via the CAP approach, which may be beneficial in improving chiral discrimination of a diverse range of racemates. In addition, film formation is surface confined, which enables the unreacted macrocross-linkers to be recycled/reused to fabricate subsequent batches of CSPs,

making the overall process economical. As a proof of concept, the CSPs were synthesized on a small-scale via CAP mediated by ring-opening metathesis polymerization (ROMP) on mesoporous silica particles. Norbornene functionalized benzylcarbamate derivatives of chitosan or amylose (**P1** or **P2**, respectively) were employed as the macrocross-linkers. The resulting CSPs were packed into thin glass columns (2.0 mm internal diameter), and the enantioselectivity of these small-scale columns was determined using a HPLC system fitted with a commercial chromatography column (Chiralcel OD-H) (**Figure 1**).

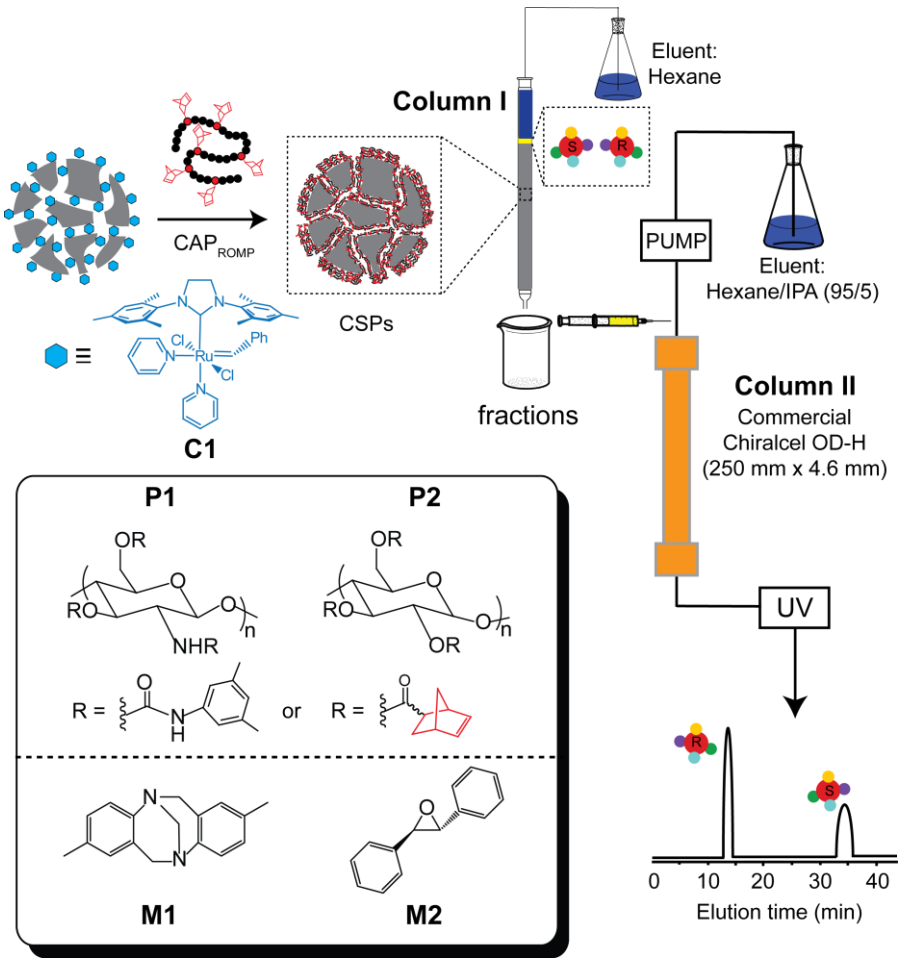

Figure 1. Fabrication of CSPs via the CAP_{ROMP} approach with macrocross-linker **P1** or **P2** and the HPLC setup for resolution of enantiomers from chiral compounds **M1** or **M2**.

2. Experimental Section

2.1 Synthesis of CAP_{ROMP} macrocross-linkers

Dried chitosan or amylose (M_w ~ 15 kDa) were suspended in dry pyridine, followed by the addition of 5-norbornene-2-carbonyl chloride, 3,5-dimethylphenyl isocyanate and dibutyltin dilaurate. The reaction mixture was stirred at 100 °C for 4 days and was precipitated into methanol. For more detailed experimental procedures, see Supporting Information (SI).

