Separation of enantiomers with magnetic silica nanoparticles modified by a chiral selector: enantioselective fishing†

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Magnetic silica nanoparticles modified by a chiral selector were demonstrated to be useful in magnetic field induced separation of enantiomers.

The two enantiomers of racemic chiral compounds, including chiral drugs, often show different pharmacological, toxicological and/or metabolic activities in living systems. Consequently, individual enantiomers of chiral compounds should be examined for their own biological activities particularly during the process of developing or marketing new chiral drugs.[1] For this purpose, methods of separating enantiomers are essential. There are various methods that can be applied for the separation of enantiomers and/or for the determination of the enantiomeric composition of chiral compounds.[2] For example, various chromatographic methods including high performance liquid chromatography (HPLC),[3] gas chromatography (GC),[4] supercritical fluid chromatography (SFC),[5] simulated moving bed (SMB) chromatography,[6] various electromigration capillary methods,[7] enzymatic or non-enzymatic dynamic kinetic resolutions[8] and classical chemical resolutions[9] have been successfully utilized. As another method of separating enantiomers, in this study, we directed our attention to the magnetic field induced separation of enantiomers with the use of magnetic silica nanoparticles (MSNPs).

MSNPs, spherical silica nanoparticles containing magnetite (Fe_3O_4), have attracted much attention because of their superparamagnetic property, low cytotoxicity, and chemically modifiable surfaces.[10] Because of the superparamagnetic property, MSNPs are attracted to a magnetic field, but retain no residual magnetism after the field is removed. Consequently, MSNPs attached to specific molecules of interest can be removed from a medium using a magnetic field. Previously, MSNPs have been shown to be useful in magnetic field induced bioseparations,[10a,b,11] and magnetite nanoparticles (MNPs) immobilized with an appropriate chiral catalyst or enzyme have also been successfully utilized for asymmetric reactions or enzymatic kinetic resolutions.[12] However, MSNPs have not been utilized in the direct separation of enantiomers to the best of our knowledge. In this study, we demonstrate that MSNPs can be used in the direct separation of enantiomers.

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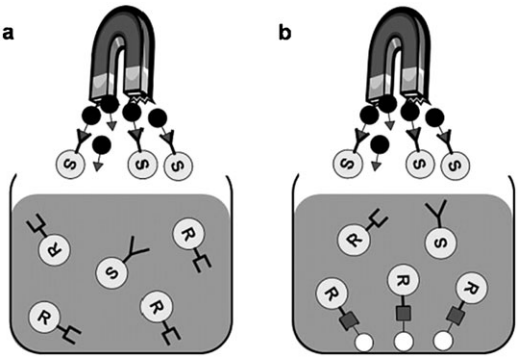

Fig. 1 Schematic representation of the separation of enantiomers using (a) surface modified MSNPs (black solid circle) only, and (b) surface modified MSNPs and surface modified NMSPs (white empty circle) at the same time.

Our new strategy for the separation of enantiomers with MSNPs is the utilization of inorganic–organic hybrid materials based on MSNPs and an appropriate chiral selector (CS). MSNPs tagged with an appropriate CS (MSNPs/CS) are expected to select a single enantiomer from a racemic mixture and the resulting MSNPs/CS–enantiomer complexes can be easily removed by using a magnet as shown in Fig. 1a. Another strategy is to use both MSNPs/CS and non-magnetic silica particles tagged to the antipode CS (NMSPs/CS) at the same time (Fig. 1b). Then the MSNPs/CS and NMSPs/CS particles are expected to enantioselectively interact with one of the two enantiomers; the resulting MSNPs/CS–enantiomer complexes are separated by using a magnet and the other NMSPs/CS–enantiomer complexes are separated by simple decantation after centrifugation. The enantiomer separation process shown in Fig. 1 looks like fishing and our new strategy for the separation of enantiomers is termed as 'enantioselective fishing'.

The MSNPs used in this study were prepared *via* a procedure which is similar to, but slightly modified from that reported previously.[10b] The average particle size of MSNPs prepared in this study was 300 nm (see Fig. S1 of the ESI†) and the measured magnetization saturation (M_S) was 15.5 emu g^{-1} (see Fig. S2 of the ESI†). NMSPs (Kromasil silica gel, 5 μm) used in this study were available from Eka chemicals (Bohus, Sweden). (*R*)- and (*S*)-*N*-(2,2-dimethyl-4-pentanoyl)-proline-3,5-dimethylanilide, which have been shown to be useful for the resolution of *N*-(3,5-dinitrobenzoyl)-α-amino acid *N*-propylamides as a HPLC chiral stationary phase (CSP) when bonded to silica gel,[13] were selected as the CS.

