ACS POLYMERS Au
AN OPEN ACCESS JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

pubs.acs.org/polymerau

Article

Enantioselective Separation of Amino Acids Using Chiral Polystyrene Microspheres Synthesized by a Post-Polymer Modification Approach

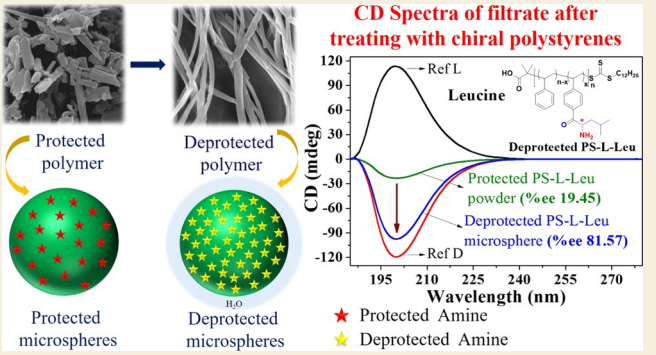
Shrikant B. Nikam and Asha S. K*

Cite This: *ACS Polym. Au* 2022, 2, 257−265

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: The enantioselective separation of a racemic mixture of amino acids was achieved by chiral amino acid-modified polystyrene (PS) that was developed by a post-polymer modification approach. Styrene was polymerized using the reversible addition−fragmentation chain-transfer (RAFT) polymerization technique and further post-polymer modification was applied by Friedel−Crafts acylation reaction with chiral *N*-phthaloyl-L-leucine acid chloride to obtain the protected PS-L-Leu. The chiral PS (protected PS-L-Leu) was assembled into microspheres using a surfactant and was used for carrying out the enantioselective separation of amino acid racemic mixtures by enantioselective adsorption followed by a simple filtration process. Compared to as-precipitated chiral PS (protected PS-L-Leu) powder, the protected PS-L-Leu microspheres exhibited a better enantioselective separation efficiency (ee %). Furthermore, the protected PS-L-Leu was deprotected to obtain the amine-functionalized deprotected PS-L-Leu chiral PS, which was also assembled into microspheres and used for carrying out enantioselective separation. Deprotected PS-L-Leu-functionalized chiral PS microspheres could achieve up to 81.6 ee % for the enantioselective separation of a racemic mixture of leucine. This is one of the first reports of the synthesis of amino acid-modified chiral PS microspheres and their application to the simple filtration-based enantioselective separation of native amino acids from their racemic mixtures.



KEYWORDS: *chiral polystyrene, polymer microspheres, circular dichroism, filtration, enantioselective separation, enantiomeric excess, hydrogen bonding*

INTRODUCTION

The synthesis of enantiomerically pure molecules is a big industry as it caters to a host of requirements in the pharmaceutical,[1] agrochemical,[2−4] food additive,[5−7] and fragrance industries.[8,9] Natural sources for enantiopure starting materials are insufficient to meet the continuously growing demand, and thus these compounds have to be synthesized in laboratories.[10] Among the accepted methods of obtaining enantiopure molecules, the widely used "chiral or asymmetric synthetic approach" requires highly enantiopure starting material and expensive and environmentally hazardous chiral catalysts, besides being very sensitive to any changes in the solvent, ligand, and so forth.[11,12] The alternate "racemic approach" method involves synthesizing chemical compounds in a racemic form, followed by their enantioselective separation. Different methods such as chiral chromatography,[13] enantioselective crystallization,[14−16] and enantioselective filtration are used in the racemic approach to separate the synthesized enantiomers.[17] Chiral chromatographic methods are most commonly used but are associated with limitations such as high-end instruments and a skilled workforce.[18] Methods such as enantioselective crystallization need enantiopure sacrificial compounds. Recently, enantioselective filtration methods using chiral selectors have gained more attention from the scientific community because these methods are simple, straightforward, energy efficient, and do not require high-end instruments.

Chiral selectors can differentiate between the enantiomers, and they include natural materials such as polysaccharides, cyclodextrins, micelles, proteins, macrocyclic glycopeptides, and crown ethers.[19−22] The use of various synthetic polymers as chiral selectors is also increasingly reported.[23−25] Our research group reported the application of chiral polyfluorenes to the enantioselective separation of racemic mixtures from

Received: January 23, 2022
Revised: March 8, 2022
Accepted: March 8, 2022
Published: March 18, 2022

https://doi.org/10.1021/acspolymersau.2c00004
ACS Polym. Au 2022, 2, 257−265

various classes of materials such as amino acids, amino alcohols, and sugars by a simple filtration method from their aqueous mixtures.[26] The enantioselective separation efficiency could be improved [enantiomeric excess (ee) of ∼95%] by applying the chiral polyfluorenes on commercially available mesoporous anodic aluminum oxide (AAO) membranes.[27] Polymeric microspheres are getting more attention in recent years due to their easy modification and processability, where shape, size, and surface functionality can be easily modified.[28] Chiral polymeric microspheres with particle sizes ranging from 1 to 200 μm with a high surface area are increasingly reported as the stationary phase in chiral chromatography.[29−31] Reports are available using polymeric microspheres or polymer coated-inorganic materials such as modified silica or metal nanoparticles as chiral selectors.[32,33] In 2007, Mastai *et al.* synthesized hollow poly(*N*-vinyl-α-L-phenylalanine microspheres using polystyrene (PS) microspheres as a template and used them to carry out the enantioselective crystallization of a racemic mixture of valine with 25% ee.[34] Zhang *et al.* reported L-phenylalanine-imprinted polymer (MIP) based on monodisperse hybrid silica particles that were utilized as chiral selectors in packed columns for the resolution of a racemic mixture of phenylalanine.[35] Song *et al.* reported helical polyacetylene microspheres modified with multicomponents such as stearyl acrylates, butyl acrylates, and β-cyclodextrin acrylate derivatives, which were used to separate racemic mixtures of menthol in 6 days.[36] Small changes in the composition were reported to have a significant impact on the separation efficiency. The enantioselective separation of racemic mixtures was achieved using magnetic microspheres immobilized with chiral substituents such as human serum albumin or β-cyclodextrins.[37,38] There are recent reports on microspheres prepared from amino acid-based acrylate polymers, amino acid-containing chiral metal−organic frameworks, and chitosan-functionalized (P(S-DVB) particles that were used to carry out the enantioselective crystallization of a racemic mixture of amino acids with 25% ee or used as chiral selectors in chiral high-performance liquid chromatography (HPLC) for the separation of several racemates with separation factors (α) of 7.55 and 5.70 for naproxen and benzoin, respectively.[28,29,39]

In the present work, we report the enantioselective separation of racemic mixtures of native amino acids achieved by chiral PS microspheres. Chiral PS was synthesized by a post-polymer modification approach starting from PS with well-defined molecular weights and molar mass distribution, synthesized by solvent-free reversible addition−fragmentation chain-transfer (RAFT) polymerization. The synthesized PS was modified with chiral *N*-phthaloyl-L-leucine acid chloride by the Friedel−Crafts acylation reaction to give protected PS-L-Leu. The polymer upon deprotection yielded chiral PS with a free amine (deprotected PS-L-Leu), making the polymer hydrophilic in nature compared to the starting PS or the protected amino acid-appended PS. Leucine is a highly abundant amino acid in protein structures with an inert aliphatic side chain that does not require protection or deprotection steps during synthesis. Additionally, when the leucine is directly connected to PS through a carbonyl (ketone) group, it is anticipated to effectively induce chirality in the PS backbone. The protected and deprotected PS were assembled into microspheres using the surfactant Tween 80 (polyoxyethylene sorbitan monooleate) and used for the filtration-based enantioselective separation of native amino acids from their aqueous racemic mixture. Higher ee % could be achieved with microspheres of deprotected PS with free amine groups. Deprotected PS-L-Leu microspheres achieved an 81.6% ee for the separation of leucine. According to our knowledge, this is the first report where amino acid-modified chiral PS microspheres have been synthesized and used to carry out simple filtration-based enantioselective separation of native amino acids from their racemic mixtures.