2.2 Fabrication of CSPs via CAP_{ROMP}

7 μm-diameter mesoporous silica particles (100 nm pore size) functionalized with catalyst **C1** were exposed to a 1:1 weight ratio of solution containing macrocross-linker **P1** or **P2** (32 $mg.mL^{-1}$ in anhydrous and degassed dichloromethane). The mixture was stirred at 25 ^oC for 25 h and the CAP_{ROMP} process was terminated by the addition of excess ethyl vinyl ether (100 μL). After 5 min, the particles were isolated by centrifugation and washed with DCM (3 × 15 mL) prior to analysis.

2.3 Column packing of the CSPs

Dried CAP-coated particles were packed into a glass column (2.0 mm i.d.) (Column I), and equilibrated using hexane as the mobile phase at 0.05 $mL.min^{-1}$ for 10 h. The racemate solution (20 $mg.mL^{-1}$ in hexane) was injected into the column (130 mm high). Fractions were collected and diluted with hexane (0.220 mL), and injected to a chiral HPLC system (Column II) for chiral discrimination analysis.

2.4 Measurement

The chiral discrimination analysis of the CSPs was conducted on a Shimadzu liquid chromatography system (Shimadzu LC-20AD) equipped with a Shimadzu SPD-20A UV-Vis

detector (λ = 254 nm) using a Chiralcel OD-H column (250 mm x 4.6 mm) (Column II) operating at 30 °C. Hexane/isopropanol (95/5) was employed as the mobile phase at a flow rate of 0.5 $mL.min^{-1}$.

3. Results and Discussion

Initially, all mesoporous silica particles (7 μm in diameter) were functionalized with an initiating layer via deposition of allyl-PEI,[30] followed by catalyst **C1** immobilization. To effect the CAP_{ROMP} process, the initiator-functionalized particles were then dispersed in a solution of macrocross-linker **P1** or **P2**. Subsequently, the CAP modified particles (i.e., CSPs) were packed into glass columns and racemic analytes were passed through the columns and fractions were collected for analysis. The chiral recognition ability of the immobilized CSPs obtained via the CAP_{ROMP} approach was evaluated by HPLC using Tröger's base (**M1**) or *trans*-stilbene oxide (**M2**) as the racemate for chitosan and amylose derivatives, respectively. These racemates were able to be resolved by the commercial columns and were chosen to demonstrate the feasibility of the CAP approach for CSP synthesis.

3.1 Enantioseparation of Tröger's base (M1) using chitosan (3,5-dimethylphenylcarbamate-norbornene) (P1).

The first polysaccharide that was investigated in this study was chitosan. Chitosan has a similar structure to cellulose with ß-(1-4) linkages with an amino group at the 2-position of the glucosamine repeat unit. The macrocross-linker **P1** consists of chitosan modified with aromatic substituents and partly functionalized with norbornene groups (19 mol%). After the

fabrication of CSPs via CAP_{ROMP} (as described previously) (Figure 1), the amount of organic coating (i.e., **P1**) on the CSPs was determined by thermogravimetric (TGA) analysis. **Figure 2b** shows that after a single CAP_{ROMP} reaction, the organic content of the resulting CSPs was *ca.* 11%, which is lower compared to the commercial column (*ca.* 20%) where distillation-precipitation polymerization was employed in the fabrication of cross-linked films.[3] To increase the **P1** film thickness on the particles, a process of reinitiation was performed.[30,34] Firstly, the film made after one CAP step was replenished with initiator **C1** through reaction with the terminal alkene, as well as the 'left-over' unreacted norbornene, in the film, followed by exposure to macrocross-linker **P1** under identical conditions to the first CAP reaction (**Figure 2a**). After two subsequent reinitiation steps (i.e., three CAP_{ROMP} reactions in total), the organic content of the CSPs increased to *ca.* 19% (**Figure 2b**), indicating an increase in film thickness.

