Enantioselective crystallization in miniemulsions based on chiral surfactants†

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In this letter we describe a new application of a chiral micellar system for the optical resolution of racemic compounds by crystallization. Chiral surfactants based on *N*-stearoyl acid were synthesized and used in order to form a chiral micellar system of calcium tartrate tetrahydrate (CaT) crystallization. It is shown for a chiral model system of CaT that crystallization in chiral miniemulsions leads to kinetic chiral resolution. In addition, crystallization in chiral miniemulsion results in crystal morphology modification.

Crystallization within a confined size and geometry has critical implications in materials science. Several chemical systems[1] can be utilized as nanoreactors, *e.g.*, zeolites, mesoporous silica or other porous inorganic structures. However, the easiest and most widely-used approach to achieve crystallization confined in size and shape is the use of miniemulsions and micelle systems.[2] Miniemulsions have been used as nanoreactors for different applications such as the synthesis of nanosized inorganic particles[3] and polymerization.[4] In addition, miniemulsion systems have been actively studied over the last two decades, with the main challenge being the production of polymeric materials with tuneable sizes and morphology.[3,5] Crystallization in miniemulsions has also been utilized to study crystallization mechanisms, *e.g.*, the process of homogeneous nucleation, crystallization kinetics[2] and the crystallization of nano materials.[1,3,5,6] It has been shown that crystallization parameters such as induction time, polymorphic occurrence, and morphology may be strongly affected by the miniemulsion or miniemulsion droplet size. Over the last few years, new applications of chiral micellar and miniemulsion systems have been reported in both chiral synthesis and separation research. Recently, chiral surfactants have been used in the separation of enantiomers. The application of chiral micellar media for the separation of enantiomers is known as micellar electrokinetics capillary chromatography (MECC).[7–11] One example of enantioseparation using this technique is a chiral micellar system based on a chiral micelle polymer of poly sodium *N*-undecylenyl-L-valinate.[12] In recent years, chiral surfactants based on amino acids were prepared and used for a variety of chiral applications.[13–15] Another example is the synthesis and use of some chiral surfactants based on (*R*,*R*)-tartaric acid and long-chain aliphatic amines in micellar electrokinetic capillary chromatography.[16] However, there are relatively few reported applications of chiral surfactants in enantioselective synthesis.[17]

In this letter we describe the new application of a chiral micellar system for the chiral resolution of racemic compounds by crystallization. Our approach is based on the formation of chiral reverse miniemulsions (water droplets in oil W/O) and the crystallization of chiral molecules in the aqueous phase of the miniemulsions. The chirality in this reverse miniemulsion system is based on the use of a chiral amino acid surfactant. In this class of miniemulsions, the head group of the chiral surfactant will adsorb at the water/oil interface, leading to the formation of a chiral mini-reactor. In this paper, we will demonstrate that the crystallization of chiral systems within the chiral miniemulsion leads to enantioselective crystallization, namely, the resolution of enantiomers. Overall, our approach is in line with the general concept of using chiral additives as an auxiliary for the resolution of racemic solutions by crystallization, such as the resolution of enantiomers with "tailor-made additives"[18,19] and chiral polymers as additives in crystallization.[20–22]

To demonstrate chiral discrimination during crystallization in a chiral micellar system, we first need to select model systems for chiral miniemulsion systems. In this work we chose to prepare pure enantiomeric surfactants, *N*-stearoyl D- or L-serine, based on a *N*-stearoyl acid with the head group of serine. *N*-stearoyl D- or L-serine form micelles in water with a unique chiral surface,[23] which may provide an effective template for selective chiral crystallization. The chiral surfactants, D- and L-stearoyl serine, were prepared by coupling a stearic acid *N*-hydroxy succinimide ester with L- or D-serine, as described by Lapidot *et al.*[24] All miniemulsions were prepared on a (W/O) basis by mixing appropriate percentages of *n*-octanol, a chiral surfactant and water (HPLC-grade) in a 20 mL vessel. The mixtures were sonicated for 2 min by a high-intensity ultrasonic probe (Sonics VCX-750 1 cm^2 titanium horn, 20 kHz, 40 W). We examined the formation of chiral miniemulsions in a broad range of W/O surfactant ratios, and found that stable reverse miniemulsions were formed at the following weight ratio; 96.66% *n*-octanol, 3.33% water and 0.03% surfactant.

