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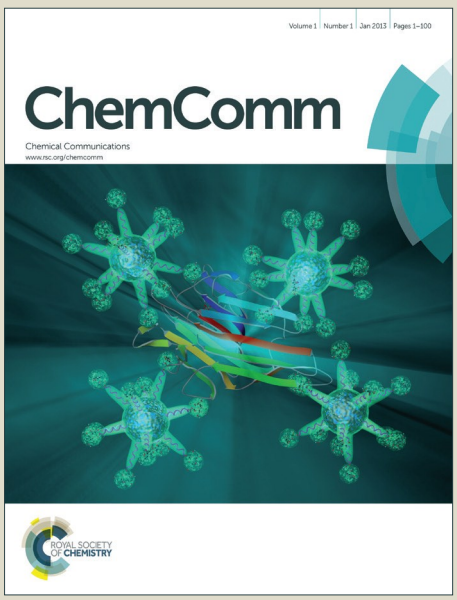

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(-)/(+)-Sparteine Induced Chirally-active Carbon Nanoparticles for Enantioselective Separation of Racemic Mixtures†

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Chiral carbon nanoparticles (CCNPs) were developed by surface passivation using the chiral ligand (-)-sparteine or (+)-sparteine (named (-)-SP/CNP and (+)-SP/CNP, respectively). The chirality of the prepared CCNPs was demonstrated by circular dichroism and polarimetry and employed as an enantioselective separation platform for representative racemic mixtures.

Chirality is an important phenomenon in materials of both synthetic and natural origin. Carbohydrates, peptides and amino acids are some of the common chiral molecules present in living systems. Chiral recognition and separation has a vital relevance in various fields like agrochemicals, consumer products and especially in pharmaceutical industry where one of the enantiomers of the same molecule may exhibit remarkable disparity in terms of pharmacological potency, pharmacokinetics, toxicity, metabolic pathways and recognition by immune system.[1] Thus it is highly desirable to separate a specific enantiomer from their racemic mixture before using for any decided application.

Considerable effort has been expended in the last few years for the development of nanoscale structures with chirality for diverse applications like asymmetric synthesis, liquid crystals and chiral recognition.[2] Chiral particles in the nanometric scale would increase the area of interaction significantly and open up various advantages associated in process of chiral separation. Metallic chiral nanoparticles and quantum dots (QDs) have been previously prepared using chiral molecules like DNA, peptides, or small organic ligands like cysteine, glutathione and penicillamine as capping agents.[3] The chirality induced in nanoparticles play a crucial role in their application as biosensors, recognition of chiral molecules and fabrication of chiroptical nanomaterials.[4] In spite of various emerging beneficial applications of the metal based nanoparticles, they are plagued with potential disadvantages related to toxicity to the environment when used in large scale and biological toxicity.[5] To avoid such disadvantages, another category of chiral nanomaterials have been reported as chiral polymeric particles that were explored for potential applications in chiral resolution by crystallization and stereo selective synthesis.[6] But restriction of using polymer based particles with many reactive racemic mixtures, directed the search for a commercially exploitable base material with better universality in regard to having lesser chances of reacting with racemic mixtures.

Carbon nanoparticles (CNPs) provide an environmentally benign alternative by virtue of their tunable biocompatibility, reactivity, ease of preparation and cost effectiveness.[7] To the best of our knowledge, an optically active, chiral carbon nanoparticles (CCNPs) for separation of enantiomer from racemic mixtures has yet to be explored. With this unmet need, our approach introduces chirality in CNPs by controlled surface passivation with chiral molecules, i.e. sparteine. In its both enantiomeric form the compound has long been used as a chiral base for various asymmetric reactions often leading to highly enantioselective transformations.[8] The choice of sparteine to induce chirality into the CNPs is due to its unique structural feature. Sparteine contains a rigid bisquinolizidine structure with the asymmetric center present adjacent to the chiral amines. Both the amines are conformationally locked and would not allow easy chiral inversion. Consequently, this property would restrict inversion of chirality of prepared CCNPs under conditions of enantiomeric separation.