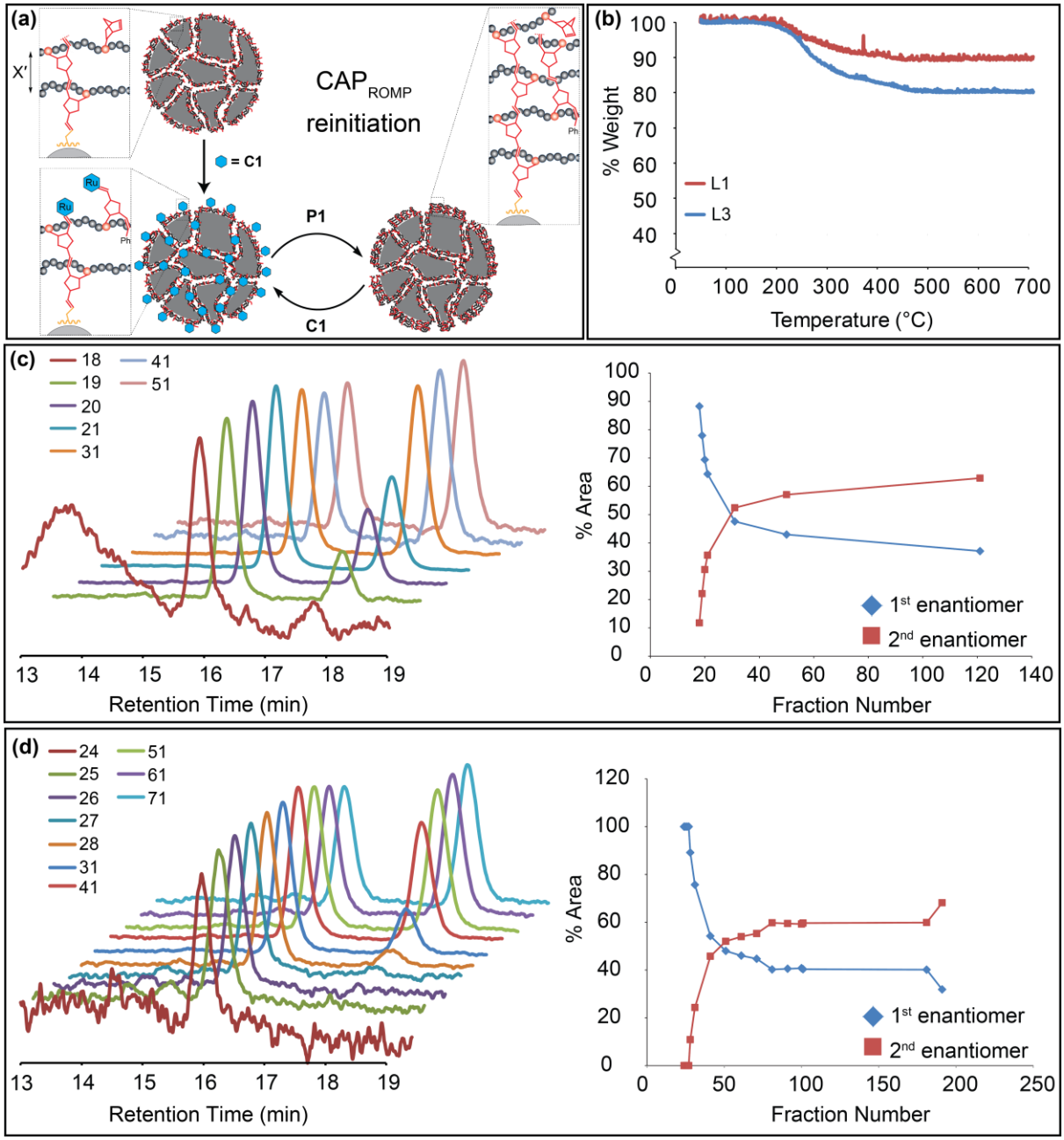

Figure 2. (a) General scheme of CAP_{ROMP} reinitiation on mesoporous silica particles with macrocross-linker **P1**. **(b)** TGA analysis of the CSPs fabricated via a single CAP_{ROMP} step (L1) (red) and after two reinitiation steps (L3) (blue) with chitosan-based macrocross-linker **P1**. The graphs indicate percentage weight loss with increasing temperature, which results from degradation of the chitosan-based **P1** films. The degradation of **P1** occurred between 200 to 480 °C. UV absorbance at 254 nm (left panel) of racemic Tröger's base **M1** after being passed through columns packed with **P1**-based CSPs; **(c)** fabricated by a single

CAP_{ROMP} reaction (L1) and **(d)** with two additional reinitiation steps (L3). Right panels in (c) and (d) show the ratio of area under the peak between the two enantiomers. Low-resolution UV traces of some fractions result from high signal to noise ratios at low sample concentrations.

