Single Wall Carbon Nanotube Dispersions Stabilised with *N*-Trimethyl-Chitosan

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We report the noncovalent complexation of a biocompatible low substituted *N*-trimethyl chitosan (TMC), a cationic chitosan derivative, onto the graphitic surface of single-walled carbon nanotubes (SWCNTs). TMC was synthesized and characterized by ^{1}H-NMR. A yield between the range of 34% and 56% was obtained with a degree of substitution of 19.7%. SWCNTs dispersed in TMC resulted in stable dispersions, which were further characterized by Atomic Force Microscopy (AFM) and Raman Spectroscopy and ζ-potential. AFM and Raman spectroscopy studies confirmed that smaller diameter SWCNTs are preferentially dispersed by TMC molecules resulting in stable dispersions. In addition TMC coated CNT have a high ζ-potential over a wide range of pH (2–12) exceeding values from 35 mV (pH 2) up to 10 mV (pH 12) respectively. The presence of TMC induces electrostatic repulsions which stabilize the CNTs against van der Waals attractions resulting in stable dispersions. Such complexes may have potential applications in nanomedicine.

Keywords: Single Wall Carbon Nanotubes (SWCNTs), *N*-Trimethyl Chitosan, Raman Spectroscopy, Atomic Force Microscopy (AFM), Nanotechnology.

RESEARCH ARTICLE

1. INTRODUCTION

Carbon nanotubes are of significant interest due to their unique properties and applications,[1] particularly in the field of nanomedicine.[2, 3] However, bundling and aggregation of individuals nanotubes combined with poor solubility in aqueous media are the main obstacles to overcome before they can be further used for biomedical applications.[4] Since stable colloidal dispersions are a prerequisite before they can be further considered for drug delivery purposes, much effort has been invested in developing approaches for reproducible dispersions of individual carbon nanotubes.[5]

In most cases, two general chemical approaches are widely employed for modification of the graphitic cylinders. The sidewalls or the defect sites can be covalently modified by various grafting reactions which give rise to more soluble nanotubes. Alternatively, the noncovalent adsorption or wrapping of various functional molecules results in the formation of supramolecular complexes and the fabrication of innovative systems.[6] For

non-covalent adsorption/wrapping, a variety of molecules including surfactants,[7] polymers[8] or phospholipids[9] have so far been used. Recently, the biocompatible polymer, chitosan (($1\rightarrow4$)-2-amino-2-deoxy-β-D-glucan) was used to increase the dispersability of multi-wall carbon nanotubes.[10, 11] Chitosan, a natural polysaccharide derived by *N*-deacetylation of chitin, is considered as a biocompatible, biodegradable polymer and is widely used in the food industry[12] and as a novel drug delivery platform for many routes of administration, e.g., nasal, oral, nasal, buccal and parenteral.[13]

Here, we report the stabilization of single wall carbon nanotubes (SWCNTs) with a chitosan derivative, namely, *N*-trimethyl chitosan (TMC). This material has been used successfully as a drug delivery system for oral delivery of macromolecules.[14, 15] In light of this, in the emerging field of nanotechnology and nanomedicine, dispersions of carbon nanotubes stabilised with chitosan derivatives could potentially be used as drug delivery systems in gene delivery. For instance, chitosan/DNA complexes are very popular vectors for gene[16] or vaccine delivery.[17] Recently, the interactions of plasmid DNA with functionalized carbon nanotubes have been studied for gene delivery

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purposes.[18, 19] In the current work, the non-covalent stabilization of the polymer onto the graphite walls has been studied by Atomic Force Microscopy (AFM) and Raman Spectroscopy.

2. MATERIALS AND METHODS

2.1. Materials

Low viscosity chitosan was obtained from Sigma-Aldrich (United Kingdom). *N*-methyl-2-pyrrolidinone and methyl iodide were obtained from Acros Organics (Geel, Belgium). Sodium hydroxide pellets, sodium iodide, sodium chloride, ethanol (96% w/v) and diethyl ether were obtained from Fisher Scientific (Leicester, UK). HipCO SWCNTs were obtained from Carbon Nanotechnology Inc. (Houston, USA). All solutions were prepared by Millipore water (conductivity $< 0.5 \ \mu$S cm^{-1}).