In order to study the stability of our micellar system, we performed time-dependent, light scattering measurements. The results from the DLS measurements show that the as-prepared miniemulsions have a typical size of *ca.* 0.15 μm with

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† Electronic supplementary information (ESI) available: Preparation of chiral surfactants. Fig. S1: Light microscopy image of a chiral miniemulsion. Fig. S2: Powder X-ray diffraction pattern of pure racemic CaT and of racemic CaT crystallized in the L-stearic acid miniemulsions. See DOI: 10.1039/b800211h

a standard deviation of 5%. With time, the average emulsion size grows gradually. For example, after 30 min the measured size of the miniemulsions is 2.2 μm. Within one hour, stable transparent miniemulsion solutions were obtained with a typical size of 4 μm (see light microscopy image, Fig. S1, ESI†) that were stable for several weeks. These results lead to the conclusion that our micellar systems can be defined as stable miniemulsions.[23] We should mention that we work at surfactant concentrations that are above the critical micelle concentration (CMC) value (*ca.* 2×10^{-5} M) of *N*-stearoyl serine, as reported in the literature.[23]

In the next stage we studied the crystallization of a chiral system in chiral miniemulsions. In general, when a chiral molecule crystallizes from solutions 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- or right-handed crystals of pure enantiomers, such as Pasteur's tartrate salt, or (c) a racemic solid solution in which the two enantiomers coexist in a disordered manner in the crystal lattice in any fraction, or at a specified concentration range. To demonstrate chiral discrimination, we selected the crystallization of calcium tartrate tetrahydrate (CaT) as a characteristic case for crystallization of a stable racemate crystals.

The crystallization of racemic CaT in the chiral miniemulsions was performed as follows: stock solutions of 50 mM of sodium hydrogen tartrate and 90 mM of $CaCl_2 \cdot 2H_2O$ at pH = 6.5 at room temperature were prepared. Equal quantities were taken from the stock solutions to make up a total amount of 0.1 g, which was immediately added to a vial containing 1 mg of *N*-stearoyl L- (or D-) serine with 2.9 g of *n*-octanol. The mixtures were sonicated for 2 min. Finally, the miniemulsion solutions were left to crystallize while stirring overnight at room temperature. In this procedure, CaT crystals crystallized within the miniemulsions and were separated by centrifugation. The chiral surfactant was removed by rinsing the crystals a few times with cold ethanol. Crystallization in chiral miniemulsions shows various effects on the crystal morphology and chiral discrimination, effects that will be discussed separately. In Fig. 1, typical morphologies, as seen in scanning electron microscopy (SEM), of the "default" crystals, *i.e.*, crystallization in bulk solutions and crystallization in miniemulsions, are shown. Slight modifications in crystal morphology were found for the CaT crystallized in the chiral miniemulsions. Racemic CaT crystallizes in pure water as needle-shaped crystals with a typical length of 30–40 μm (Fig. 1(A)), whereas crystals of 2–4 μm of CaT were obtained in the chiral miniemulsions (Fig. 1(B)). The degree of this shape transformation can be characterized by the crystal aspect ratio (*a*/*b*), which shifts from ~10 for the ordinary CaT to ~4 for crystals in miniemulsions. It should be noted that X-ray diffraction confirmed that the CaT crystallized in the chiral miniemulsions is identical to the CaT crystals crystallized from a pure water solution (see X-ray diffraction, Fig. S2, ESI†).