The CSPs obtained after CAP L1 and L3 were packed into glass capillary tubes to afford columns $\mathbf{C1_{L1}}$ and $\mathbf{C1_{L3}}$ (130 mm in height × 2.0 mm internal diameter), respectively. A 50/50 mixture of enantiomers of chiral compound **M1** (20 $mg.mL^{-1}$ in hexane, 50 μL) was injected into the columns and eluted with hexane. Fractions were collected into individual glass vials (2 drops per vial ~ 40 μL). The individual fractions collected from columns $\mathbf{C1_{L1}}$ and $\mathbf{C1_{L3}}$ were diluted with 220 μL of hexane and then injected into a HPLC system fitted with a commercial Chiralcel OD-H column to investigate the enantioseparation efficiency of the CAP-based CSPs. **Figure 2c** and **d** shows the resolution of racemate **M1**, as detected by UV absorbance at 254 nm. Although column $\mathbf{C1_{L1}}$ showed some enantioselective characteristics, under the conditions of the experiment complete separation of the enantiomers was not achieved, with the best enantio-resolved fraction contained 90% of one enantiomer and 10% of the other (**Figure 2c**). Subsequent fractions contained almost equimolar amounts of both enantiomers. In comparison, column $\mathbf{C1_{L3}}$ showed improved separation, with the first three fractions containing a single enantiomer (**Figure 2d**). This improvement in chiral separation by three fractions is attributed to an increase in film thickness after reinitiation.

3.2 Enantioseparation of *trans*-Stilbene oxide (M2) using amylose (3,5-dimethylphenylcarbamate-norbornene) (P2).

The CAP_{ROMP} reactions with the amylose-based macrocross-linker **P2** were conducted under similar conditions to those described for the chitosan-based macrocross-linker **P1**. Similar to the chitosan system, a single CAP_{ROMP} reaction (i.e., L1) provided a CSP containing a polysaccharide content to *ca.* 12%, as determined by TGA (**Figure 3b**). After two additional reinitiation steps (i.e., L3), the amylose content increased to *ca.* 56%, which is significantly higher than that observed for reinititation experiments with the chitosan macrocross-linker **P1** (19% after two reinitiation steps).