2.2. Synthesis of *N*-Trimethyl-Chitosan (TMC)

Chitosan polymer was reductively methylated to form TMC polymer with low degree of quarternisation using a reaction adapted from that described previously.[20] Chitosan (2 g) and sodium iodide (4.8 g) were dissolved in *N*-methyl-2-pyrrolidinone (80 mL) on a water bath at 60 °C with stirring. After the chitosan had dissolved, the solution was kept stirring and sodium hydroxide (11 mL, 15% w/v) and methyl iodide (11.5 mL) was added. The reaction was left stirring for 1 h at 60 °C in the presence of a Liebig condenser. The polymer product was then collected by precipitation with ethanol and ether and isolated by centrifugation using a Jouan B4i centrifuge (Thermo Electron Corporation, Waltham, USA). The product was washed twice with ethanol and diethyl ether to yield *N*-trimethyl-chitosan iodide with a low degree of quarternisation. Ion exchange was undertaken by dissolving the product in sodium chloride (40 mL, 10% w/v). The product was again precipitated with ethanol and diethyl ether and isolated by centrifugation to yield a white powder which was subsequently dissolved in deionised water (40 mL) and filtered using a dialysis membrane to remove any residual sodium chloride. The solution was then freeze dried using a Modulyo Freezedryer (Thermoelectron Corporation, Waltham, USA) to yield *N*-trimethyl chitosan chloride with a low degree of quarternisation (LDQ).

2.3. Characterization of TMC with Nuclear Magnetic Resonance (NMR)

^{1}H-NMR was used to characterise the synthesised TMC polymer. The ^{1}H-NMR spectrum was recorded in D$_2$O with a Bruker 600 MHz spectrometer (Bruker, Faellanden, Switzerland) at 80 °C. Minimum interference was observed from the water peak at this temperature and suppression of the water peak was not necessary. The degree

of quarternisation of TMC polymer from the ^{1}H-NMR spectra was calculated from Eq. (1),

$$\text{DQ (\%)} = [(\text{TM/H}) \times 1/9] \times 100 \quad (1)$$

where DQ (%) is the degree of quarternisation expressed as a percentage, TM is the integral of the trimethyl amino group peak, and H is the integral of the ^{1}H peaks.

2.4. Preparation of Stable SWCNTs-TMC Dispersions

Stable dispersions of HiPco SWCNTs (Carbon Nanotechnologies Inc., Houston, TX, USA) were obtained by mixing pristine material (0.1 mg/mL) and TMC (20 mL, 0.5%) and the mixture was ultrasonicated at 42 KHz and 100 W in an ultrasonic bath (Branson, Danbury, USA) for 2 h. As a control, SWCNTs were dispersed in distilled water (0.1 mg/mL) and subjected to sonication at identical conditions as the sample.

2.5. Atomic Force Microscopy (AFM) Studies

The sample dispersion (10 μL) was placed on freshly cleaved muscovite mica (Agar Scientific, Stansted, Essex, UK), mounted on a nickel disc (dia. 1 cm^2) with double-sided adhesive tape, and dried in a gentle stream of N$_2$. AFM studies were carried out using a Multi-Mode/NanoScope IV Scanning Probe Microscope (Digital Instruments, Santa Barbara, CA, USA) in air under ambient conditions ($T = 23$ °C, $RH = 21\%$) using the J-scanner (max. $xy = 200 \ \mu$m). Scanning was performed in Tapping Mode using Si cantilevers with integrated tips ($t = 3.5$–4.5 μm, $l = 115$–135 μm, $w = 30$–40 μm, $\nu_0 = 200$–400 kHz, $k = 20$–80 Nm^{-1}, $R < 10$ nm; Model: RTESP, Veeco Instruments, France) and an RMS amplitude of 0.8 V. Images were subsequently processed using NanoScope software (V 7.10, Digital Instruments, Santa Barbara, CA, USA).

2.6. Raman Spectroscopy

Raman spectroscopy measurements were performed using a light source of an Ar$^+$ ion laser (Spectra Physics) operating at 514.5 nm. A properly modulated metallurgical microscope (Olympus BHSM-BH2) was used for the delivery of the excitation beam as well as for the collection of the scattered light. The light was focused by a 100$\times$ objective and the backscattered light was analyzed by a Jobin Yvon T64000 Raman instrument operating at double subtractive mode and detected by a CCD cooled at 140 K. The spectral resolution was set at 2 cm^{-1}. Raman spectra measurements were carried out on the following samples: (a) raw material (SWCNTs, control), (b) non centrifuged dispersion of functionalized TMC-SWCNTs, and (c) the supernatant obtained after centrifugation of TMC-SWCNTs at 5000 rpm for 5 min. The samples were prepared by adding few drops of the samples on a glass microscope slide and drying in air at 50 °C.