EXPERIMENTAL SECTION

Materials

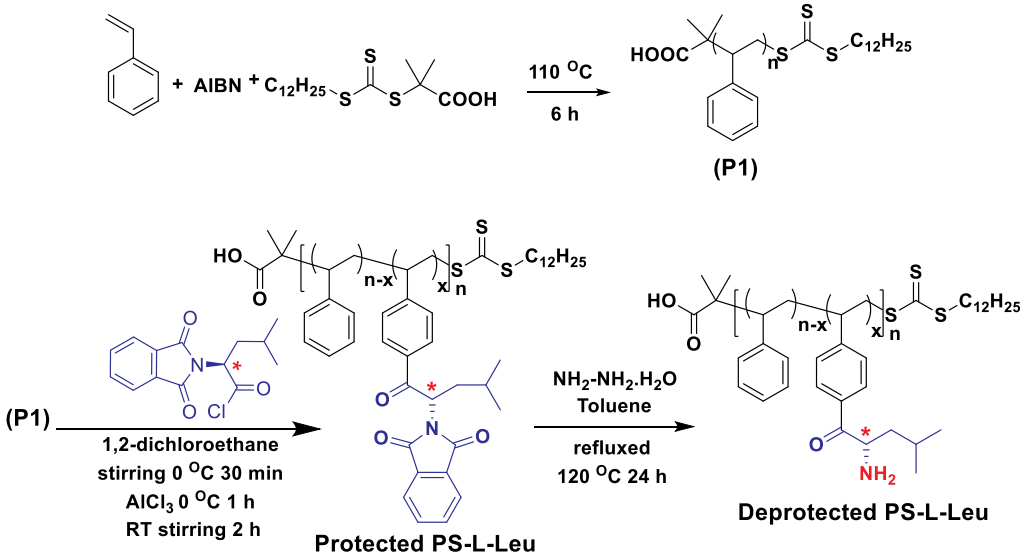
The synthetic-grade styrene monomer was purchased from Sigma-Aldrich and purified by washing with a 10% aqueous NaOH solution and water, followed by drying over anhydrous sodium sulfate. It was stirred with calcium hydride and distilled under a vacuum at 40 °C.[40] Synthetic-grade 2,2′-azobis(2-methylpropionitrile) was purchased from Sigma-Aldrich and was recrystallized in methanol before being used. The biochemistry-grade amino acids such as D/L-leucine and D/L-alanine were purchased from Spectrochem Pvt., Ltd., India. The RAFT agent was previously synthesized in the laboratory and was used directly without further purification.[41] Phthalic anhydride and hydrazine hydrate were purchased from Sigma-Aldrich. Bottle-grade tetrahydrofuran (THF), methanol, chloroform, 1,2-dichloroethane, hydrochloric acid (HCl), ethanol, and triethylamine were purchased from Merck Chemicals and were used without further purification. Toluene was purchased from Merck Chemicals and pre-dried using $CaCl_2$ and CaH_2 and dried by heating over sodium with benzophenone as the indicator.[42] Dichloromethane (DCM) was dried by distillation using CaH_2, followed by storage over activated 3 Å molecular sieves.[42] Reagent-grade sodium hydroxide (NaOH), potassium hydroxide (KOH), sodium sulfate (Na_2SO_4), calcium hydride (CaH_2), and HPLC-grade THF were purchased from Merck Chemicals. The analytical-grade Tween 80 surfactant was purchased from TCI chemicals. Extra pure anhydrous $AlCl_3$ and synthetic-grade $SOCl_2$ were purchased from Loba Chemie Pvt., Ltd., India. Analytical-grade deuterated $CDCl_3$ was purchased from Sigma-Aldrich. 0.45 μm Polytetrafluoroethylene (PTFE) and polyvinylidene fluoride (PVDF) filters were purchased from Global Nanotech. Drum-grade ethyl acetate and pet ether were purchased locally and used after distillation.

Measurements

^{1}H NMR spectra were recorded on Bruker Avance 200 and 400 MHz spectrometers in $CDCl_3$. Chemical shifts (δ) are reported in ppm at 25 °C using $CDCl_3$ as the solvent containing a trace amount of tetramethylsilane as an internal standard. Molecular weights of polymers were measured on a ThermoFinnigan-make gel permeation chromatograph, with a refractive index detector at room temperature. HPLC-grade chloroform was used as an eluent with a flow rate maintained at 1 mL/min. The gel permeation chromatography (GPC) column was standardized using PS standards with narrow polydispersity. The samples were prepared by dissolving a 3 mg of polymer in 1.5 mL of chloroform and filtered through a 0.45 μm PTFE filter. Infrared (IR) spectra were recorded on a Bruker α-T spectrophotometer. Sample pellets were prepared by mixing with 5% w/w in KBr and dried under vacuum before the spectra were recorded. The polymeric microspheres were prepared by using a polymeric solution in DCM and a 4% Tween 80 surfactant solution. The polymer and surfactant solutions were mixed in a 1:4 v/v ratio and homogenized using the RT Miccra D-9 Digitronic Homogenizer Disperser on Stand at 16,000−21,000 rpm for 15 min. The polymeric microsphere suspension was centrifuged on a Remi PR-24 research compufuge centrifuge with a 6 × 15 mL rotor head at 10,000 rpm. The average particle size (*Z*-average) and polydispersity index (PDI) of aqueous dispersion of polymeric microspheres were measured using a Zetasizer ZS 90 apparatus from Malvern Instruments at a fixed angle of 90° at 25 °C. The zeta potential (ζ) of polymeric microspheres was measured at pH 7 using 0.1 mg/mL dispersion in

https://doi.org/10.1021/acspolymersau.2c00004
ACS Polym. Au 2022, 2, 257−265

Scheme 1. Schematics for Synthesis of PS (P1), Protected PS-L-Leu, and Deprotected PS-L-Leu



0.1 mM KCl. The morphological characterizations of polymeric samples and microspheres were performed using field emission scanning electron microscopy (FE-SEM) with an FEI Nova Nano SEM 450. The polymer solution in THF (0.2 mg/mL) was drop cast on precleaned silicon wafers. In the case of polymer microspheres, the aqueous dispersion was drop cast on precleaned silicon wafers. The samples were dried overnight under reduced pressure at 45 °C. The sample drop cast on silicon wafers were directly mounted on the top of the grooved edge of the aluminum SEM specimen stub with a carbon tape. Before conducting morphological studies, the samples were coated with a 5 nm thick gold film by the sputtering method. The water contact angle of polymers was recorded using the Drop Shape Analyzer-DSA25- KRUSS GmbH. Solution-state circular dichroism (CD) measurements were made using a JASCO-815 CD spectrometer equipped with a Jasco PTC-424 S/15 Peltier system. 10 mm path length quartz cuvettes were used for a sample volume of 3 mL in Milli-Q water or THF at 25 °C. Three scans were averaged for each sample, with a scanning rate of 100 nm/min.