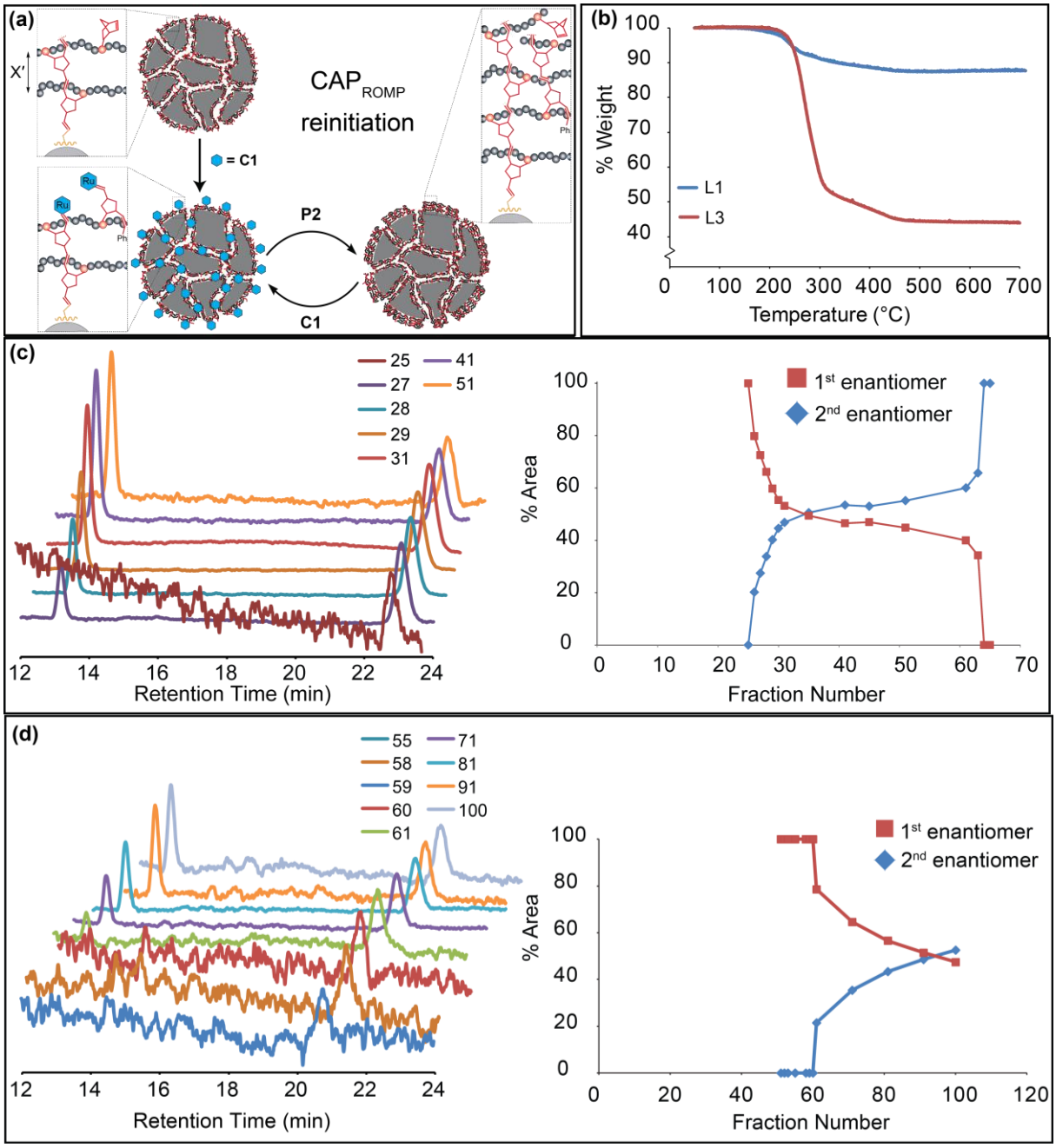

Figure 3. (a) General scheme of CAP_{ROMP} reinitiation on mesoporous silica particles with amylose-based macrocross-linker **P2**. **(b)** TGA analysis of the CSPs fabricated via single CAP_{ROMP} step (L1) (red) and after two reinitiation steps (L3) (blue) with **P2**. The graphs indicate percentage weight loss with increasing temperature, which results from degradation of the amylose-based **P2** films. The degradation of **P2** occurred between 230 to 480 °C. UV absorbance at 254 nm (left panel) of *trans*-stilbene oxide **M2** after being passed over column **C2** packed with **P2**-based CSPs; **(c)** fabricated by a single CAP_{ROMP} reaction and **(d)** after

two additional reinitiation steps. Right panels in (c) and (d) show the ratio of area under the peak between the two enantiomers. Low-resolution UV traces of some fractions are due to high noise/peak area ratio at low sample concentrations.

The amylose-based CSPs were packed into glass capillary tubes to afford columns $\mathbf{C2_{L1}}$ and $\mathbf{C1_{L3}}$ (130 mm in height × 2.0 mm internal diameter). A 50/50 mixture of enantiomers of chiral compound **M2** (20 mg.mL^{-1} in hexane, 50 µL) was then injected into the columns and eluted with hexane. Fractions were collected and injected into the HPLC system to determine the enantiomeric separation efficiency. The HPLC results measured by UV-visible spectrophotometry (at 254 nm) show that the column $\mathbf{C2_{L1}}$ is able to completely resolve the enantiomers at the start (one fraction) and end of the elution period (**Figure 3c**). In comparison, $\mathbf{C2_{L3}}$ showed significant improvement as there were seven fractions within the range of analysis that contained only a single enantiomer (**Figure 3d**).

These preliminary results demonstrate the concept that the CAP approach can be utilized to synthesize robust cross-linked chiral stationary phases because of four reasons: i) the film thickness can be tuned and the enantioseparation can be improved via reinitiation processes; ii) a diverse range of polymers can be used as macrocross-linkers to fabricate single or potentially multicomponent films; iii) the CAP process is surface confined, which allows the unused macrocross-linkers to be recycled, providing a more economical process; and iv) a wide range of eluent solvents can be used given that the cross-linked films are chemically bonded to the particle substrates. The features of CAP-derived CSPs are unique and are potentially useful to resolve a wide range of chiral compounds.

4. Conclusion

Derivatives of chitosan (3,5-dimethylphenylcarbamate-norbornene) (**P1**) and amylose (3,5-dimethylphenylcarbamate-norbornene) (**P2**) were synthesized and employed as macrocross-linkers in the fabrication of cross-linked films on mesoporous silica particles via CAP_{ROMP}. In addition, it was shown that simple reinitiation reactions (akin to chain extension reactions) can increase the polysaccharide content by 8 wt% and 44 wt% for chitosan- and amylose-based macrocross-linkers, respectively. The increase in polysaccharide content also improves the chiral discrimination performance of the corresponding CSPs where single enantiomers of Tröger's base and *trans*-Stilbene oxide were obtained in the first three and seven fractions, respectively, compared to one fraction for both CSPs made with only one CAP reaction. The results demonstrated the proof of concept that CAP can be an option in coating optically active compounds for chiral separation processes. It is anticipated that further optimization including different packing conditions and increased column length, can facilitate CAP-derived CSPs to tailor specific separation needs.