2.7. Zeta Potential Measurements

The electrophoretic mobility of the SWCNTs samples was measured as a function of pH at 25 °C using a Zetasizer (Malvern Nanosizer ZS, Malvern Instruments, UK). The ζ-potential of the dispersions was calculated by the instrument according to the Helmholtz-Smoluchowski equation

$$\zeta = 4\pi\mu\eta/D$$

where μ is the electrophoretic mobility, η is the viscosity and D is the dielectric constant of the medium in the boundary layer.

The pH values of the dispersions were measured by a combination electrode and adjusted with dropwise addition of 0.1 M NaOH or HCl solutions. An aliquot of the supernatant was placed to the cuvette and measured immediately. Each measurement repeated at least three times and the mean values were calculated. Three different samples were used for these studies

3. RESULTS AND DISCUSSION

3.1. Characterisation of TMC

A yield between the range of 34% and 56% was obtained. As described previously, the polymers synthesised were characterised by ^{1}H-NMR to determine the degree of quarternisation.[16] Equation (1) was used to determine the
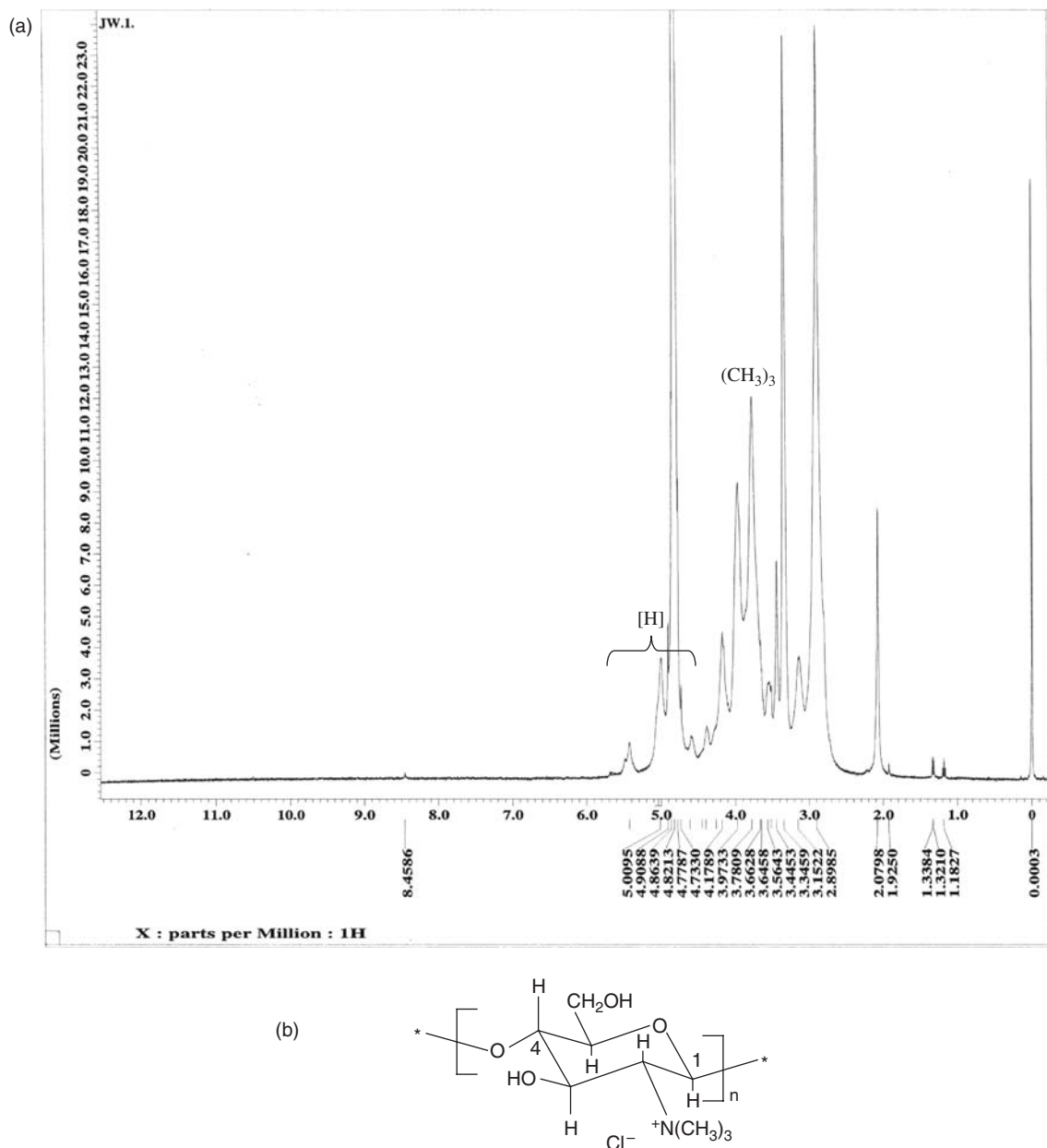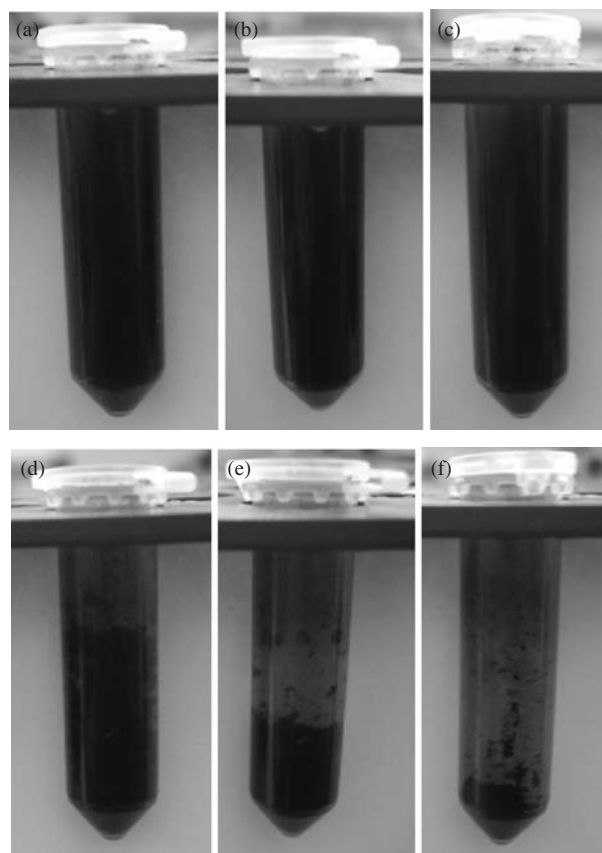

