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Easy 'Filter-and-Separate' Method for Enantioselective Separation and Chiral Sensing in Wide Range Using Biomimetic Homochiral Polymer

T. Senthilkumar*[a] and Dr. S. K. Asha*[a]

We present polyfluorene appended with protected L-glutamic acid that exhibited reversible α-helix/β-sheet-like conformation and helical porous fibrous morphology mimicking the super-structure of proteins. The new
10 **homochiral polymer probe enabled efficient heterogeneous enantioselective separation and chiral sensing of a wide variety of substrates from their aqueous racemic mixture in an easy 'Filter-and-Separate' method.**

Chirality plays an indispensible role in the molecular design of
15 naturally occurring materials like proteins and DNA.[1] Imparting homochirality is absolutely essential in these biopolymers for producing precisely defined three dimensional structures having multifunctional properties.[2] Homochiral materials with three dimensional structures can function as biomimetic materials,[3]
20 chiral sensors,[4] chiral catalysts,[5] and bioactive chiral drugs.[6] Among the various applications of homochiral materials, chirality sensing and enantioselective separation remains a significant challenge in multidisciplinary scientific research since the biological and chemical activities are highly specific for
25 particular chiral form.[7] Although there are several reports on chiral sensing probes, most of them demand high levels of enantiopurity of the chiral enantiomer to realize sensing activity.[8] Enantiomeric excess determination and enantioselective separation of racemic mixtures are important aspects that still rely
30 on tedious asymmetric synthesis involving chiral catalysts and chromatographic techniques. Homochiral metal organic frameworks (MOF) have been demonstrated for enantioselective separation[9] due to their inherent molecular adsorption property. However, the low certainty in crystal growth of the homochiral
35 MOF and narrow range of analyte window are prevailing limitations. To the best of our knowledge, a single homochiral probe for combined operations of enantioselective separation and chirality sensing for wide range of analytes is unprecedented so far.

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a) Polymer Science and Engineering Division, CSIR-National Chemical Laboratory, Dr.Homi bhabha Road, Pashan, Pune-411008, India. Tel: +91 20 2590 3186; Fax: +91 20 2590 2615 E-mail: t.senthilkumar@ncl.res.in; sk.asha@ncl.res.in;

In this communication, we introduce homochiral polyfluorene (PF-GAP) with protected L-glutamic acid appendage,
50 demonstrating for the first time, heterogeneous enantioselective separation (HES) of a wide range of racemic mixtures. Conjugated polymers find an inevitable place in the field of fluorimetric sensing applications. Synthesis of homochiral conjugated polymers can be achieved by polymerizing chiral
55 monomers, doping or blending a chiral moiety to the conjugated backbone.[10] Polyfluorenes are known for their tendency to coil helically to avoid the steric strain of their rigid planar backbone. Chiral alkyl (linear as well as branched) side chain substitution has been shown to induce helical conformation with preferred
60 handedness.[11] Chiral amino acids are very interesting molecules to be incorporated either into the backbone or side chain to induce chirality in polymers. Appending chiral amino acid to polyfluorene is expected to confer homochirality to the conjugated polymer, thereby combining the photophysical
65 characteristics of the polymer with specific conformations creating an attractive route for biomimetic design. The design of the homochiral conjugated polyfluorene appended with protected L-glutamic acid (PF-GAP) and its performance in enantioselective separation and sensing is illustrated in Figure-1.
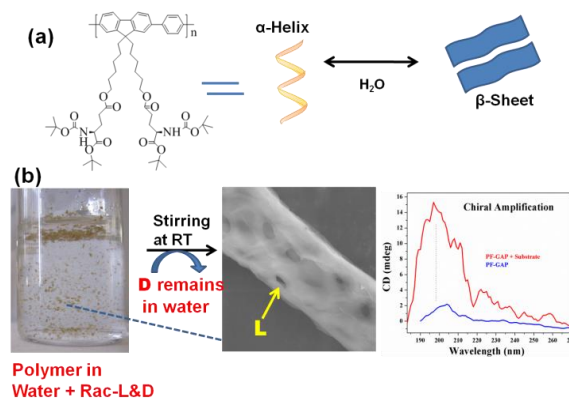
70 **Figure 1.** Schematic representation of the enantioselective separation and sensing using suspended polymer probe.

The details of the synthesis and structural characterization of the protected L–glutamic acid appended fluorene monomer and its copolymerization with 1, 4-benzene diboronic ester by Suzuki

polycondensation to afford PF-GAP polymer is given in the supporting information.