Before describing our results on chiral discrimination, it should be pointed out that CaT can crystallize only in two crystal forms, the racemic and the pure enantiomeric form. In view of this, the chiral resolution observed in our experiments can be interrelated simply by the enrichment of the pure enantiomeric crystals. The enantiopurity, namely the chiral discrimination, is reported in terms of "enantiomeric excess" (ee %). We performed a series of crystallization experiments of racemic CaT in the chiral miniemulsions. To confirm optical resolution during crystallization in chiral miniemulsions, we conducted time-dependent polarimetry experiments throughout the crystallization runs. These experiments were performed at room temperature, and the optical rotation was measured with a JASCO digital polarimeter (Model P-1010, λ = 586 nm, ± 0.05° accuracy) using a cylindrical quartz cell (6 mL). Fig. 2 shows one of these experiments, and analyses of the results verify high chiral discrimination during the crystallization stages. For example, in the crystallization of CaT in chiral *N*-stearoyl L-serine miniemulsions at the early crystallization stages, (*ca.* after 30 min) an ee of about 12% is recorded, which rises rapidly with time to a maximum value of 30% ee within 170 min of crystallization. Similar results regarding the optical resolution were observed in an identical experiment with chiral miniemulsions of the *N*-stearoyl D-serine, as shown in Fig. 2(B). In addition, based on the results of the time-dependent polarimetry, we performed an additional set of crystallization experiments in which crystals were collected at different crystallization stages where a maximum optical resolution was observed. The crystals were filtered and rinsed a few times with cold ethanol to remove

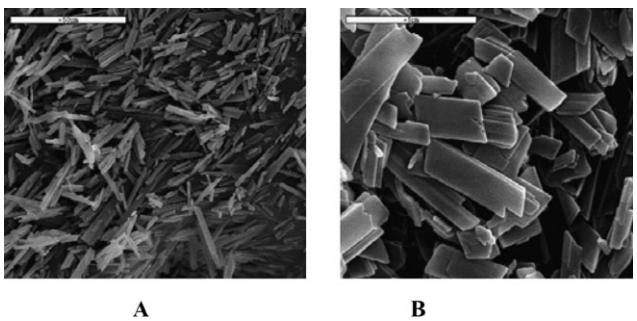

Fig. 1 Scanning electron microscopy images of: (A) racemic calcium tartrate tetrahydrate crystallized in water and (B) calcium tartrate tetrahydrate crystallized in a chiral miniemulsion (L-serine).

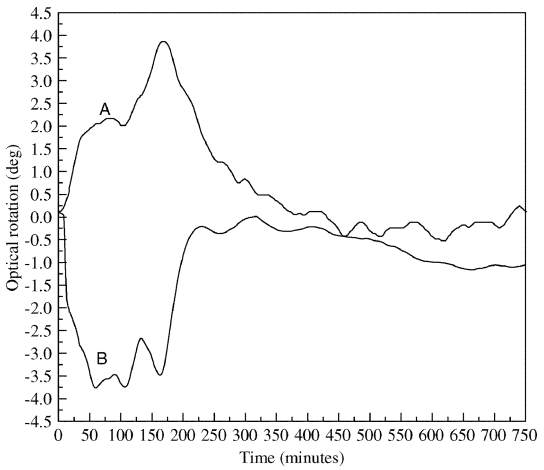

Fig. 2 Time-resolved polarimetry experiments: (A) CaT crystallization in *N*-stearoyl L-serine miniemulsions and (B) CaT crystallization in D-stearoyl L-serine miniemulsions.

the chiral surfactant. Finally, the optical rotation of the crystals was measured at room temperature by dissolving the crystals (5–10 mg) in water. The enantiomeric excess in the crystals revealed comparable results (but with a "mirror image"), as shown by time-dependent polarimetry experiments. It should be mentioned that the ee decreases at longer crystallization times as shown in Fig. 2. We have to emphasize that the "rule of reversal" concerning chiral resolution also holds in our study. The "rule of reversal"[25] refers to a crystallization process for the kinetic chiral resolution of racemic conglomerates. The general principle of the "rule of reversal" is explained in terms of the stereoselective adsorption of the resolved additive at the surface of the growing crystals of the enantiomers of the same absolute configuration, resulting in a drastic decrease in their rate of growth, thus allowing preferential crystallization of the opposite enantiomers.

Although the resulting enantiomeric excess is not very high, it can be improved by repeated crystallization. We carried out a series of repeated crystallization experiments; crystals with *ca.* a 30% enantiomeric excess from the first crystallization cycle were collected, dissolved and re-crystallized in another crystallization cycle. A maximum enantiomeric excess value of 78% (ee) is achieved after three crystallization cycles.

