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Enantioselective Crystallization on Chiral Polymeric Microspheres**

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In this article, a new approach to chiral resolution based on enantioselective crystallization on chiral polymeric microspheres is presented. To demonstrate the approach, a new synthetic method is developed for the preparation of porous and hollow chiral polymeric microspheres. Chiral microspheres based on poly(*N*-vinyl α-L-phenylalanine) (PV-L-Phe) are synthesized from uniform polystyrene (PS) microsphere templates by a single-step swelling process. The chiral microspheres display a narrow size distribution, and their properties, especially the porosity, can be controlled by varying the synthesis conditions. The chiral discrimination ability of these chiral microspheres is studied for DL-valine crystallization, as a model system for chiral racemic crystallization. X-ray diffraction (XRD) and differential scanning calorimetry (DSC) provide evidence for the crystallization of enantiopure crystals on the chiral polymeric microspheres. Optical rotation measurements during crystallization show an enatiomeric excess of ca. 25 % for L-valine in the chiral microspheres. Although this excess is not yet very high, the basic principle of chiral discrimination by enantioselective crystallization on chiral microspheres is demonstrated.

1. Introduction

The separation of chiral compounds has been of great interest primarily because of the fact that the majority of bioorganic molecules, such as amino acids, sugars, proteins, and nucleic acids, tend to be chiral. The need for efficient high-throughput techniques for the production of enantiomerically pure compounds is growing in conjunction with the increasing structural complexity of new drug molecules.[1] In the absence of methods for the synthesis of drugs in optically pure forms, the resolution of racemates using chiral selectors or auxiliaries is the only available route for obtaining chiral drugs. The resolution of enantiomers can be achieved by various methods,[2] including fractional crystallization, microbiological methods, kinetic enzymatic resolution, and chromatography. Of growing importance are methods that permit the continuous production of pure enantiomers, such as by liquid–solid partitioning in simulated moving bed (SMB) chromatography,[3] chiral-membrane-based separation techniques,[4] and polymers imprinted with chiral templates.[5]

Although all these techniques show high chiral discrimination, they are not yet applicable for the large-scale resolution of enantiomers. Therefore, large-scale separations are still typically achieved by classical crystallization methods. Crystallization is a very powerful technique, although it is far from being generally applicable, and permits common compounds that behave as conglomerates to be resolved from their equimolecular enantiomeric mixtures. Resolution by crystallization can be accomplished either by seeding the crystallization solution with crystals of one enantiomer (preferential crystallization), or by using chiral auxiliaries during crystallization. In recent years, new approaches for the optical resolution of enantiomers based on chiral recognition have been employed. For instance, Lahav and co-workers have developed a new general methodology consisting of the use of "tailor-made additives"[6] for the kinetic resolution of conglomerates and racemes. In principle, this method is suitable for large-scale applications. This separation technique relies on the addition of enantiospecific chiral inhibitors, usually low-molecular-weight chiral compounds that prevent or delay the growth of one of the enantiomorphs. Zbaida et al. have extended this work to soluble chiral polymers.[7,8] We have recently demonstrated the potential application of chiral double hydrophilic block copolymers in controlling chirality during the crystallization process.[9,10] We have shown that appropriate chiral polymers can slow down the formation of thermodynamically stable racemic crystals while favoring the formation of one of the pure enantiomeric crystals.

In this paper, we describe a new approach for chiral separation based on enantioselective crystallization on the chiral surfaces of micrometer-sized polymeric particles. The underlying principle of our approach involves the utilization of chiral particles as resolving auxiliaries in the crystallization of enantiomers. Our hypothesis is that enantiospecific chiral particles will serve as selective chiral nuclei (seeds) during crystallization, lowering the formation energy for crystals of one enantiomer. Consequently, this enantiomer will crystallize in excess on the chiral particles, thereby enabling separation of this enantiomer from the crystallization solution.

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In order to demonstrate this chiral discrimination approach on chiral polymeric surfaces, we have developed a new method for the synthesis of chiral polymeric particles. Typically, chiral particles are prepared by the chiral surface functionalization of spherical particles of polystyrene (PS)[11] or silica.[12] Although chiral particles formed by surface functionalization exhibit chiral recognition properties, it is obvious that for the chiral particles to be used as resolving auxiliaries for selective chiral crystallization, a highly chiral surface is desired. Therefore, we have developed a new synthesis route for the preparation of polymeric chiral particles, wherein the entire particle, and not just the surface, is chiral. In the present article, we describe the synthesis and characterization of porous chiral polymeric microspheres and the use of these microspheres for enantioselective crystallization.