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Supporting Information

Fabrication of Chiral Stationary Phases via Continuous Assembly of Polymers for Resolution of Enantiomers by Liquid Chromatography

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Materials

Allyl bromide (99%), 3,5-dimethylphenyl isocyanate (99%), 5-norbornene-2-carboxylic acid (mixture of *endo* and *exo*, 98%), and poly(ethylene imine) (PEI) (M_w ~25 kDa) were obtained from Aldrich and used without further purification. Metathesis catalyst $(IMesH_2)(Cl)_2(C_5H_5N)_2Ru{=}CHPh$ **C1** was prepared from 2nd generation Grubbs catalyst as described in the literature.[1] Pyridine and basic alumina (basic Al_2O_3) were obtained from Scharlau and used without further purification. Oxalyl chloride (≥ 98%) was obtained from Acros Organics and used as received. Magnesium sulfate ($MgSO_4$, anhydrous), *n*-hexane, toluene, isopropanol and ethanol were obtained from Merck and used without further purification. Sodium hydroxide (NaOH) was obtained from Chem-Supply and used without further purification. Chitosan (M_w ~ 15 kDa) was obtained from Polysciences, Inc. and used without further purification. Amylose (M_w ~ 15 kDa) was obtained from TCI Co., Ltd and used without further purification. Deuterated chloroform ($CDCl_3$), methanol (CD_3OD) and dimethylsulfoxide (d_6-DMSO) were obtained from Cambridge Isotope Laboratories. High-purity water with a resistivity greater than 18 MΩ·cm was obtained from an in-line Millipore RiOs/Origin water purification system. Mesoporous silica particles (Daisogel SP-1000-7, diameter 7 µm, pore size 100 nm) were obtained from Daiso co. Ltd (Japan).

Characterization methods

^{1}H nuclear magnetic resonance (NMR) spectroscopy was conducted on a Varian Unity 400 MHz spectrometer at 400 MHz, respectively, using the deuterated solvent as reference and a sample concentration of approximately 20 mg·mL^{-1}.

The chiral discrimination analysis of the CSPs was conducted on a Shimadzu liquid chromatography system (Shimadzu LC-20AD) equipped with a Shimadzu SPD-20A UV-Vis detector (λ = 254 nm) using Chiralcel OD-H column (250 mm x 4.6 mm) operating at 30 °C. Hexane/isopropanol (95/5) was employed as the mobile phase at a flow rate of 0.5 $mL \cdot min^{-1}$.

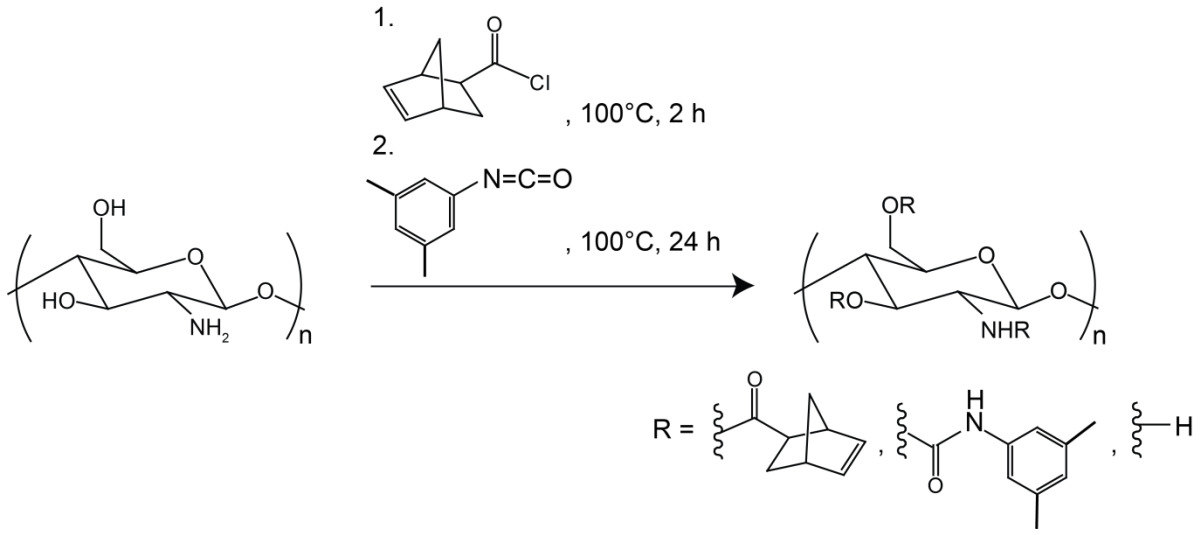
TGA was performed on a PerkinElmer Pyris-1 thermogravimetric analyzer, and the samples were heated from 50 to 700 ^{o}C at a heating rate of 10 $^{o}C \cdot min^{-1}$ under nitrogen flow (20 $mL \cdot min^{-1}$).