Both (-)-sparteine and (+)-sparteine were used to bestow chirality on the CNPs through surface passivation and generate (-)-SP/CNP and (+)-SP/CNP, respectively. Synthesized CCNPs were demonstrated to be a novel chiral recognition platform and as an efficient adsorbent for separation of enantiomers.

Sucrose was used as the carbon source for the synthesis of CNPs and the surfaces of the CNPs were passivated by the chiral

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ligands, (-)- and (+)-sparteine by pre-passivation using a facile and economical microwave irradiation route. During the course of microwave irradiation, the colorless solution changed to dark brown solution followed by evaporation of water, finally turning into a black residue (Fig. 1).

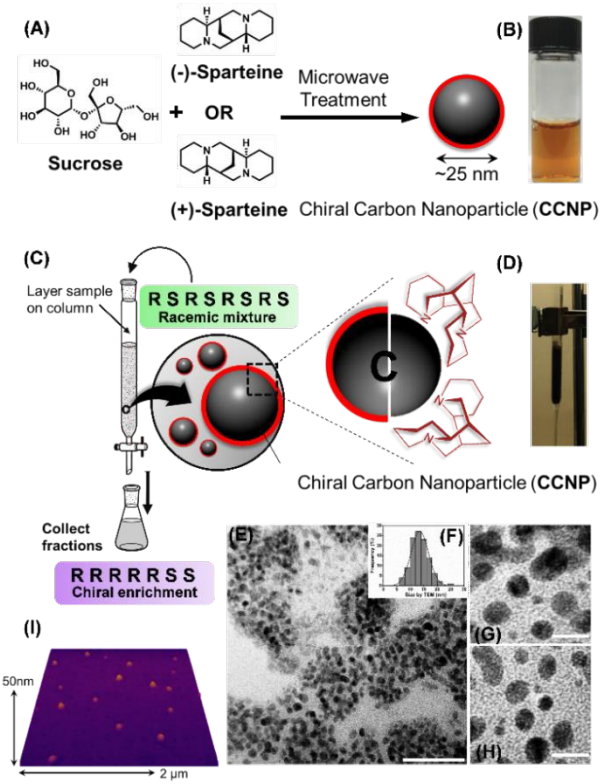

Fig. 1 Schematic representation for the (A) synthesis of (-)- and (+)-sparteine passivated CCNPs, (-)-SP/CNP and (+)-SP/CNP; (B) Optical photograph of aqueous suspension of CCNPs; (C, D) schematic representation and optical photograph of CCNPs as chiral adsorbent in a column. Representative transmission electron microscopy images of (-)-SP/CNP 1:10 (E), particle size distribution curve from TEM (F), (-)-SP/CNP 2:1 (G) and (+)-SP/CNP 2:1 (H) (scale bar 200 nm); atomic force microscopy image of (-)-SP/CNP 1:10.

The interaction between the carbon nanoparticles and (-)-/(+)-sparteine is purely physical and electrostatic in nature, presumably arising from the tertiary amines of sparteine and surface abundant carboxylic acids groups in CNPs.[7b,e,f] The (-)-SP/CNP and (+)-SP/CNP were well dispersed in aqueous medium and were stable for several months without any noticeable precipitation. The morphology and chemical integrity of the prepared (-)-/(+)-SP/CNP nanoparticles were extensively characterized by transmission electron microscopy (TEM), dynamic light scattering (DLS), atomic force microscopy (AFM), electrophoretic potential (Zeta), Raman spectroscopy, Fourier Transform infrared (FT-IR), nuclear magnetic resonance (NMR), UV-Vis, Fluorescence and circular dichroism (CD) and optical rotation measurements, which confirmed the successful preparation of CCNPs presenting chiral ligands at the nanoscale. Three different weight ratios of (-) or (+)-sparteine and sucrose (1:1, 1:2, and 1:5 respectively) were attempted for surface passivation.

