PURIFICATION OF SEMICONDUCTING SINGLE-WALLED CARBON NANOTUBES BY SPIRAL COUNTER-CURRENT CHROMATOGRAPHY

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ABSTRACT

Over the last decade man-made carbon nanostructures have shown great promise in electronic applications, but they are produced as very heterogeneous mixtures with different properties so the achievement of a significant commercial application has been elusive. The dimensions of single-wall carbon nanotubes are generally a nanometer wide, up to hundreds of microns long and the carbon nanotubes have anisotropic structures. They are processed to have shorter lengths but they need to be sorted by diameter and chirality. Thus counter-current chromatography methods developed for large molecules are applied to separate these compounds. A modified mixer-settler spiral CCC rotor made with 3 D printed disks was used with a polyethylene glycol-dextran 2-phase solvent system and a surfactant gradient to purify the major species in a commercial preparation. We isolated the semi-conducting single walled carbon nanotube chiral species identified by UV spectral analysis. The further development of spiral counter-current chromatography instrumentation and methods will enable the scalable purification of carbon nanotubes useful for the next generation electronics.

KEYWORDS carbon nanotubes, semiconductors, chiral types, mixer-settler rotors, gradient counter-current chromatography (CCC), aqueous two-phase solvent system (ATPS)

1.0 Introduction

The long awaited commercial application of single walled carbon nanotubes [1] depends on an economically viable process to produce pure materials. Methods to seed and grow homogeneous carbon nanotubes are being developed [2-3]. Nevertheless, the capability to produce chirally pure carbon nanotube dispersions for uses such as printing on bio-sensor circuits will be greatly aided by scalable chromatographic separation. Carbon nanostructures with semiconductor properties provide very fast and sensitive electronic detection in advanced applications such as field-effect transistors, nano-scale sensors, conducting films, optimized near IR fluorophores and drug conjugates [4]. These technologies are developed for diagnostics and drug delivery. Since the processes of preparing chirally pure structures of single-walled carbon nanotubes (SWCNTS) are difficult and under intense development [5], it is worthwhile that methods proposed here can serve in separations of these macromolecules to highly homogeneous preparations. CC Biotech has been developing spiral CCC technologies specialized for large molecules. This includes designs of novel separation columns/rotors for use in a planet centrifuge with the aqueous two-phase solvent systems (ATPS) heretofore studied for protein purification [6]. An initial report of separating carbon nanotubes by high-speed CCC used an aq. organic solvent system, but no details on the types of carbon nanotubes isolated were provided [7]. More recently the utilization of ATPS in spiral CCC with the mixer-settler rotor served to achieve separation of chiral species [8], an important feat which opens up opportunities to develop more analytical and preparative methods to achieve the purification needed in the important applications.

Carbon nanotubes (CNTs) are carbon-carbon extended polymers in 2 dimensions with fused sp² orbitals having aromaticity [4-5]. The sheets form long tubes, the nanotubes; when they remain sheet-like they are the graphenes. The tubular forms are of various diameters from less than 1 nm to a few hundred nm and have lengths of 500 nm to several hundreds of μ m. Nanotubes are single-walled, double-walled or multi-walled, having anisotropic structures. They exhibit conductive or semi-conductor properties