The circular dichroism (CD) spectrum of the polymer powder was characterized by an intense positive cotton effect with peak maximum around 210 nm along with negative cotton effects around 230 nm and 245 nm (figure 2). These features of the CD spectrum are characteristic of α-helix conformation.[12] However, in contrast to the CD spectrum in THF (see figure S1 and S2), in the solid state the CD signal beyond 300 nm in the range of the polymer absorption was not very significant. The dry polymer powder was suspended in water for prolonged periods of time (48 h) and subsequently dried and the CD spectrum was recorded again. Figure 2a, b compares the CD spectra of the polymer powder before and after treatment with water. It could be seen that after treatment with water the polymer conformation was altered; the typical double inflected negative band of the α-helix had disappeared. In its place a broad negative band was observed which was more characteristic of the β-sheet-like conformation. It is believed in protein unfolding studies that in presence of water, the hydrophobic moieties would collapse inside while the polar residues would remain on the surface to engage in intermolecular hydrogen bonding interaction with water molecules. In a similar way, the intermolecular hydrogen bonding interaction between the N-H groups of glutamic acid and water molecules formed the driving force for the observed change in conformation of PF-GAP upon being suspended in water.
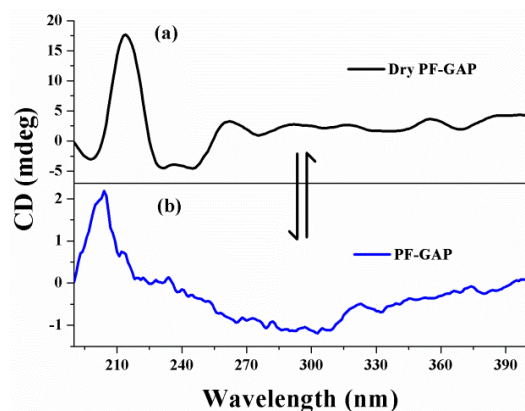
Figure 2. Solid state CD of dry (a) and 2 days water treated (b) spectra of polymer (**PF-GAP**).

FT-IR spectroscopy has been used as a reliable tool to characterize the various secondary structures of proteins and polypeptides. This is based on the fact that the secondary structure of proteins like the α-helix, β-sheet and random conformations are associated with characteristic hydrogen bonding pattern between the amide >C=O and N-H groups. Therefore, each type of secondary structure will give rise to characteristic amide I absorption in the range 1600 -1700 cm^{-1}.[13] For instance, the vibration band at 1648 cm^{-1} is characteristic for α-helix conformation, while the β-sheet exhibits vibration at lower frequencies.[14] The supporting figure S3 compares the expanded region in the FTIR spectra of PF-GAP before and after treatment with water, highlighting the characteristic vibrations. The band at 1648 cm^{-1} characteristic of α-helix conformation disappeared upon treatment with water. However, the α-helix conformation could be regained upon drying (see figure S4). The

reversible change in conformation from α-helix to β-sheet and eventually to a random one in aqueous medium is known to occur in proteins due to variation in extent of hydration.[15] Similarly, in PF-GAP polymer also the water treatment brought about a change in the hydrophilicity, which was traced by water contact angle measurements. THF solutions (10 μM) of the as-dried polymer and water-treated-and-dried polymer were drop cast on cover slip for the contact angle measurement (figure 3e and 3f). A drop in contact angle was observed from an obtuse angle of 105° for the as-dried polymer with α–helix structure to 90° for the water treated-and-dried polymer with β-sheet-like structure. The scanning electron microscopy (SEM) images of the dry PF-GAP and PF-GAP after treatment with water containing racemic mixture of various substrates are given in figure 3a-d (and figure S5) and figure S6 respectively. Dry PF-GAP revealed fibrous filaments with pores on the surface. The SEM images of PF-GAP after treatment with water containing racemic mixture (figure S6) indicated swollen fibers.
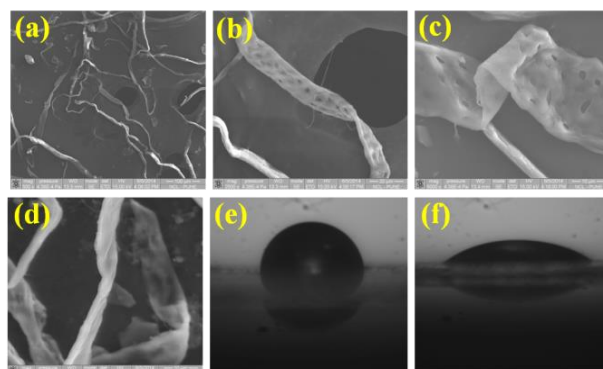

Figure 3. (a-d) Scanning electron microscopy (SEM) images of **PF-GAP** polymer particles on carbon tape. Contact angle of dry (e) and water treated (f) polymer (**PF-GAP**) drop cast from THF solutions on cover slip. [polymer]=10 μM.