DLS measurements revealed that CCNPs were monodispersed in nanopure water (0.2 µM) and there was an increment in the size of CCNPs upon increase in the concentration of sparteine on the surface (Table S1). The number-averaged hydrodynamic diameters of aqueous suspensions of (-)-SP/CNP and (+)-SP/CNP (sparteine:sugar = 1:10) were 21±5nm and 24±5 nm respectively, while the pristine CNPs without any passivation showed 50±7nm (Fig 2A). The aqueous suspensions of CCNPs prepared from higher ratios of sparteine:sugar resulted in particles in the size range of 50-200 nm. Electrophoretic mobility studies for various suspensions of CCNPs revealed that with increasing feed ratio of sparteine, the resultant CCNPs possessed more negative zeta potentials (Table S1). TEM was performed to study the morphology of the CCNPs and the anhydrous state particle diameter of (-)-SP/CNP (1:10) was measured to be 14±2nm (Fig 1E-H).

AFM images were acquired from drop casted samples on clean and polished glass slides to study the surface pattern of these CCNPs. The average height value (H_{av}) of representative samples was found to be 7±1 nm (Fig 1I). The UV-Vis absorption spectra of (-)-sparteine revealed a peak at 220 nm which is characteristic of n-σ* due to the tertiary amine. Pristine CNP displays a characteristic strong absorption peak at 282 nm related to the n-π* transition of carbonyl group. This peak is slightly red-shifted to 283 nm for (-)-SP/CNP. In the fluorescence spectra, when excited at λ_{ex} = 360 nm, (-)-SP/CNP showed a broad peak in the range of 450-600 nm.

The FT-IR spectrum for the (-)-SP/CNP exhibited stretching vibrations of C-OH at 3350 cm^{-1}, C-H at 2928 cm^{-1} and 2860 cm^{-1}, and the C-N stretch at 1280 cm^{-1}. The peak at 1064 cm^{-1} can be identified as stretching peak of the C–O–C bond. (Fig 2C) We also observed the low frequency C-H stretching bands in the region 2800-2500 cm^{-1} called "trans bands" which are typical for sparteine that depends on its conformational and configurational arrangement.[9] Raman spectra of CNP, (-)-SP/CNP and (+)-SP/CNP are shown in Figure 2D. We observed the characteristic D (~1370 cm^{-1}) and G (~1600 cm^{-1}) bands of CNPs. The G band originates due to the in-plane vibration of sp^2 carbon atoms while D (defect/disorder) band is related to a hybridized vibrational mode of graphene edges and is an indicator of some disorder in the graphene structure. We found that the intensity ratio (I_G/I_D) was slightly increased after passivation of CNPs with (-)/(+)-sparteine. The marginal increase in I_G/I_D of (-)-/(+)-SP/CNP than pristine CNP is plausibly arising from induced graphitic nature due to presence of sparteine during preparation.

To demonstrate the chiroptical properties of CCNPs, we performed circular dichroism measurements of the aqueous suspensions of (-)- and (+)-SP/CNPs. CD spectra of CCNPs suspended in water showed clear CD responses which are mirror images of each other in the region of 195-220 nm, while the aqueous CNP suspensions without passivation showed no CD signal (Figure 2E). Optical activity of the CCNPs was also probed by polarimetry. The optical rotation of (-)-SP/CNP and (+)-SP/CNP prepared using sparteine:sugar = 1:10 was found to be -0.08° and +0.108°, respectively (Table 1). With the increasing passivation by the chiral ligand, a corresponding increase in the optical rotation of the respective CCNPs has been observed. CD and polarimetry studies revealed that the achiral CNPs were transformed into CCNPs by surface passivation of chiral ligand. We also examined the effect of

microwave irradiation of (-)-sparteine by ^{1}H-NMR and found that the structural integrity was retained even after microwave treatment (Fig 2F). Figure 2G shows the ^{1}H-NMR of (+)-SP/CNP. We observed the presence of a multiplet at δ 1.1-1.2 in the