depending on their chirality. The hexagonal array of the atoms in the polymeric tubes have a left-handed or right-handed spiral pattern. The dimensions of the patterns of the hexagonal honeycomb-like lattice are described by vectors, with certain values of m , n being semi-conducting. The pattern 'zig-zag' where $m=0$ and 'arm chair' where $n=m$ (metallic ones) are the non-chiral structures. More specifically, chirality of SWCNTs are completely described by a single vector C (chiral vector). Two atoms in a planar graphene sheet are chosen and one is used as origin. The chiral vector C is pointed from the first atom toward the second one and is defined by the relation: $C = n\alpha_1 + m\alpha_2$ where n and m are integers. The values, α_1 and α_2 , are the unit cell vectors of the two-dimensional lattice formed by the graphene sheets. The direction of the nanotube axis is perpendicular to this chiral vector as described previously [9-10]. The very long CNTs are generally metallic. CNTs are very hard, strong and chemically stable materials; very fast thermal and electrical conductors. The two types of CNTs are components of different devices which are avidly researched in industry today. Semi-conducting carbon nanotubes are applied in memory devices and sensors whereas metallic carbon nanotubes are applied in electrode materials of cells and electromagnetic shields. Thus, there is a need for separating the types and isolating those of specific chirality. There are various methods of producing the CNTs such as chemical vapor deposition and arc discharge on catalysts. Some methods do not produce a high amount of the semiconductor types and there are some methods of seeding the desired types, but the yields are limited [2-3].

The semi-conducting species of carbon nanotubes have been separated by density gradient ultracentrifugation [5,11] ion exchange, gel chromatography and electrophoresis [12-15]. Separation methods also include chiral nano-tweezers [16]. The yields are small from ion exchange and results from differential gradient ultracentrifugation yield small fractions, although chiral enantiomers are separated. The nanotubes are solubilized in water by binding with anionic surfactants such as sodium dodecyl sulfate SDS [12] and DNA [13], and processes separate on the basis of surface charge which

varies with the sizes of the particles. Since CNTs can bind with dextran [15], column and ATPE (aq. 2 phase extraction) methods using dextran [17] or related materials are used. Agarose or Sephacryl and related supports achieve separation of semiconducting CNTs by size [17-19] and other ATPE methods are described [20-22]. Novel solvent systems and new agents for selectivity can be readily used in CCC. Methods to achieve mono disperse species with increased mass recoveries are needed. CCC can be investigated to achieve this.

The spiral-design CCC rotors, the spiral tubing support and mixer-settler spiral disk rotors have proved useful for the separation of proteins, the large bio-molecules [24-26]. The primary advantage of using these rotors in HS-CCC is that the stationary phase retention of the high viscosity ATPS is high, 60-80% at moderate flow rates which is much better than the other types of CCC rotors. This has made possible the application to large molecules [27]. CCC does not use expensive solid column packing that takes up volume and adsorbs mass. The higher volume of the stationary phase simply holds more sample mass. The system is versatile in that either phase can be utilized as the mobile phase to maximize the separation.

The chromatography samples were applied to the field-effect transistor (FET) circuits according to the procedures of Fuzbien Technology Institute [28-29] to determine semiconductor activity.

2.0 Material and Methods

2.1 Materials. Dextran MW 75,000 was obtained from Fisher Scientific (Boston, MA) (Tokyo Chemical Co. manufacturer) or Spectrum Chemical (Gardena, CA), polyethylene glycol MW 8000 was from Fisher Scientific or Spectrum Chemical, sodium cholate, sodium deoxycholate and sodium dodecylsulfate were from Fisher Scientific or Spectrum Chemical, carbon nanotube dispersion of an enriched nanotube preparation (designated 6,5i by the manufacturer) in 0.2% sodium deoxycholate was obtained from

South West Nanotechnologies Inc. (now Chasms Advanced Materials, Norman, OK). A sample of 6,5i powder from the same manufacturer was also used. Since the concentrations of individual components of the (6,5i) preparation have not been quantified by the manufacturer and there are no methods to quantify distinct SWCNT chiral species, we have relied on spectral scans to identify these species. A dispersion of 3% CNTs in 3% PVP (polydivinylpyrrolidone) was from Cheap Tubes, Inc. Grafton, VT. Also from the same manufacturer a powder of short SWNTs >90wt% was used. From Nano-C, Westwood, MA, a powder of as-produced SWCNT APT25, < 1 micron in length was provided. Water was purified in a Neu-Ion system (Baltimore, MD) or HPLC water was obtained from Fisher Scientific.