Overall, our approach is in line with the general concept of molecular recognition at chiral surfaces. However, we also expect additional advantages that are primarily important from the technological point of view. Micrometer-sized polymeric particles can easily be separated from the crystallizing substrate, thus allowing the recovery of both the polymers and the enantiomers. Moreover, our approach of using chiral particles for enantioselective crystallization can be efficiently coupled with existing technologies, resulting in improved chiral resolution.

2. Results and Discussion

2.1. Synthesis of Chiral Polymeric Microspheres

Micrometer-sized particles with a narrow size distribution have attracted much attention because of their potential applications in areas such as adsorbents for high-pressure liquid chromatography (HPLC), calibration standards, spacers for liquid crystals, inks, and catalysis.[13] Dispersion polymerization is the most common method used for the one-step preparation of non-porous and uniform micrometer-sized particles.[14] However, particles formed by this method possess a relatively small surface area, and their properties, such as the porosity, surface morphology, and functionality, can be difficult to manipulate. Recently, a new method for preparing micrometer-sized polymeric particles with a narrow size distribution and controllable properties (such as surface areas) has been developed, based on the single-step swelling of uniform microsphere templates.[15] We have adopted this method for the preparation of chiral micrometer-sized polymeric particles. In this study, we report the preparation of chiral microspheres of poly(*N*-vinyl α-L-phenylalanine) (PV-L-Phe). Notably, the method described here is, in principle, suitable for the preparation of a wide variety of amino acid chiral polymers.

Figure 1 depicts the synthetic pathway for the synthesis of chiral polymeric microspheres. In the first step (Fig. 1A), uniform template particles of PS (size 2.7 μm ± 7 %) are prepared according to a procedure described in the literature.[16] In the next step, the template PS particles are swollen with an initiator (divinylbenzene (DVB)) and a chiral monomer (*N*-vinyl α-L-phenylalanine)[17] using a swelling solvent, either chlorobenzene or bromochloromethane. It should be pointed out that this swelling process is accomplished in a single step, which is in contrast to multi-step swelling processes where swelling with these reagents is accomplished in two or more steps. This swelling process leads to a significant increase in the diameter of the particles, from 2.7 μm ± 7 % (Fig. 1A) to 7.6 μm ± 6.5 % (Fig. 1B). The polymerization of the chiral monomer within the uniformly swollen PS particles is induced by increasing the temperature of the swollen particles to 70 °C. The polymerization reaction leads to composite particles with a slightly smaller diameter of ca. 6.4 μm ± 2.5 % (Fig. 1C). Finally, the selective dissolution of PS with methylene chloride leads to a further slight decrease in the diameter, from 6.4 μm ± 2.5 % to 5.4 μm ± 2 % (Fig. 1D).

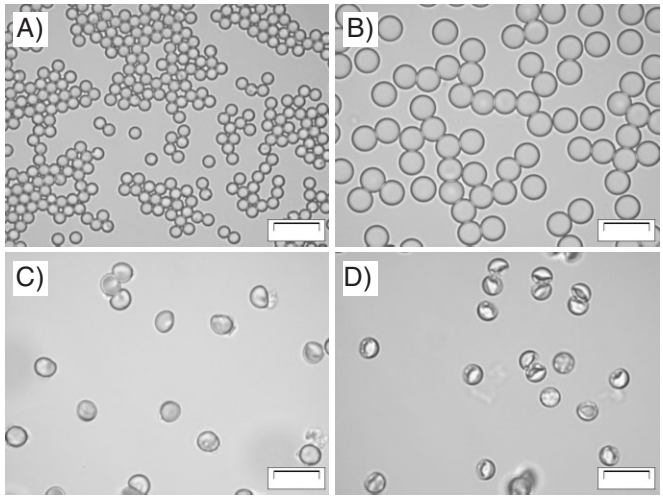

Figure 1. Light microscopy pictures illustrating the preparation of chiral polymeric particles (scale bars = 10 μm): A) PS template microspheres; B) Swelling: PS template microspheres after swelling with bromochloromethane; C) Polymerization: PV-L-Phe–PS crosslinked composite microspheres; D) After dissolution of the template: crosslinked PV-L-Phe–PS microspheres.