RESULTS AND DISCUSSION

Synthesis and Characterizations

Scheme 1 outlines the overall synthetic scheme for the synthesis of L-leucine-appended chiral PS. PS of the predetermined molecular weight was synthesized by RAFT polymerization (details are given in Supporting Information S1). The RAFT polymerization route was preferred over conventional free-radical polymerization for the synthesis of PS, as the former would result in a polymer with a controlled molecular weight. The polymer with a controlled molecular weight is anticipated to produce microspheres with controlled particle size, in contrast to one with large polydispersities. The proton NMR spectrum of PS indicated a number average molecular weight (Mn) of 20,800 (Figure S1), which was in good agreement with the theoretical calculated molecular weight of 20,500. The PS was subjected to post-polymer modification following the Friedel–Crafts acylation with *N*-phthaloyl-L-leucine acid chloride to obtain chiral PS (protected PS-L-Leu). The phthaloyl protection is advantageous as it is inert toward acidic conditions such as the Lewis acid ($AlCl_3$) during Friedel–Crafts acylation, which would have deprotected the Boc group (if used) with the stoichiometric amount of HCl that is released during the reaction. Furthermore, the deprotection of the *N*-phthaloyl group was achieved under mild conditions by refluxing the protected amino acid-containing PS in toluene with hydrazine hydrate to obtain the deprotected PS-L-Leu. The detailed synthesis and characterization of small molecules (*N*-phthaloyl-L-leucine and *N*-phthaloyl-L-leucine acid chloride), post-polymer modification, and the deprotection are given in the Supporting Information (S2–S4 and Figures S2–S10).

Figure 1 compares the expanded region in the labeled proton NMR spectra of the post-polymer-modified PS with

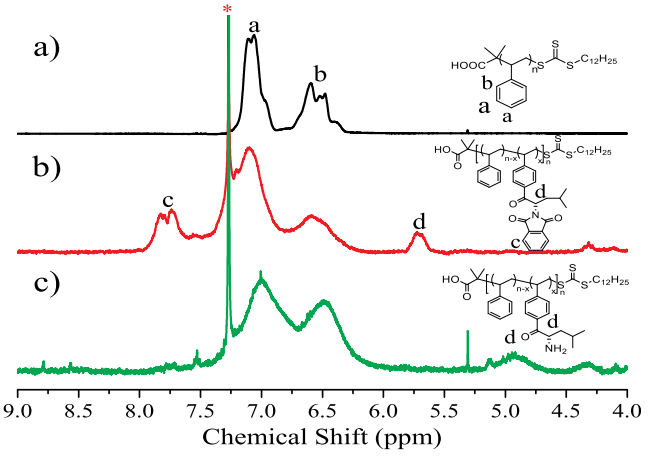


Figure 1. ^{1}H NMR spectra of (a) PS, (b) protected PS-L-Leu, and (c) deprotected PS-L-Leu.

pristine PS. PS (Figure 1a) shows two peaks in the aromatic region corresponding to the two types of aromatic protons (a and b) from the phenyl ring. The protected PS-L-Leu (Figure 1b) exhibited two additional peaks, c (7.79 ppm) and d (5.74 ppm), corresponding to four aromatic protons of phthaloyl protection and one proton on chiral carbon, respectively. Figure 1 also compares the proton NMR spectra of the deprotected PS-L-Leu (Figure 1c). The deprotection could be confirmed by the disappearance of the peaks corresponding to phthaloyl protection, that is, the peak labeled "c" at 7.79 ppm. The peak labeled "d", corresponding to the single proton on

https://doi.org/10.1021/acspolymersau.2c00004
ACS Polym. Au 2022, 2, 257–265

the chiral carbon, was shifted downfield in the deprotected PS, which also confirmed complete deprotection. Quantitative estimations of the percentage modification of PS with L-leucine were performed by taking the ratio of integrations of peak "b" from PS and peak "d" coming from the proton on the chiral carbon of the deprotected polymer. The percent modification was found to be 34% for deprotected PS-L-Leu. The ^{1}H NMR and Fourier transform IR (FTIR) spectroscopic characterization details of the protected and deprotected PS samples are given in Supporting Information Figures S7−S10.

FTIR spectra also provided clear evidence of post-polymer modification with the appearance of peaks at 1709 and 1713 cm^{-1}, corresponding to ketone carbonyl (C═O) for protected PS-L-Leu and deprotected PS-L-Leu polymers, respectively. Figure S11 compares the FTIR spectra (range 1800−1450) of the modified PS polymers (protected PS-L-Leu and deprotected PS-L-Leu) with that of the protected amino acids (*N*-phthaloyl-L-Leu). Compared to the pristine amino acid (protected), a shift was observed in the carbonyl stretching frequency in the modified polymers, which could be taken as additional evidence for the post-polymer modification.

The change in % elemental composition can be an additional evidence of post-polymer modification. The % elemental composition of PS, protected PS-L-Leu. and deprotected PS-L-Leu were determined by energy dispersive X-ray analysis (EDAX) using a field emission scanning electron microscope with a FEI Nova Nano SEM 450 (Supporting Information S5 and Figure S12). The pristine PS contained 98.66 wt % of carbon and a trace amount of sulfur (0.48 wt %) from the RAFT agent at the chain end (Figure S12a). The Protected PS-L-Leu shows the appearance of nitrogen (7.63 wt %) and enhancement in oxygen (16.22 wt %) along with a reduction of carbon (75.96 wt %) (Figure S12b). The deprotection of the phthaloyl group gave deprotected PS-L-Leu, which showed 80.42 wt % carbon, 6.09 wt % nitrogen, 12.88 wt % oxygen, and 0.61 wt % sulfur (Figure S12c). The appearance of nitrogen and enhancement in the oxygen wt % in the case of modified PS (protected and deprotected PS-L-Leu) gave evidence of successful modification with L-leucine, which induced the chirality in the polymer structure.

The average molecular weights of the polymers were determined using GPC in $CHCl_3$ (Figure S13). The number and weight average molecular weight (Mn and Mw) and molar mass distribution ($Đ_M$) of the polymers are given in Table 1.

Table 1. Average Molecular Weights (Mn and Mw), PDI ($Đ_M$), and Yield

polymers	Mn (g/mol)	Mw (g/mol)	$Đ_M$	% yield
polystyrene	22,800	30,100	1.3	65
protected PS-L-Leu	18,500	32,300	1.7	68
deprotected PS-L-Leu	16,700	29,400	1.8	74

The post-polymer-modified PS samples exhibited a reduced number-average molecular weight compared to the pristine PS, which could be attributed to the different hydrodynamic volumes of the amino acid-appended PS.