2.2 Rotor and Instrument. Planetary Centrifuge (CentriChrom, Inc. Buffalo, NY) was mounted with one mixer-settler rotor (17.5 cm OD, cat. no. 205-10001, CC Biotech, Rockville MD)[30]. The early experiments were done with the previously reported rotor, made of stacked poly-carbonate disks [10]. Each disk has 4 interleaved spirals that the flow goes through serially, then to the next disk. The flow channel groove in the disk has mixing and settling sections with a glass bead in every 4th section. The total volume in the rotor with 6 layers is 84 ml. We performed experiments with the rotor made with 7 or 5 layers at times. There were difficulties with leaking and the channel underneath each disk was made too shallow such that another gasket was designed and inserted to space out the sealing gasket to allow the liquid flow. However, there were problems with high back pressure and air getting in the rotor.

To correct these problems we designed and made a new rotor as shown in Fig. 1. It has thicker top and bottom metal plates to apply stronger even pressure on the plastic disks. The mixer-settler plastic disk had the return-channels underneath deepened so an extra sheet or gasket was eliminated. The segment divisions or pins in the flow channels were straightened out as it was discovered these were not centered throughout the channel. The new disk is shown in Fig. 2. The volume of 6 layers was around 60 ml. The lower volume could be due to the 'pins' or protrusions in the channels which are

slightly larger in these printed disks. The holes in the disks and gaskets were made slightly wider, as well. As a result the performance was a lot better, with lower pack pressure and no difficulty with air bubbles forming. The rotor weighs 2.2 Kg which is 0.6 Kg more than the first rotor.

The inlet and outlet PTFE flow tubing, 1/32-in ID (Zeus Industrial Products, Orangeburg, SC) are connected to the top flange (metal plate) and bottom plate, respectively with metal nuts (compression screws, each with a stainless steel flange and plastic ring, IDEX Health and Science, Chicago, IL) and passed into the rotor shaft and out the bottom into the central axis and out the top of the chamber where they are clamped to prevent twisting. The other side of the rotor arm has counterbalancing metal rings equal in weight to the mixer-settler rotor, placed the same distance from the center axis as described previously [30].

The solvent is pumped from a gradient dual pump system (DGS-200 automated delivery system, D-Star Instruments, Manassas, VA, controlled with Clarity software). The flow passes through a manifold with a 10-ml sample loop valve and another valve with Helium for changing the flow to gas pressure to clear out the rotor contents. The solvent flow is then connected to the in-flow tubing to the CCC instrument. The out-flow from the instrument central axis goes to a fraction collector (Pharmacia or Bio-Rad).

2.3 Solvent system. The aqueous two-phase solvent system consisted of polyethylene glycol MW 8000 (PEG) and dextran MW 75,000 (DEX) containing sodium deoxycholate (SDC). The gradient of sodium dodecylsulfate (SDS) from 0 to 0.7% in the upper PEG rich phase as the mobile phase served to elute the carbon nanotubes in the chromatography. The volumes of the stock solutions listed in Table 1 were mixed in a separatory funnel and the phases separated after some time, 45 min. The resulting phases distributed to 351 ml upper phase (UP) and 129 ml lower phase (LP) for SS#1. The solvent composition, except for a numeric error in the solvent system preparation that was corrected, and the gradient method as reported previously were followed [8, 21].

2.4 Sample preparation. The best method developed for sample preparation of powdered CNTs was to sonicate (Branson 450 UltraSonic) with a probe, short pulses in 2 min segments approximately 3 times, a 1% solution of CNT powder followed by 5 min in a water bath sonicator. A suspension of 10 mg CNTs in 0.5 ml 5% sodium cholate (SC) and 0.5 ml 5% SDC is thus sonicated and 0.25 ml is removed and added to 3 ml UP SS#1 and 3 ml LP SS# 1, vortexed and allowed to settle into 2 phases, The upper phase is removed and 1 ml UP SS#1 is added and the total is loaded into the CCC. This was the procedure for the 2.5 mg sample load.