In order to eliminate the possibility that the enantioselective crystallization is achieved by the adsorption of chiral surfactants during the crystallization process, (*i.e.*, the chiral surfactants function as common chiral additives in resolution crystallization), we performed experiments in which the chiral surfactants were added to crystallization solutions. In a set of CaT crystallization experiments in water, chiral surfactants were added at various concentrations (0.5–2 mg ml^{-1}), and chiral discrimination during the crystallization process and at the end of the crystallization was measured. The results from these experiments showed zero chiral discrimination. These results exclude the possibility that the enantioselective crystallization seen in chiral miniemulsions is due to the simple effect of chiral additives in crystallization.

Further evidence for enantioselective crystallization in the chiral miniemulsions can be obtained from differential scanning calorimetry (DSC) of the crystals crystallized in miniemulsions. DSC is a preferred technique that provides chiral information in the solid state.[25] Racemic compounds are characterized by the fact that the presence of small amounts of pure enantiomers lower the melting point of the racemate. The DSC curves of crystals collected from crystallization in miniemulsions and those crystallized at solutions are shown in Fig. 3.

Pure racemic CaT displays two melting peaks at 149 and 189 °C, while pure enantiomeric CaT displays two melting peaks at 115 and 166 °C. In both cases, the first peak is attributed[26] to the loss of water hydration, while the second peak is due to the decomposition of the anhydrous calcium tartrate into calcium oxalate. The DSC scan of CaT crystals crystallized in miniemulsions shows two broad melting peaks at 116 and 160 °C. These peaks are due to the decomposition of hydrate calcium tartrate into anhydrous calcium tartrate, and they are indicative of the presence of pure enantiomeric CaT crystals.

In conclusion, it has been shown for chiral model systems of CaT that crystallization in chiral miniemulsions is indeed

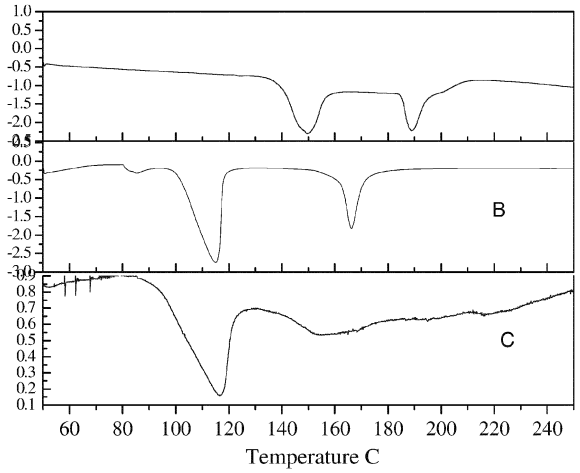

Fig. 3 Differential scanning calorimetry (DSC): (A) pure racemic CaT, (B) pure L-enantiomeric CaT crystals and (C) CaT crystals collected from crystallization in *N*-stearoyl L-serine miniemulsions.

enantioselective. We described the preparation of a reverse chiral miniemulsion system based on a chiral *n*-stearoyl serine surfactant. Measurements of the optical rotation during crystallization and DSC of the crystals crystallized in the chiral miniemulsion showed high ee during crystallization. Our results can be explained based on basic principles of kinetic chiral resolution in which the two enantiomers crystallized at different rates, depending on the chirality of the miniemulsions. Although the resulting enantiomeric excess was not yet very high, the basic principles of chiral discrimination by crystallization in chiral miniemulsions could be demonstrated, so that future studies with optimized chiral surfactants and chiral functions might result in significant improvements in optical resolution. Additional crystallization in the chiral miniemulsions resulted in modifications in crystal size and morphology. 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 present approach of using chiral miniemulsions may provide further insight into chiral discrimination processes in crystallization. The possibility of creating a variety of chiral miniemulsions with a range of size, architecture and chemical functionalities opens new experimental and application opportunities. Chiral micellar media are believed to be able to offer a viable alternative to more traditional methods of accomplishing many organic reactions. Micelles can concentrate the reactants within their small volumes; stabilize substrates, intermediates, or products; and orient substrates. Thus, they can alter the reaction rate, mechanism, and stereochemistry of a process. Furthermore, they can be prepared at low cost, and can be applied to a range of different reactions. They are also recyclable. The use of chiral surfactants can offer an economic alternative to traditional chiral solvents, while simultaneously reducing organic waste.

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