Fig. 1. (a) ^{1}H-NMR spectrum of *N*-trimethyl-chitosan (TMC), (b) the chemical structure of TMC.

degree of quarternisation by identification of the $[(CH_3)_3]$ and $[H]$ peaks respectively. The degree of quarternisation indicates the percentage of *N*-groups which are methylated to quarternary ammonium groups. The structure and the spectrum for TMC are illustrated in Figure 1. A clear relationship is demonstrated between the degree of quarternisation and the height of the peak at ca. 3.4 ppm, such that the height of the peak increases with the degree of quarternisation, particularly with respect to the $[(CH_3)_3]$ peak seen at 3.2 ppm. The integrals for the $[(CH_3)_3]$ and $[H]$ were 208.67 and 117.90, respectively. The degree of substitution was 19.7%. The values observed fall within the range typically observed for a one step synthesis reported previously.[20]

3.2. Stabilization of SWCNTs with TMC

Optical photographs of the TMC-treated and untreated carbon nanotubes samples are shown in Figure 2. It can be seen that in th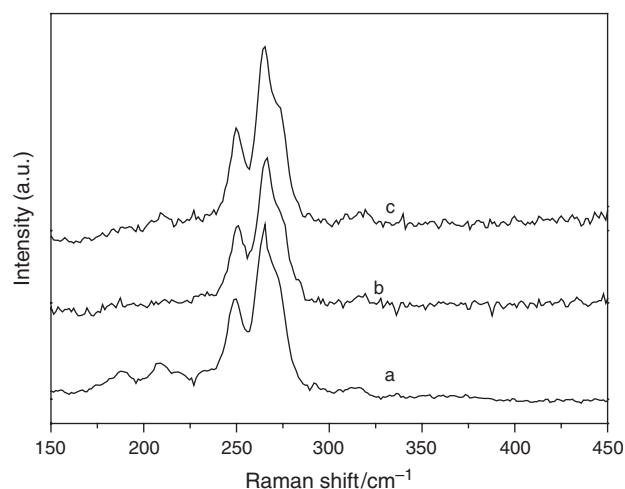e presence of the TMC, the nanotubes were homogeneously dispersed (Figs. 2(a–c)). In particular, the TMC-treated dispersion was stable after standing for 48 h (Fig. 2(b)) and showed an excellent stability even after centrifugation for 5 min to 5000 rpm (Fig. 2(c)) suggesting that the polymer acts as a surfactant inducing their solubility and functionalizing the nanotubes non-covalently. In contrast, in the absence of the polymer, SWCNTs start to precipitate immediately after the ultrasonication (Fig. 2(d)) and increases on standing for 48 h (Fig. 2(e)); further precipitation occurs after centrifugation (Fig. 2(f)).