In general, the nature of the swelling solvent can lead to different pore structures within the particles. Figure 2 shows scanning electron microscopy (SEM) images of chiral PV-L-Phe microspheres prepared with different swelling solvents. The major difference is that PV-L-Phe microspheres prepared with

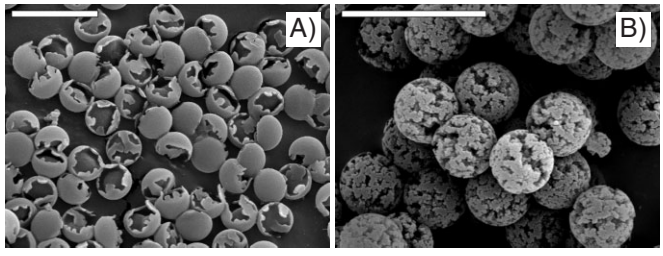

Figure 2. Scanning electron microscopy images of chiral PV-L-Phe microspheres prepared with different swelling solvents: A) bromochloromethane and B) chlorobenzene (scale bars = 10 μm).

bromochloromethane have a hollow structure, whereas those prepared using chlorobenzene as a swelling solvent are spherical in shape. In both cases, the average size of the microspheres is similar (ca. 5.5 μm), although the surface areas are remarkably different.

We have utilized Fourier transform infrared (FTIR) spectroscopy to follow the different stages during the growth of the chiral microspheres. The FTIR spectra of the chiral PV-L-Phe microspheres (Supporting Information, Fig. S1) show typical absorption peaks at ca. 1000–1300, 1220, and 1736 cm^{-1}, corresponding to ester carbonyl stretching bands; at 1669 and 1530 cm^{-1}, corresponding to amide stretching bands; from 2800 to 3100 cm^{-1}, corresponding to CH (aromatic and aliphatic), CH_2, and CH_3 stretching bands; at 1444 cm^{-1}, corresponding to the C–C stretching band of aromatic rings; and at ca. 3400 cm^{-1}, corresponding to N–H stretching modes. The selective dissolution of PS by methylene chloride can also be verified by FTIR measurements; specifically, the characteristic PS peaks at 398, 540, and 766 cm^{-1} (C–C bending/stretching) are weakened to almost zero, whereas the characteristic peaks for PV-L-Phe increase in intensity. Finally, the UV-vis absorption spectrum of the chiral PV-L-Phe microspheres is characterized by a strong absorption peak at 207 nm and significantly weaker absorption peaks at 252, 259, 264, and 269 nm, all of which correspond to the aromatic ring of PV-L-Phe, as reported in the literature for similar compounds.[18]

In summary, we have described the preparation and characterization of new porous and hollow chiral microspheres based on crosslinked PV-L-Phe. The method reported here can be extended to the preparation of chiral microspheres of any desired α-amino acid.

2.2. Chiral Crystallization

To demonstrate the chiral discrimination of the polymeric microspheres in the crystallization process, we have chosen to work with select model systems for chiral crystallization. In general, when a chiral molecule crystallizes from solution it can form either a) racemic crystals, which contain equal numbers of left- and right-handed molecules in the same crystal, b) conglomerates of separate left- and right-handed crystals of the pure enantiomers, such as Pasteur's tartarate salt, or c) a racemic solid solution in which the two enantiomers coexist in a disordered manner over a specified concentration range in the crystal lattice.

It is worth noting that racemic crystals tend to greatly outnumber conglomerates in nature. For example, Jacques et al.[19] have reported that statistically, only 5 to 10 % of all racemates form conglomerate crystals. With regards to chiral resolution by crystallization, conglomerate crystals are easier to resolve without the aid of a resolving agent, such as by spontaneous resolution. In this work, we examine amino acids as model systems for chiral crystallization, mainly because of their biological importance and the extensive literature available pertaining to their crystallization and crystal structures. We have selected DL-valine as a model system for studying racemic crystallization. The crystallization experiments are performed from supersaturated solutions of DL-valine. Since the supersaturation levels of the solution have a strong impact on the crystallization rate, growth mechanisms, and crystal size, we work under low supersaturation conditions with a typical supersaturation index of $\sigma = 0.5–2$.