MSNPs/(*S*)-CS and NMSPs/(*R*)-CS shown in Fig. 2 were prepared by treating MSNPs and NMSPs with (*S*)- or

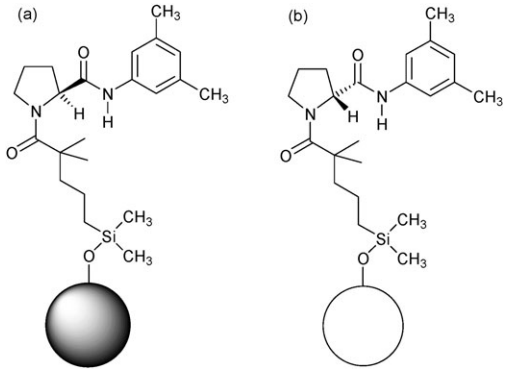

Fig. 2 Schematic presentation for the structures of (a) MSNPs/(*S*)-CS and (b) NMSPs/(*R*)-CS.

(*R*)-*N*-(2,2-dimethyl-5-ethoxydimethylsilylpentanoyl)-proline-3,5-dimethylanilide, which in turn were prepared from (*S*)- or (*R*)-proline in refluxing toluene for 72 h, *via* a previously reported procedure.[13] Elemental analysis of MSNPs/(*S*)-CS (C, 13.76%; H, 2.22%; N, 2.32%) and NMSPs/(*R*)-CS (C, 6.47%; H, 1.50%; N, 1.40%) showed a loading of 0.52 mmole of (*S*)-CS per gram of MSNPs/(*S*)-CS and a loading of 0.25 mmole of (*R*)-CS per gram of NMSPs/(*R*)-CS, respectively.

MSNPs/(*S*)-CS was utilized in the separation of the two enantiomers of *N*-(3,5-dinitrobenzoyl)-α-amino acid *N*-propylamides. As per the method shown in Fig. 1a, MSNPs/(*S*)-CS (400 mg) and a 10 mL solution of racemic *N*-(3,5-dinitrobenzoyl)-α-amino acid *N*-propylamide (0.5 mg mL^{-1} or 2.0 mg mL^{-1}) dissolved in 2-propanol–hexane (10 : 90, 30 : 70, 50 : 50 or 80 : 20, v/v) were added to a 20 mL vial. The mixed heterogeneous solution was shaken with a vortex mixer for 5 min at room temperature and then MSNPs/(*S*)-CS–enantiomer complexes were collected by using a magnet. The collected MSNPs/(*S*)-CS–enantiomer complexes were washed with ethanol in order to retrieve the separated enantiomer. The enantiomeric composition of the separated enantiomers present in the ethanol solution was analyzed by HPLC with a (*R*,*R*)-Whelk-O 1 chiral column (Regis Tech., USA).

The enantiomer separation results are summarized in Table 1. Racemic *N*-(3,5-dinitrobenzoyl)alanine, valine and leucine *N*-propylamides were subjected to separation. In each case, the (*S*)-enantiomer was selectively taken by MSNPs/(*S*)-CS even though the enantioselectivity was not so great. The selectivity for the (*S*)-enantiomers by MSNPs/(*S*)-CS might be rationalized by the enantioselective π–π and two hydrogen bonding interactions between the (*S*)-CS and two enantiomers as proposed previously for the resolution of *N*-(3,5-dinitrobenzoyl)-α-amino acid *N*-propylamides on an HPLC CSP.[13] When the percentage of 2-propanol in hexane was decreased, the enantiomer separations were improved (see entry 1, 2, 3, and 4 in Table 1), but solubility problems were encountered for valine and leucine derivatives. Under less-polar conditions, the stereoselective interaction between the (*S*)-CS and the (*S*)-enantiomers seems to be more significant. When the concentration of racemic *N*-(3,5-dinitrobenzoyl)alanine *N*-propylamide in 30% 2-propanol in hexane was increased, the enantiomer separation for alanine derivative was also improved (see entry 5 in Table 1), but solubility problems were again encountered for valine and leucine derivatives.