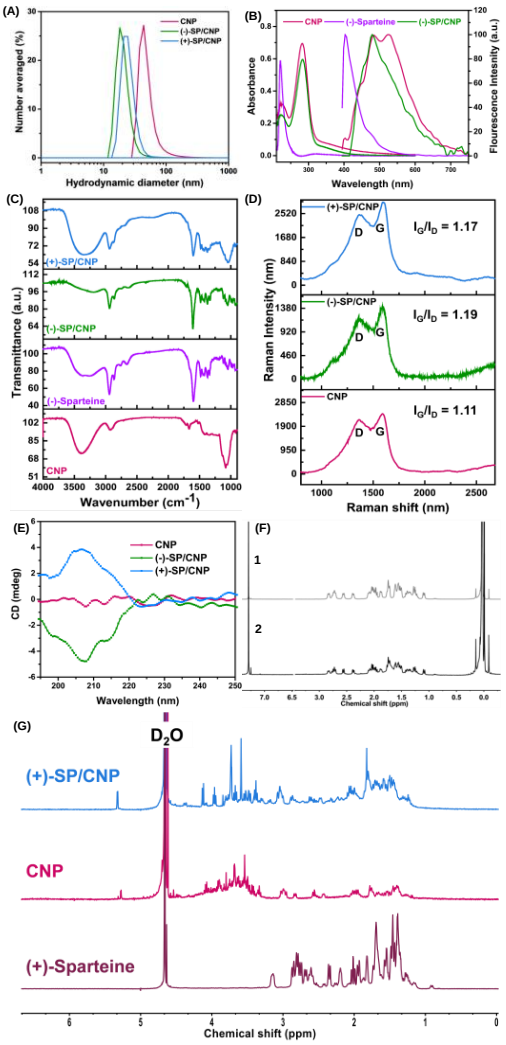

Fig. 2 Physico-chemical characterization of the chiral carbon nanoparticles (CCNPs) prepared from sparteine:sugar = 1:10. (A) Hydrodynamic diameter; (B) UV-Vis spectroscopy and Normalized fluorescence emission spectra (λex = 360 nm) (C) FT-IR; (D) Raman spectra and (E) Circular dichroism spectra (F) 1H-NMR spectra of (-)-sparteine before (1) and after (2) microwave treatment showing no significant effect and (G) 1H-NMR of different prepared formulations.

spectrum of (+)-sparteine which retained in (+)-SP/CNP, revealing the presence of (+)-sparteine in CCNP formulation. However, other important peaks of sparteine got overlapped by those of CNPs.

Then we proceeded to investigate the enantioselective interactions of chiral molecules with CCNPs. In this study, L- and D-cysteine were chosen as a representative model system to demonstrate the chiral recognition ability of the CCNPs. In order to quantify the adsorption of each enantiomer of L-/D-cysteine by CCNPs individually, chiral adsorption studies were performed. Adsorption studies were performed by preparing aqueous solutions of L- or D-cysteine (2 mM) and measured their CD responses. Then (-)-SP/CNP or (+)-SP/CNP was added such that the final concentration of CCNPs was 0.5 mg/mL and the mixture was stirred for 12 h. The CCNPs were separated from the reaction mixture by ultra-centrifugation (95,000 rpm). The CD spectrum of supernatant was measured and the amount of adsorption was calculated from the change in the peak intensity at 202 nm. Alternatively, a prototype column with the solid CCNPs was prepared through which a solution of L-/D-cysteine was passed and comparable result was obtained in both the cases.