To use chiral microspheres as selective chiral seeds for crystallization, we first need to determine the conditions under which valine only crystallizes inside the microspheres and not in the bulk solution. We have performed a series of experiments to crystallize DL-valine in the presence of these microspheres. In a typical crystallization experiment, an appropriate amount of DL-valine is suspended in deionized water. The solution is heated to 70 °C and stirred until valine is completely dissolved. Subsequently, 10 $mg\,mL^{-1}$ of the chiral microspheres are introduced into the solution and stirred for ca. 5 min. The solutions are then allowed to cool spontaneously to room temperature. After complete crystallization has taken place (ca. 10–24 h), the crystallization solutions are filtered and the solid crystals are collected. In general, under high supersaturation conditions ($\sigma = 0.9–1.1$), the co-crystallization of free crystals from solution is observed along with crystallization on chiral microspheres. These experiments are not discussed further since we are unable to separate the free crystals in solution from the chiral microspheres. Therefore, here we focus only on crystallization conditions wherein crystallization in bulk solution is restricted. Under relatively low supersaturation conditions ($\sigma = 0.7–0.8$), crystallization occurs only on the surfaces and within the chiral microspheres, and the crystallization of free crystals from solution is not observed by light and electron microscopy.

Figure 3 shows SEM images of hollow PV-L-Phe microspheres after crystallization from DL-valine solutions. These images clearly show that the surfaces of the chiral microspheres are covered with fine needle-like valine crystals with typical sizes of ca. 4.5 μm × 1.5 μm (Fig. 3A and B). In some cases, as shown in Figure 3C, it appears that crystallization also occurs within the chiral microspheres. In other words, the hollow chiral microspheres are filled with crystals.

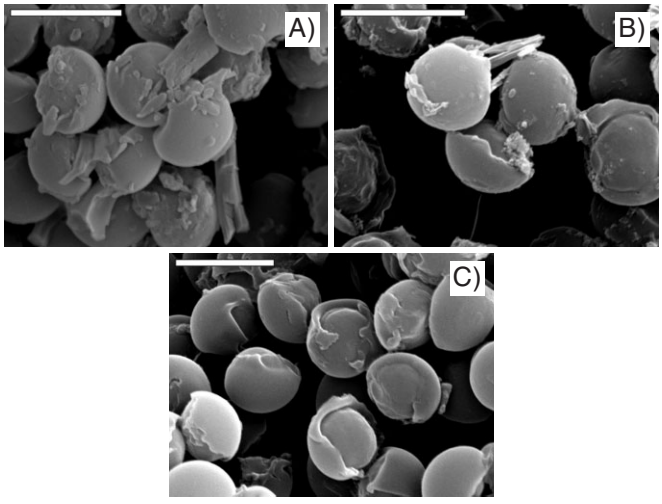

Figure 3. SEM images of chiral PV-L-Phe microspheres after crystallization of DL-valine (scale bars = 5 μm).

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In order to analyze enantioselective crystallization on the chiral microspheres, we have employed powder X-ray diffraction (XRD) and differential scanning calorimetry (DSC). Here, we should emphasize that in the crystalline state enantiomers and the corresponding racemic compound can differ considerably in their properties.[20] For example, the XRD pattern of enantiomers is different from that of the corresponding racemic compound owing to their different crystalline structures.[21] In addition, the thermal behavior of the racemates and pure enantiomers is different, and accordingly, traces of enantiomers can be examined by DSC.[22]