As an alternative method, shown in Fig. 1b, MSNPs/(*S*)-CS (300 mg), NMSPs/(*R*)-CS (300 mg) and 10 mL solution of racemic *N*-(3,5-dinitrobenzoyl)alanine *N*-propylamide (0.5 mg mL^{-1} or 2.0 mg mL^{-1}) in 2-propanol–hexane (10 : 90 or 30 : 70, v/v) were added to a 20 mL vial. The mixed heterogeneous solution was shaken with a vortex mixer for 5 min at room temperature. MSNPs/(*S*)-CS–enantiomer complexes were collected by a magnet and the NMSPs/(*R*)-CS–enantiomer

Table 1 Enantiomeric excess values of *N*-(3,5-dinitrobenzoyl)alanine (Ala), valine (Val) and leucine (Leu) *N*-propylamide enantiomers separated with MSNPs/(S)-CS in 2-propanol–hexane[a]

Entry	Percentage of 2-propanol in hexane	Ala	Val	Leu
1	10% 2-propanol (0.5 mg mL^{-1})[b]	50.1% ee in (*S*)	—	—
2	30% 2-propanol (0.5 mg mL^{-1})[b]	38.2% ee in (*S*)	29.3% ee in (*S*)	—
3	50% 2-propanol (0.5 mg mL^{-1})[b]	29.6% ee in (*S*)	28.1% ee in (*S*)	—
4	80% 2-propanol (0.5 mg mL^{-1})[b]	18.9% ee in (*S*)	16.4% ee in (*S*)	13.8% ee in (*S*)
5	30% 2-propanol (2.0 mg mL^{-1})[b]	63.8% ee in (*S*)	—	—

[a] Enantiomeric excess was checked with HPLC chiral column [(*R*,*R*)-Whelk-O 1 (250 × 4.6 mm ID]. Mobile phase: 30% 2-propanol in hexane. Flow rate: 1.0 mL min^{-1}. Temperature: 20 °C. Detector: 254 nm UV. [b] Concentration of racemic *N*-(3,5-dinitrobenzoyl)-α-amino acid *N*-propylamides in 2-propanol–hexane.

Table 2 Enantiomeric excess values of *N*-(3,5-dinitrobenzoyl)alanine *N*-propylamide enantiomers separated by using MSNPs/(S)-CS and NMSPs/(*R*)-CS at the same time in 2-propanol–hexane[a]

Entry	Percentage of 2-propanol in hexane	MSNPs/(*S*)-CS	NMSPs/(*R*)-CS
1	10% 2-propanol (0.5 mg mL^{-1})[b]	62.0% ee in (*S*)	77.2% ee in (*R*)
2	30% 2-propanol (0.5 mg mL^{-1})[b]	51.1% ee in (*S*)	66.2% ee in (*R*)
3	30% 2-propanol (2.0 mg mL^{-1})[b]	68.1% ee in (*S*)	80.1% ee in (*R*)

[a] Enantiomeric excess was checked with HPLC chiral column [(*R*,*R*)-Whelk-O 1 (250 × 4.6 mm ID]. Mobile phase: 30% 2-propanol in hexane. Flow rate: 1.0 mL min^{-1}. Temperature: 20 °C. Detector: 254 nm UV. [b] Concentration of racemic *N*-(3,5-dinitrobenzoyl)alanine *N*-propylamide in 2-propanol–hexane.

complexes were collected by decantation after centrifugation. The MSNPs/(*S*)-CS–enantiomer complexes and NMSPs/(*R*)-CS–enantiomer complexes were washed with ethanol in order to retrieve the absorbed enantiomers. Each ethanol solution was analyzed by HPLC with an (*R*,*R*)-Whelk-O 1 chiral column and the enantiomer separation results are summarized in Table 2. The trends of the enantioselectivity with the variation of the percentage of 2-propanol in hexane and the concentration of racemic *N*-(3,5-dinitrobenzoyl)-alanine *N*-propylamide are consistent with those of the first method as shown in Table 2. The enantioselectivity by MSNPs/(*S*)-CS was improved when NMSPs/(*R*)-CS was used at the same time, but the enantioselectivity by NMSPs/(*R*)-CS is greater than that by MSNPs/(*S*)-CS. During the process of separating MSNPs/(*S*)-CS–enantiomer complexes from NMSPs/(*R*)-CS–enantiomer complexes by a magnet, a small amount of NMSPs/(*R*)-CS–enantiomer complexes seems to be embedded in MSNPs/(*S*)-CS–enantiomer complexes and consequently, the enantiomeric purity of the enantiomer separated by MSNPs/(*S*)-CS might be lower than that of the enantiomer separated by NMSPs/(*R*)-CS.

In conclusion, we have demonstrated that MSNPs tagged to an appropriate chiral selector can be utilized in magnetic field induced separation of enantiomers. The separation of enantiomers were improved when both MSNPs/(*S*)-CS and NMSPs/(*R*)-CS were used at the same time. Even though the complete separation of the two enantiomers was not achieved in this study, the method of magnetic field induced separation of enantiomers with the use of MSNPs tagged to an appropriate chiral selector is expected to be developed further and utilized as a successful enantiomer separation technique in the future.

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