The chirality of the amino acid-appended PS was determined with the help of CD. Figure 2 compares the CD spectra for protected PS-L-Leu and deprotected PS-L-Leu samples. The CD spectrum was also collected for pristine PS, which did not exhibit any peaks as expected. The CD spectrum of protected PS-L-Leu exhibited four peaks at 286, 307, 322, and 338 nm, while the CD spectrum of deprotected PS-L-Leu showed peaks at 287, 300, 318, and 345 nm. From these CD spectra, it could be concluded that the post-polymer modification of PS with protected L-amino acid induced chirality in the polymers.

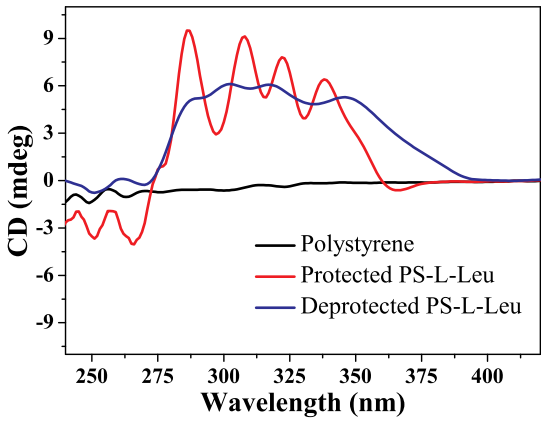


Figure 2. CD spectra of protected PS-L-Leu and deprotected PS-L-Leu plotted along with pristine PS at a concentration of 0.1 mg/mL in THF.

Polymer Microspheres

Polymer microspheres were assembled using both protected and deprotected PS samples. A solution of the polymer in DCM was added to deionized (DI) water containing a 4% Tween 80 surfactant. The solution was homogenized, and the DCM was slowly evaporated off; the precipitated polymer microspheres were repeatedly washed with DI water and collected by centrifugation. The details of polymer microsphere preparation are given in Supporting Information S6. The average sizes, PDI, and zeta potential (ζ) of polymeric microsphere samples were determined using dynamic light scattering (DLS) and zeta potential setup (Supporting Information S7).

The average particle sizes of all the microsphere samples were determined by plotting the DLS histogram at 0.25 mM concentrations (Figure S14). The average particle size for pristine PS was found to be 1.38 μm. The average size of protected PS-L-Leu was found to be 1.15 μm, and that for the corresponding deprotected microsphere samples was found to be 1.04 μm. The average size and PDI of all these polymers are tabulated in Supporting Information Table ST 1.

The shape, size, and morphology of polymeric samples (powder and microspheres) were determined with the help of micrographs recorded on a field emission scanning electron microscope. Figure 3 compares the SEM micrographs of the pristine PS, protected PS-L-Leu, and deprotected PS-L-Leu polymer samples. Pristine PS and protected PS-L-Leu did not exhibit any specific morphology (Figure 3a,c), while the deprotected polymer exhibited fibrous morphology (Figure 3e).

This change in morphology from irregular to fibrous nature upon deprotection could be understood by measuring the water contact angle of all the polymers (Figures 4 and S8). The pristine PS and the protected amino acid-appended PS had a water contact angle >95° (Figure 4a,b). After deprotection, the contact angle was reduced from 95.6 to 53.4° for deprotected PS-L-Leu (Figure 4c). This reduction in the water contact angle upon deprotection of the amino acid indicates an enhancement in hydrophilicity.

https://doi.org/10.1021/acspolymersau.2c00004
ACS Polym. Au 2022, 2, 257−265

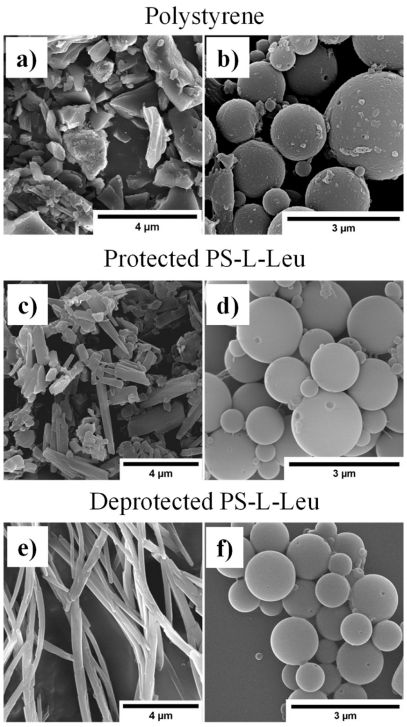


Figure 3. FE-SEM micrographs of (a) pristine PS (powder), (b) pristine PS (microspheres), (c) protected PS-L-Leu (powder), (d) protected PS-L-Leu (microspheres), (e) deprotected PS-L-Leu (fibers), and (f) deprotected PS-L-Leu (microspheres).

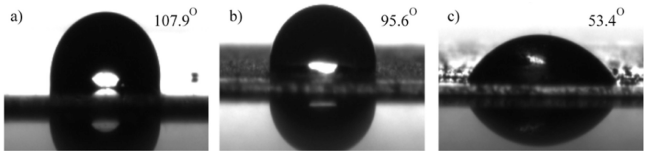


Figure 4. Water contact angles of (a) PS, (b) protected PS-L-Leu, and (c) deprotected PS-L-Leu.

The deprotected polymers could be anticipated to rearrange themselves so that the hydrophilic polar amine groups of the chiral amino acid are projected toward the hydrophilic environment, resulting in a fibrous morphology. Figure 3 also compares the spherical morphology of the pristine PS and the protected and deprotected PS. The particle sizes of these polymeric spheres were computed using ImageJ freeware. The average particle size ranged from ∼0.5 to 2 μm and was in reasonably good agreement with average sizes obtained from the DLS instrument.

The stability of the deprotected PS-L-Leu microspheres upon prolonged exposure to an aqueous environment was tested by suspending the microsphere in DI water with continuous stirring for 48 h. The deprotected polymer suspension was then drop cast on precleaned silicon wafers and dried overnight in a vacuum oven at 50 °C. The FE-SEM micrographs of these particles were recorded (Figure S15). From the micrographs, it could be observed that the microspheres retained their spherical morphology and no disassembly was observed.

Enantioselective Separation Experiments

Racemic mixtures of native amino acids such as alanine, aspartic acid, glutamic acid, leucine, lysine, phenylalanine, serine, and valine were prepared by dissolving 10 mg of each D- and L-enantiomers in 10 mL of DI water (1 mg/mL). A 5 mg of protected PS-L-Leu powder was added to each vial and the racemic mixtures were stirred along with the polymer suspension using a magnetic stirrer for 24 h at room temperature. After 24 h, the mixtures from the vials were filtered with the help of Whatman filter paper to remove the solid polymeric particles. The CD spectra of the filtrates were recorded for each sample. It was observed that the filtrate of leucine and alanine exhibited CD signals for corresponding D-enantiomers when treated with protected PS-L-Leu powder, while the filtrate of other amino acids did not exhibit any measurable signal. From this observation, it could be concluded that protected PS-L-Leu powder exhibited enantioselective separation of leucine and alanine by selective adsorption of the L-isomer from their racemic mixture leaving behind D-enantiomers in the filtrate. The CD spectrum of these filtrate solutions was then plotted against the CD spectrum of a reference solution (1 mg/mL) of L- and D-enantiomers of corresponding amino acids (Figure S16). Figure 5 shows the FE-SEM micrographs of the deprotected PS-L-Leu microsphere, which was used for enantioselective separation followed by filtration, showing adsorbed particles on the surface.