Figure 4 compares the XRD patterns of DL-valine crystallized from a pure solution and DL-valine crystallized on chiral microspheres. DL-valine crystallizes in a triclinic space group $P\bar{1}$,[23] with lattice parameters $a = 0.5222$ nm, $b = 0.5406$ nm, $c = 0.838$ nm, $\alpha = 90.89°$, $\beta = 92.34°$, $\gamma = 110.02°$, and $Z = 2$. The XRD pattern of DL-valine crystallized from a pure solution exhibits an intense peak at 8.14°, corresponding to a (001) reflection, and a series of peaks at higher 2θ angles, as reported previously in the literature.[23] The XRD pattern of valine crystals crystallized on chiral microspheres is characterized by a set of peaks corresponding to DL-valine, as well as two diffraction peaks at $2\theta = 21.9$ and 29.57° that match the diffraction from the (003) and (11–3) crystal planes of the pure enantiomer of valine.[24] It should be mentioned that the chiral microspheres exhibit an amorphous diffraction pattern. The observation that the XRD patterns of DL-valine crystals crystallized on the chiral microspheres display diffraction peaks corresponding to the pure enantiomer of valine clearly demonstrates that enantioselective crystallization has been achieved on the chiral microspheres.

Further evidence for the generation of pure enantiomeric crystals on the chiral microspheres is obtained from DSC, which provides chiral information for solid samples.[25] In racemic compounds, the presence of a small amount of pure enantiomers lowers the melting point of the racemate. The DSC curves of pure DL-valine crystals and the chiral microspheres are shown in Figure 5. Pure racemic DL valine displays a melting peak at 305 °C, while the DSC scan of DL-valine crystals crystallized onto chiral microspheres shows a shift in the melting peak to 244 °C. This shift is indicative of the presence of pure enantiomeric valine crystals. The DSC scan of pure chiral microspheres displays an endothermic peak at approximately 350 °C due to the decomposition of the polymer (Supporting Information, Fig. S2). Additionally, thermogravimetric analysis demonstrates a weight loss of approximately 20 % between 220 and 260 °C due to the decomposition of the enantiomeric and racemate mixtures of valine crystals. Although the above characterization techniques, XRD and DSC, clearly reveal enantioselective crystallization onto the chiral microspheres, it is difficult to obtain quantitative information about chiral separation from these techniques.

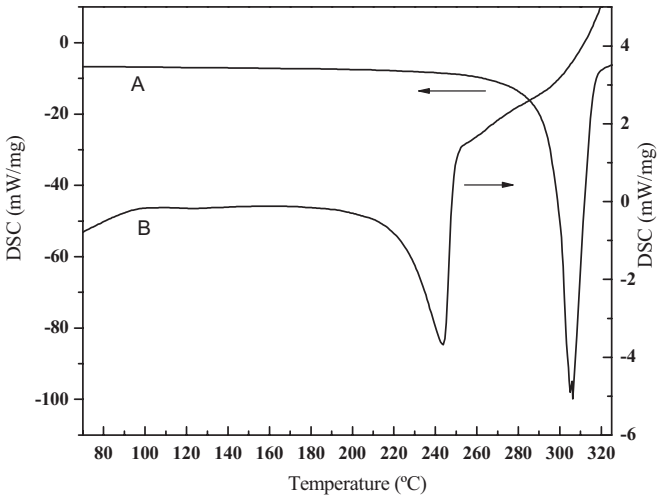

Figure 5. Differential scanning calorimetry (DSC) of A) pure crystals of DL-valine, B) valine crystals crystallized on chiral PV-L-Phe microspheres.

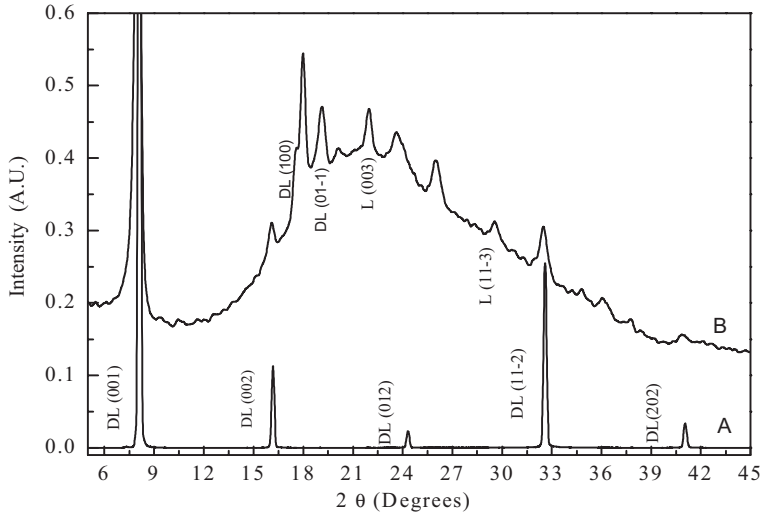

Figure 4. X-ray diffraction patterns of DL-valine. A) DL racemic phases crystallized from pure solution (0.87 g mL^{-1}, room temperature). B) Valine crystals crystallized on chiral microspheres.