The suspension of the polymer powder in water for prolonged periods with concurrent conformational change did not seem to bring about solubility in water, which was advantageous since the polymer powder could be simply filtered and removed after the enantioselective separation. A typical heterogeneous enantioselective separation experiment involved dissolving 10 mg each of the D and L enantiomer in 10 ml of distilled water, into which 5 mg of fine powdered polymer was suspended. After 48 hours of stirring at room temperature the polymer powder was filtered, dried at ambient condition and analyzed. Figure 4a-c compares the solid state CD spectra of the polymer powder collected after filtration from racemic mixtures of various substrates. The CD spectrum of the β-sheet-like structure is also included in the plots for comparison. The chemical structure of the various classes of D and L substrates screened in the present study, which included various amino acids including glutamic acid, sugars such as mannitol, amino alcohol, hydroxy acids, camptothecin and ascorbic acid is given in figure S7 and the corresponding solid state CD spectra of adsorbed polymers are given in figure S8. It was observed that in all cases the chirality of the polymer PF-GAP was significantly amplified. The amplification of the CD signal was obtained from the area under the corresponding CD peak maxima (spectra in figure 4 and S8).

The enhancement was highest for glutamic acid which exhibited 11 fold increase in the intensity of the CD signal. Table-1 summarizes the percentage enhancement in the CD intensity of the polymer upon interaction with various racemic mixtures. The filtered solution remaining after removal of the polymer powder was also analyzed for the chiral signature. Figure S9 compares the solution state CD signal of the filtered solution along with the corresponding reference D- and L-enantiomers. In almost all examples, the filtered solution showed the signature of D-enantiomer confirming the fact that the polymer had selectively separated the L-enantiomer from the racemic mixture. The uptake of the L-enantiomer by the polymer was quantified based on the ratio between the area under the CD curve for the pure reference enantiomer (figure S9, 10 mg/10 ml) and filtered solution and is listed in table-1.
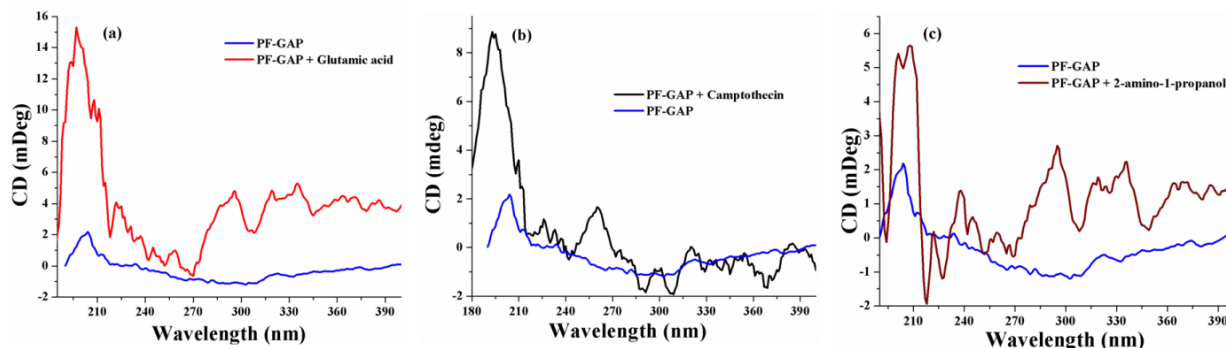
Figure-4 (a, b) Circular dichroism (CD) spectra of **PF-GAP** upon treatment with aqueous solutions of (a) Glutamic acid and (b) 2-amino-1-propanol.

Table 1. Enatioselective separation, chiral sensing and % L-Enantiomeric excess determined from CD and HPLC data.

Entry	± Substrate	Enhancement of Solid state CD of polymer (PF-GAP)	% L-Enantiomeric excess [a]	
			From CD	From HPLC
1	Glutamic acid	11.08	83.4	84.34
2	Threonine	5.3	58.12	
3	Histidine	2.37	49.55	
4	Quinnic acid	10.73	24.2	
5	Ascorbic acid	1.14	53.4	
6	2- Amino-1-propanol	3.43	94.5	
7	Phenylalanine	4.4	85.59	81.27
8	Leucine	8.34	38.2	39.16
9	Tyrosine	5.5	32.1	33.2
10	Proline	4.86	36.0	34.89
11	Mannitol	11.1	53.1	58.66
12	Tryptophan[b]	7.84	75.2	
13	Camptothecin[b]	8.45	75.62	

[a] area under the CD spectra shown in S9 and HPLC chromatogram were used to determine the ee. [b] only S or L-type of isomer was used.