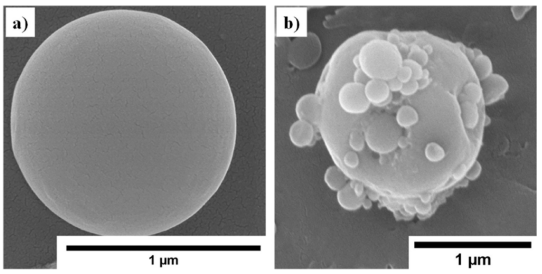


Figure 5. FE-SEM micrographs of chiral deprotected PS-L-Leu microspheres (a) before and (b) after enantioselective separation with particles adsorbed on the surface.

The area under the curve in the CD signal of the filtrate is proportional to the concentration of excess amount of the D-enantiomer in the solution compared to L-enantiomers and thus taken into consideration for determining the % ee of separation.[43] The % ee for enantioselective separation was calculated by taking the ratio of the area under the curve for the filtrate and the corresponding reference D-enantiomer solution (1 mg/mL) as shown in the formula below.

$$\%\ \text{ee} = \frac{\text{area under curve of filtrate}}{\text{area under curve of reference D solution (1 mg/mL)}} \times 100$$

The % ee values are given in Table 2. PS-L-Leu powder exhibited a 19.5% ee toward leucine. The ee % could be almost

Table 2. % ee Values for Enantioselective Separation Calculated Taking the Ratio of the Area under the Curve of the Filtrate and the Corresponding Reference D-Enantiomer (1 mg/mL)

	% enantiomeric excess values (% ee)	
racemic mixture	protected L-Leu-PS powder/microspheres	deprotected L-Leu-PS fibers/microspheres
alanine	16.5/35.2	40.6/53.8
leucine	19.5/42.1	49.5/58.2

https://doi.org/10.1021/acspolymersau.2c00004
ACS Polym. Au 2022, 2, 257−265

doubled by using the microspheres instead of the as-precipitated polymer powder. Thus, the ee % was enhanced from 19.5 to 42.1% for the separation of leucine using protected PS-L-Leu.

Figure S16 compares the CD signal of the filtrate using the as-precipitated polymer powder and the assembled microspheres for the enantioselective separation. Similar enantioselective separation experiments were set up with 5 mg each of the deprotected polymer fibers and assembled microspheres and CD spectra of the filtrate was plotted against the reference enantiomers (Figure S17). Table 2 also provides the % ee for the separation of the racemic mixtures of leucine and alanine using deprotected PS-L-Leu. A significant improvement in the % ee values was observed in the case of the deprotected polymer compared to the protected analogue (powder and microspheres) (Table 2 and Figure S17). Upon deprotection, the fibrous polymer sample exhibited an ee % of 40.6 and 49.5%, respectively, for the separation of alanine and leucine using PS-L-Leu, compared to only 16.5 and 19.5% using the protected polymer powder. Upon assembling into microspheres, further enhancement in ee % was observed with a maximum separation efficiency of 53.6% for alanine and 58.2% for leucine using deprotected PS-L-Leu.

Figure 6 shows a representative example of enhancement in the enantioselective separation of a racemic mixture of native

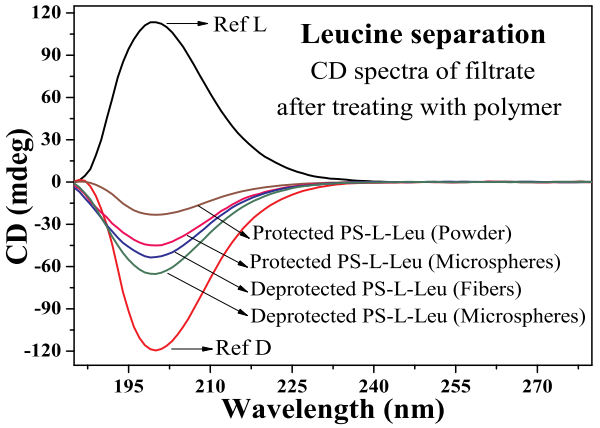


Figure 6. Representative example showing enhancement in the enantioselective separation of a racemic mixture of native leucine using deprotected PS-L-Leu (fibers and microspheres) compared to protected PS-L-Leu (powder and microspheres).

leucine from 19.5 to 58.2% ee by using 5 mg of the polymer in different forms. Increasing the amount of polymer microspheres used for the separation resulted in increased ee %. For instance, increasing the amount of deprotected PS-L-Leu microspheres from 5 mg to 9 mg for the enantioselective separation of a racemic mixture of leucine resulted in an enhancement of ee % from 58.2 to 72.8, which was further increased to 81.6% upon using 12 mg of polymer microspheres (Figure 7). The availability of larger amounts of deprotected PS-L-Leu microspheres for carrying out separation resulted in this enhancement in % ee from 58.2 to 81.6.

An approximate amount of 0.76 mg of L-leucine per milligram of chiral deprotected PS microspheres was estimated to be adsorbed on the surface using calculations based on the area under the CD curve (Supporting Information S9). This is a reasonably good achievement given the fact that separation could be achieved using a commodity polymer without any

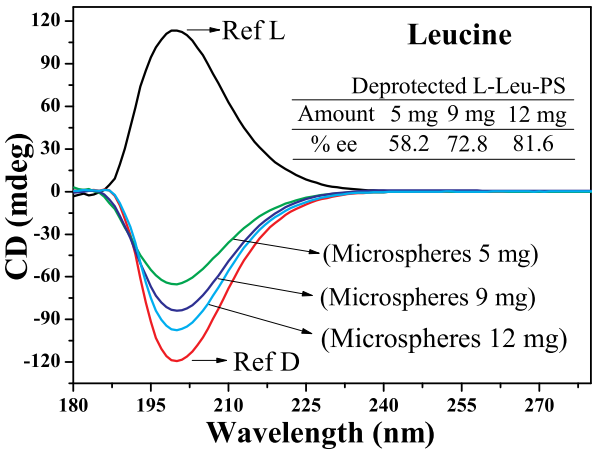


Figure 7. CD spectra for the separation of the leucine racemic mixture using 5, 9, and 12 mg of deprotected PS-L-Leu microspheres.

necessity for its application on any template or membrane such as AAO membranes, making the process commercially viable. The % ee value is anticipated to be further improved by achieving a higher % chiral modification of PS or by polymerizing chiral styrene monomers.