In order to determine the chiral efficiency of the microspheres during crystallization, we have conducted time-dependent optical rotation experiments during the crystallization process. These have been performed as follows: a 10 mg mL^{-1} solution of the chiral microspheres is added to a supersaturated DL-valine solution at $T = 60$ °C. The optical activity of the crystallization solutions are subsequently monitored by withdrawing 1 mL aliquots from the solutions at 2 h time intervals. Subsequently, the chiral microspheres and the solution phase are rapidly separated by filtration (0.2 μm) in order to obtain the clear solution phase. After this step, the solutions are transferred to a polarimeter cell with a 5 cm path length and the optical rotation (α) is measured at $\lambda = 586$ nm in an automatic polarimeter. The optical rotation has been identified with an accuracy of $\alpha = \pm 0.05°$. Figure 6 shows data from one of these experiments. Analyses of the optical activity spectra

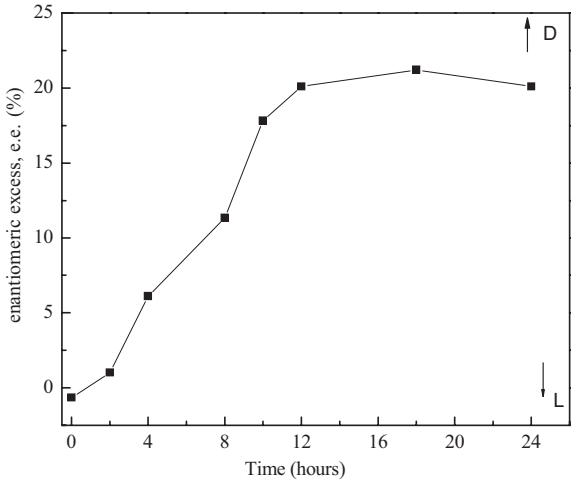

Figure 6. Time-resolved polarimetry experiment for DL-valine crystallization in the presence of 10 mg mL^{-1} of chiral PV-L-Phe microspheres.

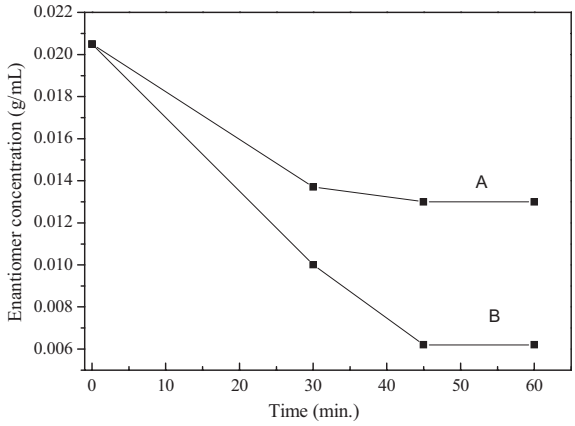

Figure 7. Chiral adsorption measurements showing the stereoselective uptake of the L and D enantiomers of caline by the chiral microspheres: A) D-valine; B) L-valine.

verify chiral discrimination on the chiral microspheres during crystallization.

An enantiomeric excess of about 5 % is observed (ca. 10 % crystal yield) during the early stages of crystallization, which increases with time to a final value of ca. 20 % at the end of the process (ca. 90 % crystal yield). By monitoring the optical activity of the crystallization solutions, we have determined a high enantiomeric excess of the D-enantiomer, indicating that the solid phase is chirally enriched with the L-valine enantiomer. In analogous experiments, the optical activity of crystals crystallized on the chiral microspheres, instead of the "mother solution", is measured; the results obtained are consistent with the above conclusion. In other words, an enantiomeric excess of ca. 20 % of the L enantiomer is observed for crystals crystallized on the chiral microspheres.