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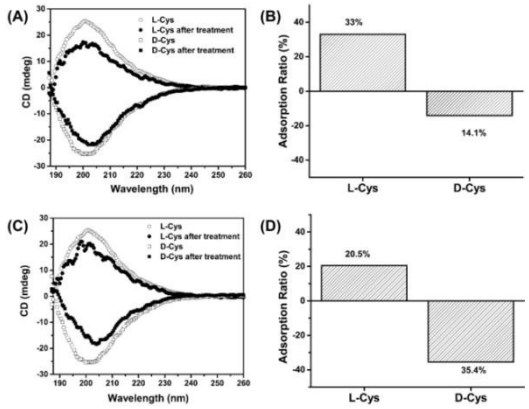

Fig. 3 Circular dichroism spectra and adsorption ratio of L-/D-cysteine by (A, B) (-)-SP/CNP and (C, D) (+)-SP/CNP. Absorbance of L- and D- cysteine solution (circles and squares, respectively) by (-)-SP/CNP (A) and (+)-SP/CNP (C). Blank symbols correspond to signals from the solution of 2 mM concentration before treatment while filled symbols correspond to signals of the spectra after treatment with (-)-SP/CNP or (+)-SP/CNP. Chiral CNPs were prepared from (-)- or (+)-sparteine: sugar = 2:1. Bar chart of the adsorption ratio for both the chiral CNPs (B, D).

Fig. 3A shows that (-)-SP/CNP has adsorbed 33% of L-cysteine enantiomer from the solution, whereas only 14% of D-cysteine was adsorbed. This results in an enantiomeric excess of 19% ee for L-cysteine. In the case of (+)-SP/CNP, the selectivity of adsorption was found to be in opposite direction. (+)-SP/CNP showed enantiomeric excess of 15% ee for D-cysteine enantiomer (Fig 3B). As a control, we also checked if the unmodified CNPs bind to L-/D-cysteine. We found there was no binding by pristine CNPs (data not shown). We chose two other ratios of (-)- or (+)-sparteine:sugar (1:5 and 1:10) during the preparation of (-)-SP/CNP and (+)-SP/CNP to study the effect of surface passivation of CNPs on the specific binding of the enantiomers. We found that enantiospecific adsorption by the chiral carbons decreased with the decrease in the amount of the chiral ligand deposited on the CNPs (Fig. S3). As the initial ratio of (-)-sparteine:sugar varied from 1:5 to 1:10, % ee for L-cysteine reduced from 19.3% to 11%. Similarly, (+)-SP/CNP showed enantiomeric excess of 9% and 4.7% towards D-cysteine for the ratios of (+)-sparteine:sugar = 1:5 and 1:10 respectively. However, when an excess of the chiral passivating agent was used as in the case of (+)-SP/CNP prepared from an initial ratio of (+)-sparteine:sugar of 10:1, there was only a marginal improvement in the % ee for D-cysteine up to 17.4% (Fig. S4). To further verify the enantiospecific adsorption capability of CCNPs, we measured the optical rotation before and after treatment of the L-/D- cysteine with the chiral CNPs (Table 1). L-Cys (25 mM) showed an optical rotation of +0.021° which reduced to +0.015° after its treatment with (-)-SP/CNP showing the adsorption of 28.1%. However, D-Cys induced an optical rotation of -0.022° and -0.019° before and after treatment with (-)-SP/CNP, showing a lesser adsorption of 11.4%. In order to check the versatility of the enantioselective

binding of CCNPs, chiral adsorption measurements of proline enantiomers were also performed onto the (-)- and (+)-SP/CNP (Fig. 4). The adsorption pattern and ratio were similar to those of cysteine. (-)-SP/CNP resulted in 17% ee of L-proline enantiomer whereas (+)-SP/CNP resulted in 14% ee for D-proline enantiomer. These results were also supported by polarimeter studies (Table 1).