The enhanced performance of the deprotected polymer could be attributed to the increased hydrophilicity of the polymers due to free amine groups, which significantly improved the surface wettability. Additionally, it is anticipated that the hydrophilic amine groups improve the enantioselective separation ability by self-assembling with the chiral amino acids exposed to the polymer surface. Chiral groups on the microspheres can form secondary transition-state complexes of different energies with enantiomers by a three-point interaction model.[44] Because of the difference in the stability of transition-state complexes, separation is achieved. An experiment was set up to probe the mechanism of interaction of the chiral selector with the analyte. 15 mg of deprotected PS-Leu as a chiral selector was suspended in a racemic mixture (10 mg of D/L-leucine in 10 mL of DI water) of leucine for 24 h. After enantioselective separation, the polymeric microspheres were filtered through filter paper and dried overnight at 50 °C in a vacuum oven. Our attempts at tracing the interaction using the ^{1}H NMR spectra in $CDCl_3$ did not yield much information as the deprotected PS-L-Leu got completely solubilized in $CDCl_3$, but the adsorbed amino acid was insoluble in $CDCl_3$ and precipitated inside the NMR tube. Supporting Information Figure S18 compares the proton NMR spectra of the deprotected PS-L-Leu before and after enantioselective separation. Although no change in the peak position was observed, some additional peaks were observed around 8.15−8.08 and 4.10−3.65 ppm in the deprotected PS-L-Leu after separation. The peaks at 8.15−8.08 and 4.10−3.65 ppm could be assigned to the hydrogen on amine and chiral carbon of adsorbed L-leucine, respectively. In comparison, the other peaks were merged with the polymeric aliphatic region.

FTIR spectroscopy is a very useful tool to trace hydrogen bonding-based interactions between a chiral selector and an analyte. Figure 8 compares the solid-state FTIR spectra of deprotected PS-Leu microspheres before and after the enantioselective separation of L-leucine along with that of pristine L-leucine. Figure 8 compares the expanded region in the FTIR spectra focusing on the ketonic carbonyl (C═O), methylene (−CH_2−), and amine (−NH_2) stretching frequen-

https://doi.org/10.1021/acspolymersau.2c00004
ACS Polym. Au 2022, 2, 257−265

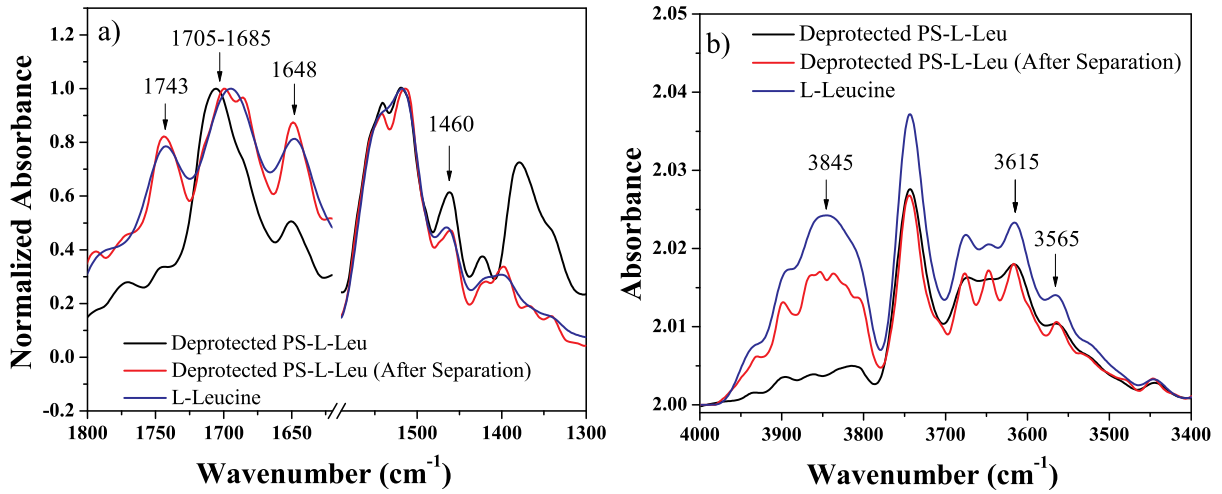


Figure 8. Normalized FTIR spectra of L-leucine and deprotected PS-L-Leu before and after enantioselective separation of (a) carbonyl stretching frequency and methylene bending vibrations and the (b) amine stretching frequency region.

cies. The peaks observed around 1705 and 1648 cm^{-1} for deprotected PS-L-Leu corresponded to the non-hydrogen-bonded and hydrogen-bonded ketonic carbonyl (C═O) stretching frequency, respectively.[45] In the FTIR spectra of deprotected PS-L-Leu after enantioselective separation, peaks appeared in the same region as those for pristine leucine, but differed in the ratio of non-hydrogen-bonded and hydrogen-bonded peak intensity. The increment in the hydrogen-bonded carbonyl (1648 cm^{-1}) peak intensity compared to the deprotected PS-L-Leu before separation gave evidence for the interaction of the chiral selector with the adsorbed L-leucine through intermolecular hydrogen bonding.

Additionally, a new peak was observed at the 1743 cm^{-1} region, which could be assigned to the carbonyl from the adsorbed amino acid, as the stretching frequency of carboxyl carbonyl appeared in the range of 1710−1760 cm^{-1}.[46] L-leucine had peaks in the same region (1743 cm^{-1}). Bending vibrations of a methylene group ($-CH_2-$) appeared at around 1460 cm^{-1}, which could be seen for all the samples. Although no apparent change was observed in its peak position, small changes in the peak intensity were observed after enantioselective separation. According to dispersion theory, the intensity of an absorbance band is proportional to the number of oscillators per unit volume and also proportional to the squared effective charge of the moving atom, which can be influenced by the surrounding.[47] The hydrogen bonding between deprotected PS-L-Leu and amino acids (analytes) could be expected to bring about a change in the net effective charge on the methylene proton, which, in turn, could influence the intensity of the vibration of the methylene bending peak. Thus, the change in the peak intensity around 1460 cm^{-1} can be considered an indication of hydrogen bonding. The enlarged region around 3615 and 3565 cm^{-1} in Figure 8b corresponded to the asymmetric and symmetric stretching frequencies of the primary amine. The appearance of a peak at 3845 cm^{-1} in deprotected PS-L-Leu after enantioselective separation is assigned to the O−H stretching frequency of the adsorbed amino acid, which was absent in the deprotected PS-L-Leu before enantioselective separation. From these FTIR studies, it could be concluded that carbonyl, methylene, and amine groups around the chiral center in deprotected PS-L-Leu were involved in chiral recognition by forming a diastereomeric complex, which differed in their binding energy and helped in the enantioselective resolution of racemic mixtures.

CONCLUSIONS

In summary, chiral PS was designed and synthesized using a post-polymer modification approach. The modification of PS with protected L-leucine induced chirality in PS. The protected and deprotected chiral PSs in the powder form and assembled into microspheres were used to carry out the enantioselective separation of the native amino acid racemic mixture by a simple filtration-based enantioselective separation method. Protected and deprotected PS-L-Leu exhibited enantioselective separation efficiencies for leucine and alanine from their racemic mixture. Better separation efficiency was achieved with microspheres compared to as-precipitated polymers in both protected and deprotected PS. Among the tested amino acids, the highest separation was achieved using microspheres of deprotected polymers. The highest % ee of 81.6 was achieved for the separation of the leucine racemic mixture using 12 mg of deprotected PS-L-Leu microspheres. The enhanced % ee value in the deprotected polymer microsphere is attributed to the rearrangement of the polymer structure, improved hydrophilicity, and increased surface area.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acspolymersau.2c00004.