In the early experiments, dispersions provided by others were extracted according to the previously published method using the solutions described in Table 3. A 0.5 ml or 1 ml amount of the CNT dispersion (approximately 0.1% CNTs 6,5i, in 0.2% SDC) is extracted and loaded via the sample loop. Generally, the sample prep was done by combining 0.5 ml carbon nanotube dispersion (CNTs) + 0.5 ml 10% SC + 4 ml UP SS#3 + 0.5 ml LP SS#3, vortex, centrifuge 1000 rpm, 3 min. The upper phase is removed and to this is added 1 ml UP SS#3 and all is loaded into the system.

2.5 Elution mode and other operating conditions. The rotor is filled with lower phase at a rate of 0.5 ml/min. Sample is loaded in a 10-ml loop; centrifugation at 990-1000 rpm is started and then sample in the loop is injected online. Next the upper phase #1 is pumped with flow of 0.5 ml/min. The gradient is started when the dark colored sample has fully entered the rotor. A gradient of UP SS#1 A to UP SS#2 B is made over an 8-hr period for a 5 layer rotor or more for increased layers in the rotor (Table 2). Fractions of 8 min are collected. Elution mode = U o H, (CCW centrifugation) which means sample and mobile phase flow enter through the bottom of rotor in head to tail direction [30].

2.6 Analysis of Fractions. Generally a 0.5 ml aliquot is taken out of the vortexed fraction to include both phases and 0.5 ml 2% aq. SDS is added to dissolve all phases and make a clear solution. Absorbance at 2 wavelengths are read with water as the blank in a Cary 3E VC-Vis Spectrophotometer

(Varian, division of Agilent, Santa Clara, CA). The absorbance is plotted of the fractions. Fractions containing black solids (soot) or dark solutions and colors are noted. When concentrated fractions were achieved in the CCC runs, spectra of these were measured from 300 to 900 nm to analyze the peaks indicating the chiral species.

2.7 Semiconductor activity. The SWCNTs are applied to circuits printed on standard 4'' silica semiconductor wafers (92 circuit size) [28-29]. For measuring field effect transistor impedance, the CNT coated circuits are treated with 2 ul Di water for 20-30 sec to obtain baseline values for each circuit. After baseline accrual, 2 ul polybrene (50 ul/ml in water) is added and an increase in impedance is measured to saturation. The levels of saturation are compared to a standard curve for the polybrene. Normalized impedance as a function of voltage applied is determined as a measurement of semiconductor activity of the SWCNTs in the CCC fraction.

3.0 Results

3.1 Early experiments.

For a good characterization of the spiral CCC process, it is best to perform separations of the purest carbon nanotubes available, so we started on a liquid dispersion 6,5i from SouthWest Nanotechnologies that was composed of mostly 6,5 chiral species with a range of lengths of the nanotubes. The 0.1% CNTs in 0.2% SDC solution/dispersion was dark, mostly black, with some passage of light. Extracts of 2 x 0.5 or 1 ml dispersion were made and run in CCC. The pre-load extraction procedure was modified from using SS#3 to SS#4 in various experiments. The result of a sample without the extraction is shown in Fig. 3. This run had more mass of sample and included right after the solvent front, an absorbing peak that had not been extracted away.

Two ml CNT solution and 1 ml 10%SC and 2 ml LP#1 were sonicated and the total volume was loaded. The convex gradient (Table 2) was run. In Fig. 3 the dotted line indicates % B or UP of SS#2. After the

solvent front the fractions were grey and a strong UV absorbing peak was measured. Nevertheless, green fractions are noted at fraction 47 and purple starting at 50. Subsequent runs with different extractions and more volume looked like this run with green fractions followed by purple fractions. The colored fractions had CNT-FET reactivity (data not shown). After the chromatography the fractions settled into distinct phases with color in the lower phase, which contain the separated carbon nanotubes. A great deal of impurities were removed as denoted by soot and dark colors in upper phase at the solvent front and more dark particles in the stationary phase left in the rotor, eluted after the pump out. After chromatography runs it is necessary to wash the rotor with many column volumes of water to remove SDS that may remain. The SDS is harder to remove from the new rotor made with a different plastic than the original polycarbonate. The bright green fractions followed by purple fractions indicated that the 6,5 chiral species was the major type and the chiral type of green color was removed. We did not have concentrated enough solutions to perform spectral scans.