It can be seen from table-1 that PF-GAP exhibited the highest % uptake of 95 % for 2-amino-1-propanol. Enantiomeric excess (ee) of more than 80% was observed for the amino acids – glutamic acid (83 %) and phenylalanine (86 %). Although amino alcohol exhibited the highest ee, other alcohol substrates like mannitol and ascorbic acid exhibited ee of around 50 % only. This value of the % enantiomeric excess obtained from the CD data was verified for a couple of samples by quantification using HPLC. In order to perform the HPLC experiment, the polymer with the adsorbed sample was filtered and removed from water, dried and then dissolved in toluene. The PF-GAP polymer remained soluble in toluene whereas the adsorbed substrates which were insoluble in toluene were precipitated out. The precipitated substrate was washed repeatedly with toluene to remove all traces of polymer, dried and used for the quantification experiments using HPLC. Prior to the quantification experiments, pure D and L enantiomers of one of the samples – phenylalanine was injected into a chiral HPLC column (CHIRALCEL OJ-H, mobile phase: isopropanol/pet ether = 10:90 with 0.1 % TFA) and analyzed for their retention times. It was observed that the pure D-enantiomer had a retention time of 4.575 minutes, while the pure L-enantiomer had a retention time of 5.033 minutes. The adsorbed phenylalanine from the polymer sample exhibited a retention time of 5.025 minutes clearly demonstrating that it was the L-enantiomer, with complete absence of any trace of the D-enantiomer (supporting figure S10). Having demonstrated the absence of the D-enantiomer in the adsorbed sample, the quantification of the L-enantiomer adsorbed on the polymer was carried out in the analytical HPLC instrument after derivatization of the amino acids following standard literature procedure.[16] The details of the derivatization as well as the various columns used for the samples glutamic acid, phenylalanine, leucine, tyrosine and mannitol are provided in the supporting information. Table-1 compares the % enantiomeric excess obtained from the HPLC data. The values were in good agreement with that calculated using CD measurements.

The enantioselective uptake by the polymer could also be confirmed by analyzing dry polymer containing adsorbed substrate that was filtered from the separation process. The absorption spectra (in DMSO) had peaks of the substrate along with the polymer (supporting figure S11) indicating the uptake of the substrates. The ^{1}H NMR spectra (in DMSO-d6) of the PFGAP+phenylalanine showed the broadening of the entire aromatic region confirming interaction between the polymer and substrate, unlike a simple physical mixture where the peak shape would not be affected. Thermogravimetric analysis (TGA) of PF-GAP+Phenylalanine showed a higher (> 5 wt %) weight loss than pristine polymer indicating loss of adsorbed material. The DSC thermogram exhibited a lowering of the glass transition temperature upon uptake of substrate (see detailed description and respective figures in supporting information S11-14). All these evidences indicated adsorption of substrate in an enatioselective manner into the polymer matrix during the stirring process in water. The amplification of the chiral signal of the polymer upon enantioselective adsorption suggested a 'Sergeants and Soldiers' principle,[17] involving organization of the adsorbed enantiomer in the homochiral confines of the porous polymer fiber. Although both enantiomers entered the porous fibrous channels of the polymer along with water, the homochiral channels retained only the L-enantiomer following a 'like-dissolves-like' rule. Unlike a membrane based separation where only selective materials are allowed to pass through, the porous fibers of the polymer resembled 'cellular' uptake. The specific folding of the homochiral polymer could be expected to stabilize only the enantiomers with similar chiral identity through non-covalent interactions, just like the polypeptide folds which are able to perform specific biological function solely due to the specific sequence of amino acids having one type of chirality. Among the various substrates, glutamic acid exhibited the highest enhancement of CD signal due to structural similarity between the substrate and the appendage unit. The degree of enhancement of the CD signals varied from substrate to substrate depending on the extent of non-covalent interactions with the homochiral polymer channels. The amplification of chirality demonstrated here proved the potential of PF-GAP polymer as an effective enantioselective separation media for racemic mixtures.

In conclusion, we have developed homochiral biomimetic helical polyfluorene by appending protected L-glutamic acid. The polymer (PF-GAP) depicted the characteristic α-helix conformation of the proteins that changed reversibly from α-helix to β-sheet-like conformation upon treatment with water. The polymer exhibited helical fibrous morphology with pores on the walls that mimicked the protein super-structure. Heterogeneous enantioselective separation of a wide range of racemic mixtures of amino acids, sugar, amino alcohol, hydroxy acid, ascorbic acid and aromatic drug in water was successfully accomplished using PF-GAP as probe. The chiral recognition ability of the polymer resulted in the enantioselective uptake of the L-form of enantiomer from their racemic mixture in water. Enantioselectively adsorbed substrates lead to an amplification of chirality with the highest value of 11-fold enhancement based on the 'Sergeant Soldier principle'. This work demonstrates a very efficient design of a biomimetic polymer probe for the combined operation of enantioselective separation and sensing with wide applicability.

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