Synthesis of PS, small molecules and post-polymer modification, preparation of microspheres, DLS, water contact angle, structural characterizations using ^1H NMR spectra, FTIR spectra, GPC, zeta potential, and FE-SEM, tables for particle size, particle size distribution, and zeta potential, and CD spectra indicating enantioselective separation (PDF)

AUTHOR INFORMATION

Corresponding Author

Asha S. K − *Polymer Science and Engineering Division, CSIR-National Chemical Laboratory, Pune 411008, India; Academy of Scientific and Innovative Research, Ghaziabad*

https://doi.org/10.1021/acspolymersau.2c00004
ACS Polym. Au 2022, 2, 257−265

201002 Uttar Pradesh, India; orcid.org/0000-0002-3999-4810; Email: sk.asha@ncl.res.in; Fax: 0091-20-25902615

Author

Shrikant B. Nikam − *Polymer Science and Engineering Division, CSIR-National Chemical Laboratory, Pune 411008, India; Academy of Scientific and Innovative Research, Ghaziabad 201002 Uttar Pradesh, India;* orcid.org/0000-0001-6718-9002

Complete contact information is available at: https://pubs.acs.org/10.1021/acspolymersau.2c00004

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work has been financially supported by the Science and Engineering Research Board funded Project EMR/2017/001656. S.B.N. thanks Council of Scientific and Industrial Research (CSIR) for the senior research fellowship. We acknowledge Dr. Moneesha Fernandes, CSIR-National Chemical Laboratory (CSIR-NCL), Pune, for the CD spectrometer facility. The authors also thank Dr. Kadhiravan Shanmuganathan and Prashant Yadav, CSIR-NCL, Pune, for the microsphere preparation facility.

■ ABBREVIATIONS

PS	polystyrene
CD	circular dichroism
FE-SEM	Field emission scanning electron microscopy
% ee	% enantiomeric excess
PDI	polydispersity index

■ REFERENCES

(1) Calcaterra, A.; D'Acquarica, I. The market of chiral drugs: Chiral switches versus de novo enantiomerically pure compounds. *J. Pharm. Biomed. Anal.* **2018**, *147*, 323−340.
(2) Jeschke, P. Current Status of Chirality in Agrochemicals. *Pest Manag. Sci.* **2018**, *74*, 2389−2404.
(3) Sekhon, B. S. Chiral Pesticides. *J. Pestic. Sci.* **2009**, *34*, 1−12.
(4) Mannschreck, A.; Kiesswetter, R.; von Angerer, E. Unequal Activities of Enantiomers via Biological Receptors: Examples of Chiral Drug, Pesticide, and Fragrance Molecules. *J. Chem. Educ.* **2007**, *84*, 2012−2017.
(5) Engel, K.-H. Chirality: An Important Phenomenon Regarding Biosynthesis, Perception, and Authenticity of Flavor Compounds. *J. Agric. Food Chem.* **2020**, *68*, 10265−10274.
(6) Schäfer, U.; Kiefl, J.; Zhu, W.; Kempf, M.; Eggers, M.; Backes, M.; Geissler, T.; Wittlake, R.; Reichelt, K. V.; Ley, J. P.; Krammer, G. Authenticity Control of Food Flavorings - Merits and Limitations of Chiral Analysis. *Importance of Chirality to Flavor Compounds*; ACS Symposium Series; American Chemical Society, 2015; Vol. *1212*, pp 3−12.
(7) Zawirska-wojtasiak, R. Chirality And The Nature Of Food Authenticity Of Aroma. *Acta Sci. Pol., Technol. Aliment.* **2006**, *5*, 21−36.
(8) Schmid, T.; Daiss, J. O.; Ilg, R.; Surburg, H.; Tacke, R. Enantiopure Chiral Derivatives of the Fragrance Materials Majantol and Sila-Majantol: A Bioisosteric Carbon/silicon Switch with Drastic Effects on the Sensory Characteristics. *Organometallics* **2003**, *22*, 4343−4346.
(9) Abate, A.; Brenna, E.; Fuganti, C.; Gatti, F. G.; Giovenzana, T.; Malpezzi, L.; Serra, S. Chirality and Fragrance Chemistry: Stereoisomers of the Commercial Chiral Odorants Muguesia and Pamplefleur. *J. Org. Chem.* **2005**, *70*, 1281−1290.
(10) Zhou, Y.; Wu, S.; Zhou, H.; Huang, H.; Zhao, J.; Deng, Y.; Wang, H.; Yang, Y.; Yang, J.; Luo, L. Chiral Pharmaceuticals: Environment Sources, Potential Human Health Impacts, Remediation Technologies and Future Perspective. *Environ. Int.* **2018**, *121*, 523−537.
(11) O'Brien, P. Sharpless Asymmetric Aminohydroxylation: Scope, Limitations, and Use in Synthesis. *Angew. Chem., Int. Ed.* **1999**, *38*, 326−329.
(12) Verendel, J. J.; Pàmies, O.; Diéguez, M.; Andersson, P. G. Asymmetric Hydrogenation of Olefins Using Chiral Crabtree-Type Catalysts: Scope and Limitations. *Chem. Rev.* **2014**, *114*, 2130−2169.
(13) Ward, T. J.; Ward, K. D. Chiral Separations: Fundamental Review 2010. *Anal. Chem.* **2010**, *82*, 4712−4722.
(14) McDonald, M. A.; Salami, H.; Harris, P. R.; Lagerman, C. E.; Yang, X.; Bommarius, A. S.; Grover, M. A.; Rousseau, R. W. Reactive Crystallization: A Review. *React. Chem. Eng.* **2021**, *6*, 364−400.
(15) Yao, Q.; Xie, J. Pasteur-like Separation of Silver Nanocluster Racemates by Conglomerate Crystallization. *ACS Cent. Sci.* **2020**, *6*, 1862−1865.
(16) Qiu, J.; Stevens, J. M. High-Throughput Classical Chiral Resolution Screening of Synthetic Intermediates: Effects of Resolving Agents, Crystallization Solvents, and Other Factors. *Org. Process Res. Dev.* **2020**, *24*, 1725−1734.
(17) Ward, T. J.; Ward, K. D. Chiral Separations: A Review of Current Topics and Trends. *Anal. Chem.* **2012**, *84*, 626−635.
(18) Tarafder, A.; Miller, L. Chiral Chromatography Method Screening Strategies: Past, Present and Future. *J. Chromatogr., A* **2021**, *1638*, 461878.
(19) Yu, R. B.; Quirino, J. P. Chiral Selectors in Capillary Electrophoresis: Trends During 2017-2018. *Molecules* **2019**, *24*, 1135.
(20) Berkecz, R.; Németi, G.; Péter, A.; Ilisz, I. Liquid Chromatographic Enantioseparations Utilizing Chiral Stationary Phases Based on Crown Ethers and Cyclofructans. *Molecules* **2021**, *26*, 4648.
(21) Adhikari, S.; Lee, W. Chiral Separation Using Chiral Crown Ethers as Chiral Selectors in Chirotechnology. *J. Pharm. Invest.* **2018**, *48*, 225−231.
(22) Suraj Koorpet, R.; Akshay, N.; Nishanth, G.; Chandan, R. S.; Kumar Tengli, A. A Review on Chiral Columns/Stationary Phases for HPLC. *Int. J. Res. Pharm. Sci.* **2020**, *11*, 2466−2480.
(23) Zhang, Q.; Xue, S.; Li, A.; Ren, S. Functional Materials in Chiral Capillary Electrophoresis. *Coord. Chem. Rev.* **2021**, *445*, 214108.
(24) Shen, J.; Okamoto, Y. Efficient Separation of Enantiomers Using Stereoregular Chiral Polymers. *Chem. Rev.* **2016**, *116*, 1094−1138.
(25) Zhou, C.; Ren, Y.; Han, J.; Xu, Q.; Guo, R. Chiral Polyaniline Hollow Nanotwists toward Efficient Enantioselective Separation of Amino Acids. *ACS Nano* **2019**, *13*, 3534−3544.
(26) Senthilkumar, T.; Asha, S. K. An easy "Filter-and-Separate" method for enantioselective separation and chiral sensing of substrates using a biomimetic homochiral polymer. *Chem. Commun.* **2015**, *51*, 8931−8934.
(27) Nikam, S. B.; Asha, S. K Enantioselective Separation Using Chiral Amino Acid Functionalized Polyfluorene Coated on Mesoporous Anodic Aluminum Oxide Membranes. *Anal. Chem.* **2020**, *92*, 6850−6857.
(28) Abuaf, M.; Mastai, Y. Synthesis of Multi Amino Acid Chiral Polymeric Microparticles for Enantioselective Chemistry. *Macromol. Chem. Phys.* **2020**, *221*, 2000328.
(29) Yu, Y.; Xu, N.; Zhang, J.; Wang, B.; Xie, S.; Yuan, L. Chiral Metal-Organic Framework d-His-ZIF-8@SiO2 Core-Shell Microspheres Used for HPLC Enantioseparations. *ACS Appl. Mater. Interfaces* **2020**, *12*, 16903−16911.
(30) Prosuntsova, D. S.; Plodukhin, A. Y.; Ananieva, I. A.; Beloglazkina, E. K.; Nesterenko, P. N. New Composite Stationary Phase for Chiral High-Performance Liquid Chromatography. *J. Porous Mater.* **2021**, *28*, 407−414.