3.2 Separation of SouthWest Nanotechnologies Powders.

During the sample preparation, we observed that the spectrum of the solubilized powder corresponded with a 6,5i product spectrum (on the website of Chasm Advanced Materials) and shows the presence of chiral species of CNTs. Figure 4 shows that the spectrum has the same peaks with the exception that the peak corresponding to 6,4 is higher. The complex peak labeled as 7,5 and 7,6 can also include others such as 8,3 [8]. A 5 mg amount of powder from SouthWest Nanotechnologies, SWeNT® SG6,5i-L43 was suspended at 10mg/ml and sonicated as described above. A volume of 250 µl of the CNTs was taken and added to SS#1, 3 ml of each phase, as described. After equilibration the upper phase was removed and another amount of 1 ml upper phase was added and the total loaded. A linear gradient from 0 to 100 % B was run at 0.5 ml/min over 540 min in the new mixer-settler rotor with 6 layers. The absorbance of the fractions was read and plotted in Fig. 5. All of the tubes were photographed and are shown in the Supplementary Material. The fractions after the solvent front had gray upper phase and

black fluffy precipitate at the interface and comprised the highest UV peak in Fig. 5. This was followed by clear fractions, two pink fractions came out, 48-49, followed by clear fractions then light blue fractions, a fraction that was dark yellow or green then purple fractions. Figure 6 shows photos of the colored fractions which have settled into 2 phases with the lower dextran phase containing the colored chiral CNTs. The carbon nanotubes with surfactants bind to dextran in the stationary phase so all elute as micelles [28]. Upon standing these fractions separate into two phases with the CNTs in the lower phase. This is why the fractions containing the analytes have significant stationary phase as seen in the pictured test tubes. Usually the fractions have only the mobile phase (upper phase).

These data suggest that the separation of the CNTs into enriched chiralities was successful. To further examine the CNTs in each fraction, we performed spectral analysis. Shown in figure 7 is the overlaid spectra of those fractions. From the spectral analyses, the purple fractions are the major chiral species of 6,5 and fraction 58 could be a mixture of 7,4 metallic with the 6,5 semiconducting CNTs. The peak maxima corresponding to the 6,4 species was not present in these fractions thus, was separated away.

We assessed the impedance of the above fractions and those are shown in Table 4. The highest level of impedance is not with the mass of CNTs at the solvent front, but at the colored fractions. In making a rough specific activity determination, the highest activity corresponds to the blue fractions 54-57. The major 6,5 chiral species (570 nm) are not as strongly semiconducting as the other fractions.

Interestingly, the peak activities are in fractions 56 and 57, which are blue. Figure 7 shows these 2 fractions and there are 2 major peaks at 670 and 835 nm, respectively. The 670 nm absorbance peak could correspond to 7,5 or 7,6 or other chiral species identified to have similar maxima. Fraction 58 could be mixed with 6,5 and metallic CNTs, resulting in less activity.

3.3 Separations of CNTs provided by Cheap Tubes, Inc. and Nano-C

Separations of CNT powders provided by other suppliers were done in both rotors. Samples of dispersions and powders were analyzed. The results of using the new rotor with the same linear gradient are shown (Fig. 8). Such clear separated colors were not noted which indicates that besides the composition being different, the samples were not pre-processed to concentrate any chiral species. In the case of samples from Cheap Tubes, these are processed to provide short length of single wall-double wall CNTs 90% wgt. From Cheap Tubes we first obtained a dispersion of 3% CNTs in 3% PVP (polydivinylpyrrolidone) which was very thick. We made the sample preparation with a small sample size of 100 ul, added to SS#4. Much black color was in the upper phase which was removed but still black color was at the solvent front. An intriguing broad retained peak of fractions 46-54 with 2 maxima at fractions 48 and 50 may represent some separation. The early part of the peak had a yellow color and may be a chiral species.