Finally, we have performed chiral adsorption measurements to demonstrate the stereoselective uptake of the L- and D-enantiomers of valine by chiral microspheres. Solutions of 20 mg mL^{-1} of the L- and D-enantiomers are prepared and their optical activity is measured. 10 mg mL^{-1} of the chiral microspheres are added to both solutions and the optical activity of the solutions is probed as a function of time. The results of these experiments are shown in Figure 7.

As can be seen from this figure, the chiral microspheres display a significant difference in the adsorption kinetics of the enantiomers. The chiral microspheres recognize L-valine better than they recognize D-valine. For example, after 30 min, approximately 68 mg of D-valine is adsorbed; in contrast, after the same period of time, 108 mg of L-valine is adsorbed. The chiral adsorption equilibrium for both enantiomers is reached after ca. 50 min. Based on equilibrium concentrations for L- and D-enantiomers, an equilibrium discrimination ratio of 2.16 has been deduced. Although this enantioselectivity value is not very high as compared to other chiral materials,[26] it is important to view this number in a broader context by recalling that in chiral chromatography a relative retention (R) factor of 1.5 is sufficient for achieving successful enantiomeric separation.

In conclusion, our experimental data clearly proves that chiral discrimination and enantioselective crystallization indeed occur on the chiral microspheres. The mechanism for enantioselective crystallization observed on the surface of the chiral microspheres can be rationalized based on principles of chiral recognition and nucleation/crystallization theory. As suggested by our data from chiral adsorption measurements, the chiral microspheres possess a high molecular recognition capability for the L-enantiomer. This selective interaction of the enantiomer with the chiral microspheres is expressed significantly during crystallization. Crystal nucleation and growth on the chiral microspheres possibly starts at the chiral polymeric surfaces. However, owing to greater molecular recognition of the L-enantiomer, the overall crystal nuclei of the L-enantiomer overwhelm the number of racemic or D-enantiomers. Therefore, during the crystal growth stage, those L-enantiomer nuclei serve as chiral seeds for enantioselective crystallization.

3. Conclusions

Enantioselective crystallization will remain an important economic process for the industrial production of enantiomers. In this paper, we describe the preparation and use of chiral microspheres with appropriate molecular functionalities to resolve optically-active isomers. Chiral microspheres of PV-L-Phe are synthesized from a uniform microsphere template by a single-step swelling process. The chiral microspheres display narrow size distributions, and their properties, especially the porosity, can be controlled by varying the synthesis conditions. The chiral discrimination ability of the chiral microspheres has been studied for DL-valine crystallization as a model system for racemic crystals. XRD and DSC provide evidence for the crystallization of enantiopure crystals on the chiral surfaces. Optical rotation measurements during crystallization show an enantiomeric excess of ca. 20–25 %. Although this value is not very high, the basic idea of chiral discrimination by enantioselective crystallization on chiral microspheres is demonstrated. Future

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studies with optimized polymer architectures and chiral functionalities will potentially result in significant improvements in enantiomeric excess. The possibility of optimized enantioselective crystallization on chiral microspheres might be achieved, for instance, by optimizing the chiral microspheres (such as the chiral functionality and porosity), or by varying the crystallization conditions such as the temperature and supersaturation level. The possibility of creating chiral microspheres with a variety of sizes, architectures, and chemical functionalities provides experimental opportunities for developing new chiral resolution methods. Although experiments on chirality go back to the middle of the last century, knowledge in this field is still rather limited. We believe that the proposed approach of using chiral microspheres as chiral resolving auxiliaries will provide further insight into chiral discrimination during the crystallization process, and will help in understanding chiral recognition on polymeric surfaces.

4. Experimental

Chemicals: The following analytical-grade chemicals were purchased from Aldrich and used without further purification: DL-valine, benzoyl peroxide (98 %), sodium dodecyl sulfate (SDS), polyvinylpyrrolidone (PVP) with a molecular weight of 360 000, phenyl alanine, 2-methoxy ethanol (HPLC), ethanol (HPLC), bromochloromethane, and chlorobenzene. Styrene (Aldrich, 99 %) was passed through activated alumina (ICN) to remove the inhibitor before use. Deionized water was purified by passing it through an Elgastat spectrum reverse osmosis system (Elga, High Wycombe, UK).