(31) Mutalikdesai, A.; Pagidi, S.; Hassner, A.; Gedanken, A. Microspheres of Biomolecules/macromolecules for Enantioseparation Applications. *Eur. Polym. J.* **2021**, *142*, 110145.
(32) Wang, P.-X.; Hamad, W. Y.; MacLachlan, M. J. Polymer and Mesoporous Silica Microspheres with Chiral Nematic Order from Cellulose Nanocrystals. *Angew. Chem., Int. Ed.* **2016**, *55*, 12460−12464.
(33) Paik, P.; Gedanken, A.; Mastai, Y. Enantioselective Separation Using Chiral Mesoporous Spherical Silica Prepared by Templating of Chiral Block Copolymers. *ACS Appl. Mater. Interfaces* **2009**, *1*, 1834−1842.
(34) Medina, D. D.; Goldshtein, J.; Margel, S.; Mastai, Y. Enantioselective Crystallization on Chiral Polymeric Microspheres. *Adv. Funct. Mater.* **2007**, *17*, 944−950.
(35) Zhang, Z.; Zhang, M.; Liu, Y.; Yang, X.; Luo, L.; Yao, S. Preparation of L-Phenylalanine Imprinted Polymer Based on Monodisperse Hybrid Silica Microsphere and Its Application on Chiral Separation of Phenylalanine Racemates as HPLC Stationary Phase. *Sep. Purif. Technol.* **2012**, *87*, 142−148.
(36) Chen, B.; Song, C.; Luo, X.; Deng, J.; Yang, W. Microspheres Consisting of Optically Active Helical Substituted Polyacetylenes: Preparation via Suspension Polymerization and Their Chiral Recognition/release Properties. *Macromol. Rapid Commun.* **2011**, *32*, 1986−1992.
(37) Wu, J.; Su, P.; Guo, D.; Huang, J.; Yang, Y. Cationic *β*-cyclodextrin-modified hybrid magnetic microspheres as chiral selectors for selective chiral absorption of dansyl amino acids. *New J. Chem.* **2014**, *38*, 3630−3636.
(38) Wu, J.; Su, P.; Yang, Y.; Huang, J.; Wang, Y.; Yang, Y. Immobilization of HSA on Polyamidoamine-Dendronized Magnetic Microspheres for Application in Direct Chiral Separation of Racemates. *J. Mater. Chem. B* **2014**, *2*, 775−782.
(39) Cong, H.; Xing, J.; Ding, X.; Zhang, S.; Shen, Y.; Yu, B. Preparation of Porous Sulfonated Poly(styrene-Divinylbenzene) Microspheres and Its Application in Hydrophilic and Chiral Separation. *Talanta* **2020**, *210*, 120586.
(40) Matsas, G. M.; Faliagas, A. C.; Simitzis, J. C. No Title. *Angew. Makromol. Chem.* **1995**, *227*, 35−42.
(41) Roy, M.; Rajamohanan, P. R.; Ravindranathan, S.; Asha, S. K. Self-Assembly of Bispentadecylphenol Substituted Perylenediimide with PS-b-P4VP for Structure-Property Insight into the Core of Core-Shell Micelles. *ACS Appl. Polym. Mater.* **2020**, *2*, 805−816.
(42) Williams, D. B. G.; Lawton, M. Drying of Organic Solvents: Quantitative Evaluation of the Efficiency of Several Desiccants. *J. Org. Chem.* **2010**, *75*, 8351−8354.
(43) Joyce, L. A.; Maynor, M. S.; Dragna, J. M.; Da Cruz, G. M.; Lynch, V. M.; Canary, J. W.; Anslyn, E. V. A Simple Method for the Determination of Enantiomeric Excess and Identity of Chiral Carboxylic Acids. *J. Am. Chem. Soc.* **2011**, *133*, 13746−13752.
(44) Gogoi, A.; Mazumder, N.; Konwer, S.; Ranawat, H.; Chen, N.-T.; Zhuo, G.-Y. Enantiomeric Recognition and Separation by Chiral Nanoparticles. *Molecules* **2019**, *24*, 1007.
(45) George, K. M.; Ruthenburg, T. C.; Smith, J.; Yu, L.; Zhang, Q.; Anastasio, C.; Dillner, A. M. FT-IR Quantification of the Carbonyl Functional Group in Aqueous-Phase Secondary Organic Aerosol from Phenols. *Atmos. Environ.* **2015**, *100*, 230−237.
(46) Barth, A. The Infrared Absorption of Amino Acid Side Chains. *Prog. Biophys. Mol. Biol.* **2000**, *74*, 141−173.
(47) Jones, R. N.; Ramsay, D. A.; Keir, D. S.; Dobriner, K. The Intensities of Carbonyl Bands in the Infrared Spectra of Steroids1. *J. Am. Chem. Soc.* **1952**, *74*, 80−88.

https://doi.org/10.1021/acspolymersau.2c00004
ACS Polym. Au 2022, 2, 257−265