In Fig. 9 is a CCC run in the previous rotor of a dispersion made of a powder (As-produced SWCNT APT25, < 1 micron in length, Nano-C). A 1% solution in 10% SDC was sonicated and combined with equal volume of 10%SC. A small volume, 300ul was added to 1.5 ml UP and 1.5 ml LP SS#1, sonicated in a bath and loaded. The major peak of CNTs with yellow/green at beginning and the rest dark green elutes earlier than the other samples. There is less absorbance in the 40-50% part of the gradient where CNTs come out in the SouthWest Nano samples.

4.0 Discussion

In summary, we are excited about these results and believe that with applying this linear gradient with an appropriate mass load there is a worthwhile comparative analysis of the preparations from the various sources. The gradient can be modified to maximize separations between the carbon nanotube chiral species. Also, with the availability of an instrument that provides low temperature which is known to optimize the partitioning of chiral species, the separations may be further optimized. The CNT

powders and dispersions supplied from various manufacturers gave different results. The SouthWest Nanotechnologies product was already pre-fractionated to an enriched content of a particular chiral species that was significantly purified here. The other samples require processing step(s) to increase the concentration of major chiral species that could be isolated in high purity in this chromatographic step. The spiral CCC instrument is a convenient method to purify the many species of carbon nanotubes for their investigation in research laboratories. The recent redox sorting extraction [32] and ionic liquids [33] can be adapted in this versatile technology. The CCC method can serve as an analytical technique as well as a preparative method.

A very interesting result is that the major species purified, the 6,5 chiral species did not have the most potent semiconducting activity in field-effect transistors. Instead a light blue chiral type seems to be more potent. Further analyses by UV near IR spectroscopy will identify which chiral type. It will be interesting to study as many chiral species as possible in the future to apply the ones most suitable for a device.

The new mixer-settler rotor made in this project is performing better than the first rotor. Thus this improved development in CCC will be beneficial to introduce in materials science research. To this end fabrication of a scaled-up instrument with cooling in the rotor chamber is underway at present.

Acknowledgement

This work was supported by National Science Foundation grant 1548957 and the authors would like to thank Dr. Ming Zheng of the Materials Science and Engineering Division of the National Institute of Standards and Technology for helpful discussions. Current affiliation of S. Stefansson is HeMemics Biotechnologies Inc, Rockville MD 20850.

The mixer-settler spiral disk rotor is manufactured by CC Biotech LLC under a license L 144-2007 from the National Institutes of Health.

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Figure Legends

Figure 1 Mixer-settler spiral disk CCC rotor, ver.2. The assembled separation rotor, left; view without the gear. Next to shaft is seen an opening for the compression screw where the liquid flow enters. Six disks are sandwiched between black Viton gaskets. Right; bottom view is seen where circumference screws are held with double nuts to provide more even pressure.

Figure 2 New mixer-settler spiral disk fabricated by high density stereo-lithography with epoxy-like resin (SOMOS NeXT) with wet-dry blast surface treatment. Left is top surface showing the 4 interweaved flow channels with the retaining 'pins' defining segments where glass beads (not shown) are placed in every 4th segment (mixing section). Right is the underside showing the channel from each spiral going to the next spiral or to the hole for flow to disk beneath. A Viton gasket (not shown) with the one hole over the opening to lower disk is placed between the disks. The photos are not the same scale.

Figure 3 Counter-current separation of a dispersion of single-walled carbon nanotubes using the polymer phase solvent system with the SDS surfactant convex gradient (upper phase of SS#2, shown as % B). Further details are in the 'early experiments' section 3.1. The previous mixer-settler CCC rotor was used [8].