Table 1. Polarimetry study of adsorption of cysteine enantiomers into (-)-SP/CNP.[a]

	Optical Rotation	% adsorbed
L-Cys only	+0.021 ± 0.001	
L-Cys (after treatment with (-)-SP/CNP)	+0.015 ± 0.002	28.1
D-Cys only	-0.022 ± 0.001	
D-Cys (after treatment with (-)-SP/CNP)	-0.019 ± 0.001	11.4
L-Pro only	-0.126 ± 0.001	
L-Pro (after treatment with (-)-SP/CNP)	-0.094 ± 0.003	27.1
D-Pro only	+0.119 ± 0.001	
D-Pro (after treatment with (-)-SP/CNP)	+0.106 ± 0.002	11.6

[a]A solution of [L-/D-Cys] or [L-/D-Pro] = 25 mM was treated with 6 mg of (-)-SP/CNP prepared from (-)-sparteine:sugar = 2:1.

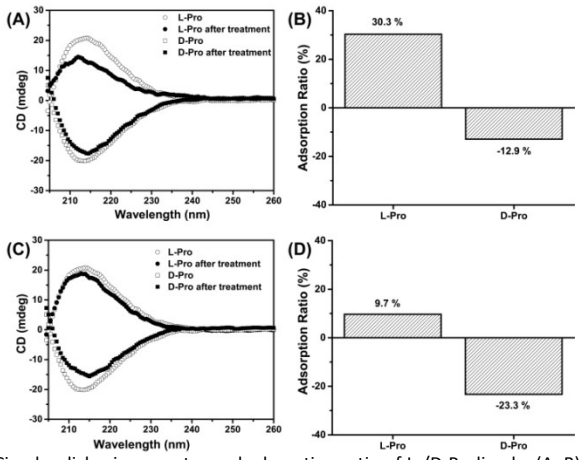

Fig. 4 Circular dichroism spectra and adsorption ratio of L-/D-Proline by (A, B) (-)-SP/CNP and (C, D) (+)-SP/CNP. Absorbance of L- and D- cysteine solution (circles and squares, respectively) by (-)-SP/CNP (A) and (+)-SP/CNP (C). Blank symbols correspond to signals from the original solution of 2mM concentration before treatment while filled symbols correspond to signals of the spectra after treatment with (-)-SP/CNP or (+)-SP/CNP. Chiral CNPs were prepared from (-)- or (+)-sparteine:sugar = 2:1. Bar chart of the adsorption ratio for both the chiral CNPs (B, D).

The observed enantioselectivity is presumably due to the preferential binding of the optically pure amino acids with the chiral surfaces of carbon nanoparticles induced by the abundance of (-) or (+)-sparteine functionalities. In order to experimentally confirm this, we studied the effect of incremental additions of L-Cysteine on (-)-sparteine by CD spectroscopy (**Fig. S5**). A shift at 215 nm for (-)-sparteine to 208-209 nm was noticed after the addition of L-Cys (Fig. S5A). A negligible shift was observed for (+)-sparteine demonstrating that L-Cys has significantly higher enantioselective interaction with (-)-sparteine than (+)-sparteine. In conclusion, we report the development of chiral CNPs by surface passivation with a chiral ligand of high asymmetric response with restricted points of chiral inversion, (-)-/(+)-sparteine. The physico-chemical integrity of the obtained CCNPs was investigated by UV-Vis, fluorescence, FT-IR, NMR, Raman, DLS, AFM, TEM and zeta potential studies. As a result of the passivation using (-)-/(+)-sparteine, the CNPs were rendered chiral. Their chiroptical response was confirmed using circular dichroism and polarimetry techniques. We demonstrated that these CCNPs could be used as a chiral adsorbent for enantiospecific binding. The % ee obtained in this work is better or comparable than that of similar adsorbants.[7g] The method excels due to its simplicity and low cost of the materials used. The general method to prepare chiral CNPs described here opens a new route to prepare a broad variety of chiral CNPs with other chiral passivating agents and we can envision enantiomeric enrichment and separation of racemic mixtures.

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