Experimental Methods

Synthesis of 5-norbornene-2-carbonyl chloride

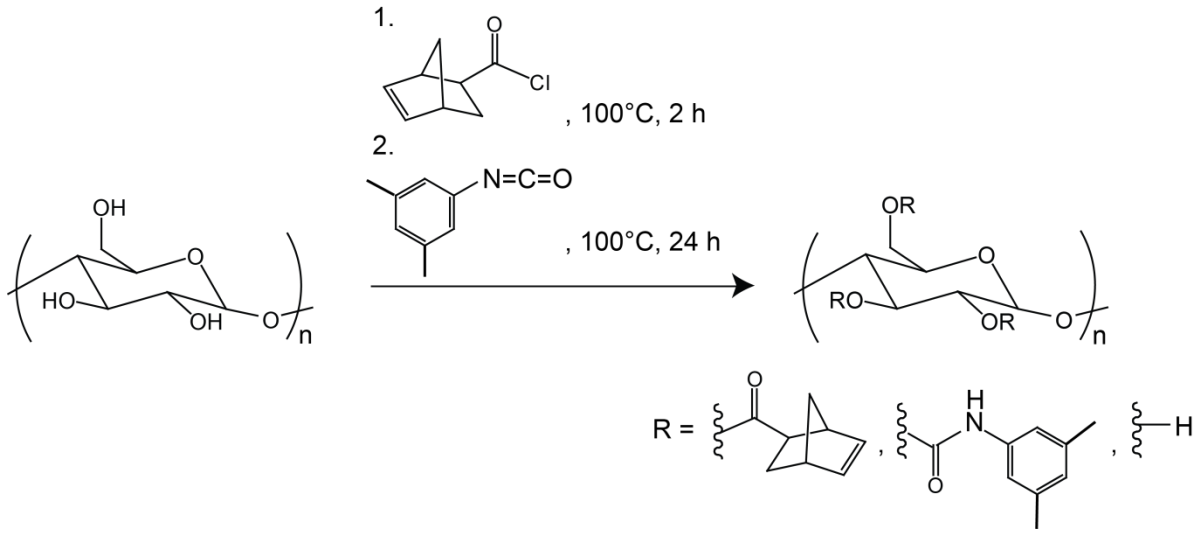
5-norbornene-2-carboxylic acid (4.00 g, 0.030 mol) was first dissolved in DCM (50 mL). The mixture was bubbled with argon for 15 minutes. Oxalyl chloride (4.90 mL, 0.060 mol) was added to the flask under argon atmosphere and the reaction mixture was bubbled with argon for a further 2 h and stirred for an additional 10 h at 25 ^{o}C. Solvent and excess oxalyl chloride were removed *in vacuo* to afford 5-norbornene-2-carbonyl chloride as a pale yellow liquid, 3.0 mL (75%). The product was used in the next step without further purification.

Synthesis of macrocross-linker P1 chitosan 3,5 dimethylphenylcarbamate-norbornene



Dried chitosan (M_w ~ 15 kDa, 1.00 g, 6.20 mmol per repeat unit) was dissolved in dry pyridine (20 mL). The reaction flask was submerged in a 100 °C oil bath. 5-norbornene-2-carbonyl chloride (0.320 mL, 2.48 mmol) was added into the suspension and the reaction mixture was stirred at 100 °C for 12 h. Then, 3,5-dimethylphenyl isocyanate (4.02 mL, 29.0 mmol) and dibutyltin dilaurate (50 µL) were added to the flask. The reaction mixture was stirred at 100 °C for another 4 days and subsequently precipitated into methanol (300 mL). The precipitate was collected via centrifugation and redissolved in chloroform (20 mL). The insolubles were filtered and the filtrate was reprecipitated into methanol (200 mL). The precipitate was again collected via centrifugation, dried *in vacuo* to afford **P1** as a brown solid, 0.7 g (19%). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ_H 8.59-8.62 (*br*, N**H**, 3H), 6.33-7.57 (*br*, Ar**H**, 7H), 5.77-6.26 (*br*, =C**H**, 2H), 3.25-5.44 (*br*, glucosamine protons, 7H), 2.71-3.06 (*br*, C**H**, 1H), 1.55-2.71 (*br*, Ar-C**H**$_3$, 15H), 1.01-1.55 (*br*, CH**H**, 2H), 0.63-0.92 (*br*, CH**H**, 1H). Pendant norbornene functionality was *ca.* 19 mol% as determined by ^{1}H NMR spectroscopic analysis.