Figure 4 Ultraviolet wavelength reading from 300 to 900 nm of the SWCNT solution (6,5i) prepared as described in the text, section 3.2. The peaks are labeled with the chiral species (in parenthesis) that are known to have that maxima according to the company's website (www.chasmadvancedmaterials.com).

Figure 5 Plot of the absorbance measurements of the fractions of the separation of the So West Nano CNTs prepared from a powder as described in section 3.2. The sample load of 2.5 mg was separated in the new mixer-settler rotor using a linear gradient of % B in the mobile phase. .

384

385 Figure 6 Close up photo of the colored fractions. The colors were bright and concentrated enough to
386 provide spectra that is presented in Figure 7. This was the most clear looking results that were found,
387 due to not overloading the system and showed the presence of various colored CNT species.

388

389 Figure 7 Overlay of spectra taken of the colored fractions. The major peak of purple color (570 nm)
390 corresponding to 6,5 has a smooth symmetrical peak, missing the hump in the unpurified sample (Fig.
391 4). The blue fractions, 54 through 58 have peaks corresponding to other chiral species, such as 8,4
392 (small amount around 605 nm) and 7,5 or 7,6 (670 nm) that are removed from the 6,5 chiral species.

393

394 Figure 8 Plot of the spCCC separation of the dispersion of CNTs in PVP, 100ul or approximately 0.3mg
395 in the new mixer-settler rotor using a linear gradient. Results are described in section 3.3.

396

397 Figure 9 Counter-current separation of a CNT dispersion made from a Nano-C powder in the first mixer-
398 settler rotor as described in section 3.3. The major peak emerging after fraction 26 was mostly green
399 indicating a different composition of CNTs.

400

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

SPECTRA FOR FRACTIONS 54 to 60 (spCCC 06/29/16)

Figure 7

Figure 8

Figure 9

Table 1

<u>Preparation of the solvent systems for gradient spiral countercurrent chromatography</u>		
<i>Stock solutions By weight g/g water</i>	<i>Solvent system #1 (SS#1)</i>	<i>SS#2</i>
PEG 10%	300 ml	300 ml
DEX 16%	200 ml	200 ml
SDC 10%	1 ml	1 ml
SDS		3.5 g

Table 2

<u>Gradient conditions for CNT separation in polymer phase system</u>		
Stationary phase = LP SS#1		
Mobile phase A = UP SS#1 B = UP SS#2		
Rotor	Conditions	Type of gradient
5 layer mixer-settler spiral disk	0 to 100% B	480 min linear gradient
7 layer mixer-settler spiral disk	0 to 100% B	660 min linear gradient
6 layer mixer-settler spiral disk <i>Mixer-settler rotor v.2</i>	0 to 100% B	540 min linear gradient
7 layer mixer-settler spiral disk	0 min 0% B 220 min 50% B 440 min 80% B 660 min 100% B	convex gradient

Table 3

<u>Solvents for gradient CCC and for sample prep</u>				
Stock solutions By weight g/g	Solvent system #1 (SS#1)	SS#2	Sample pre-load extraction SS#3 SS#4	
PEG 10%	300 ml	300 ml	4 ml	
DEX 16%	200 ml	200 ml	2.5 ml	2.5 ml
SDC 10%	1 ml	1 ml		
SDS		3.5 g		
PEG 14%				4 ml

Table 4

Biosensor responsiveness of CNT fractions isolated by CCC.

Starting material had the responsiveness of 1.1.

ND = no active circuits detected.

Fraction Number	Normalized Impedance	Absorbance (285 nm)	Impedance/Abs.
9	0.9	2.851	0.31
10	1.2	0.992	1.21
11	0.8	0.691	1.16
12	0.9	0.313	2.88
13	ND	0.343	
48	ND	0.194	
49	ND	0.206	
55	1.57	0.290	5.41
56	1.97	0.296	6.42
57	2.26	0.323	7.0
58	2.05	0.460	4.46
59	1.16	0.557	2.08
60	0.95	0.390	2.44
62	ND	0.335	
63	ND	0.355	
64	ND	0.296	