Synthesis of PS Template Microspheres: PS template microspheres were prepared according to a procedure described in the literature [16]. Briefly, PS microspheres with an average diameter of 2.7 μm ± 7 % were formed by introducing into a 1 L reaction flask, a solution containing PVP with a molecular weight of 360 000 (3.75 g, 1.5 % w/v of total solution) dissolved in a mixture of ethanol (150 mL) and 2-methoxy ethanol (62.5 mL). The temperature of the mechanically-stirred solution (200 rpm) was set at 73 °C. Nitrogen was bubbled through the solution for ca. 15 min to exclude air, and then a blanket of nitrogen was maintained over the solution during the polymerization period. A deaerated solution containing the initiator benzoyl peroxide (BP) (1.6 g, 0.6 % w/v of total solution) and styrene (38.0 mL, 17 % w/v of total solution) was then added to the reaction flask. The polymerization reaction was allowed to proceed for 24 h, and was then stopped by cooling. The formed microspheres were washed by extensive centrifugation with ethanol and water. The particles were then dried by lyophilization. PS microspheres with various diameters were prepared by changing the polymerization conditions such as the monomer concentration, stabilizer concentration, and molecular weight.

Single-Step Swelling of PS Template Microspheres: In a typical experiment, PS template microspheres that were about 2.7 μm ± 7 % in size were swollen with a swelling solvent, bromochloromethane, by adding the solvent to a 20 mL vial containing the microspheres, 10 mL of an aqueous SDS solution (1.5 % w/v), and 1.6 mL bromochlorobenzene containing 0.71 g VphA and 0.2 mL DVB. A bromochloromethane emulsion (droplet size < 0.4 μm) was formed by sonication of the former mixture for 1 min. 3.5 mL of an aqueous suspension of the PS template microspheres (7 % w/v) was then added to the stirred solvent emulsion. The diameter of the swollen microspheres was measured after the swelling was complete, when the mixture did not contain any droplets of the emulsified solvent (as verified by optical microscopy). A similar swelling process was also performed with chlorobenzene as the swelling solvent; 3.2 mL chlorobenzene was used instead of the 1.6 mL solvent volume used for bromochloromethane.

Synthesis of Uniform Micrometer-Sized PV-L-Phe–PS Composite Particles Based on a Single-Step Swelling Process: For the co-polymerization of VphA and DVB in the swollen particles, the temperature of the vials containing the swollen particles was raised to 70 °C and the mixture was shaken for 20 h. The formed composite particles were washed by extensive centrifugation with water and ethanol. The particles were then lyophilized.

Synthesis of Uniform Micrometer-Sized Crosslinked PV-L-PhA Particles: Micrometer-sized crosslinked PVPhA particles were prepared by dissolving the PS template polymer from the PV-L-PhA–PS composite particles with methylene chloride. Briefly, the PV-L-PhA–PS composite particles prepared as described in the previous paragraph were shaken at room temperature with methylene chloride for ca. 12 h. The dispersed particles were then centrifuged, and the supernatant containing the dissolved PS template polymer was discarded. This procedure was repeated four times with methylene chloride, ethanol, and water, followed by the lyophilization of the crosslinked particles.

Chiral Crystallization: All crystallization experiments were conducted in supersaturated solutions of DL-valine in deionized water at room temperature. A 0.72 mg sample of DL-valine was suspended in 10 mL of deionized water. The solution was heated to 70 °C and stirred until valine was completely dissolved. Subsequently, desired amounts of the chiral microspheres were added to the solution and stirred for ca. 5 min. The solutions were then allowed to cool spontaneously to room temperature. Specific light rotation was measured with a Jasco digital polarimeter (Model P-1010 $\lambda = 586$ nm $\alpha \pm 0.05°$ accuracy) using a cylindrical quartz cell (5 mL) at room temperature.

Experimental Techniques: A Bruker AXS D8 Advance Diffractometer (using Cu Kα $\lambda = 1.5418$ Å radiation) operating at 40 kV and 40 mA with a graphite reflected beam monochromator and variable divergence slits was used for powder XRD analysis. SEM images were acquired on a JEOL 840 instrument at an accelerating voltage of 20 kV. FTIR measurements were performed with a Varian spectrophotometer at room temperature with KBr pellets. Each pellet contained 3 mg of the polymers and 200 mg of KBr (FTIR grade).

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