Synthesis of macrocross-linker P2 amylose 3,5-dimethylphenylcarbamate-norbornene



Dried amylose (M_w ~ 15 kDa, 1.21 g, 7.45 mmol per repeat unit) was dissolved in dry pyridine (24.3 mL). The reaction flask was submerged in a 100 °C oil bath. 5-norbornene-2-carbonyl chloride (0.371 mL, 2.98 mmol) was added into the suspension and the reaction mixture was stirred at 100 °C for 12 h. Then, 3,5-dimethylphenyl isocyanate (4.83 mL, 34.3 mmol) and dibutyltin dilaurate (50 µL) were added to the flask. The reaction mixture was stirred at 100 °C for another 4 days and subsequently precipitated into methanol (300 mL). The precipitate was collected via centrifugation, dried *in vacuo* to afford **P2** as a pale brown solid, 3.61 g (82%). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ_H 8.98-9.59 (*br*, N**H**, 3H), 5.87-7.79 (*br*, Ar**H**, 7H), 3.41-5.69 (*br*, glucose protons, 7H), 2.77-2.99 (*br*, C**H**, 1H), 1.54-2.51 (*br*, Ar-C**H**$_3$, 15H), 1.12-1.52 (*br*, CH**H**, 2H), 0.78-0.92 (*br*, CH**H**, 1H). Pendant norbornene functionality was *ca.* 17 mol% as determined by ^{1}H NMR spectroscopic analysis.

Synthesis of hyperbranched poly(N-allyl ethylene imine) (allyl-PEI)

This compound was prepared according to a previously published procedure.[1] ^{1}H NMR (400 MHz, CD_3OD) δ_H 5.85 (*br s*, CH_2=C**H**CH_2N), 5.17-5.22 (*m*, C**H**$_2$=CHCH_2N), 3.11-3.20 (*m*,

CH_2=CHC**H**$_2$N), 2.57 (*br s*, N(C**H**$_2$)$_2$N) ppm. Allyl functionality was 30% as determined by ^{1}H NMR spectroscopic analysis.

Fabrication of CSPs via CAP$_{ROMP}$

2 mL of particles (8 wt%) were charged into an Eppendorf tube, washed with Milli-Q water (2 × 2 mL) and added to 2 mL of allyl-PEI solution (1 mg·mL^{-1} in 0.5 M NaCl, passed through a 0.45 μm filter). The solution was agitated overnight with an orbital shaker at room temperature, isolated by centrifugation and subjected to gradient washing with Milli-Q water (2 × 2 mL), THF (2 × 2 mL), and DCM (1 × 2 mL). Then, 2 mL of catalyst **C1** stock solution (1 mM in DCM) was combined with the particles and stirred for 30 min at 25 ^{o}C. The catalyst functionalized particles were isolated by centrifugation, followed by gradient washing with DCM (2 × 2 mL) and were used immediately in CAP reactions.

The suspension of catalyst-functionalized particles (2 mL, 8 wt%) were transferred to a 5 mL containing **P1** or **P2** (32 mg·mL^{-1} in DCM) in a round bottom flask equipped with 3-way stopcock via a degassed syringe. The mixture was stirred at 25 ^{o}C for 25 h and the CAP$_{ROMP}$ process was terminated by the addition of excess ethyl vinyl ether (100 μL). After 5 min, the particles were isolated by centrifugation, washed with DCM (3 × 15 mL) and packed into a glass column (130 mm × 2.0 mm). Reinitiation experiments were conducted via the repetition of the above procedure (without allyl-PEI deposition step).

References

[1] T. K. Goh, S. N. Guntari, C. J. Ochs, A. Blencowe, D. Mertz, L. A. Connal, G. K. Such, G. G. Qiao, F. Caruso, *Small* **2011**, *7*, 2863.