3.3. Raman Spectroscopy Studies

Raman spectra of the different samples tested are shown in Figure 3. In the spectrum of the untreated SWCNT material (Fig. 3(a)), several distinct low frequency radial breath mode (RBM) peaks can be observed at ca. 188, 207, 249, and 265 cm^{-1}. Using Eq. (2), which correlates the diameter and the RBM frequencies,[21]

$$\omega_{\text{RBM}} \ (\text{cm}^{-1}) = (223.5/d_{\text{t}} \ (\text{nm})) + 12.5 \qquad (2)$$

the calculated tube diameters d_{t} of the unmodified sample range between 0.88 and 1.27 nm. The TMC-SWCNT non-centrifuged sample (Fig. 2(b)) shows two RBM peaks at ca. 249 and 265 cm^{-1} corresponding to 0.94 and 0.88 nm, respectively. Finally, the supernatant (Fig. 3(c)) shows three RBM peaks at 209, 249, and 265 cm^{-1}, corresponding to 1.13, 0.94, and 0.88 nm, respectively. It is clear that in the TMC samples (both centrifuged and non-centrifuged), the lower frequency RBM bands (<220 cm^{-1}) corresponding to larger diameter tubes are substantially reduced in intensity relative to the higher ones, compared with the control (raw material). The above observations suggest an enrichment of small diameter

Fig. 2. Photographs of the CNTs dispersions. Upper panel: (a) TMC modified single wall carbon nanotubes (SWCNTs) after 1 h of sonication, (b) TMC modified single wall carbon nanotubes (SWCNTs) after 48 h of sonication, and (c) TMC modified single wall carbon nanotubes (SWCNTs) after centrifugation at 5000 rpm for 5 min, respectively. Lower panel: (d) pure SWCNTs in distilled water after 1 h of sonication, (e) pure SWCNTs in distilled water after 48 h of sonication, and (f) pure SWCNTs in distilled water after centrifugation at 5000 rpm for 5 min, respectively.
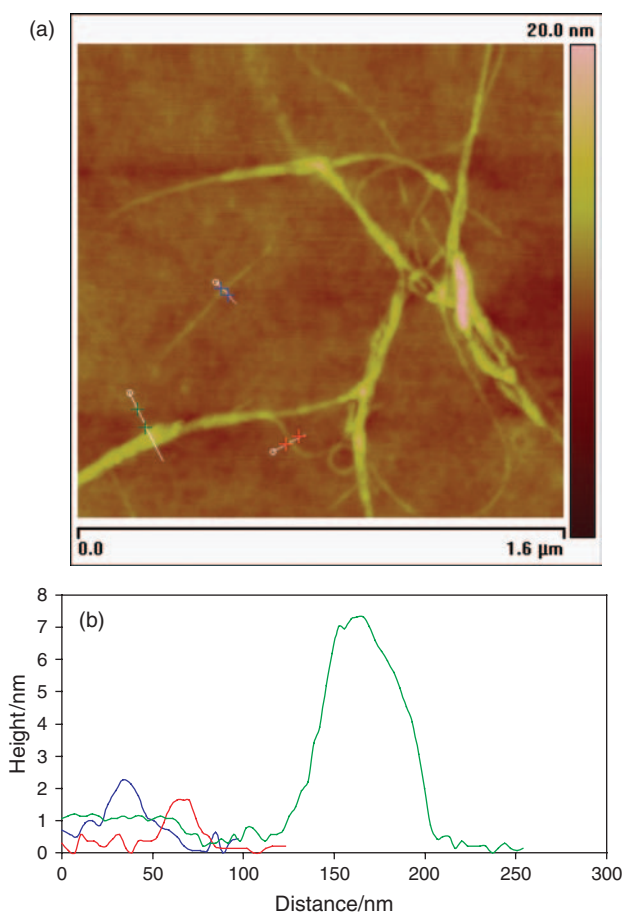

Fig. 3. Raman spectra in RBM region of: (a) raw SWCNT material, (b) non-centrifuged TMC modified SWCNTs (0.1 mg/mL) in TMC solution (0.5% w/v), and (c) TMC modified SWCNTs (0.1 mg/mL) in TMC solution (0.5% w/v) centrifuged for 5 min at 5000 rpm.

tubes in both the non-centrifuged and the TMC-SWCNT dispersion, in good agreement with a previous study.[10]

3.4. AFM Studies

AFM was used to cross-verify the previous measurements. TMC coated SWCNTs showed variable diameters exhibiting smoother areas and uneven surfaces (Fig. 4(a)). Diameter measurements were obtained in multiple points in both areas. The calculated height values (less susceptible to tip-sample artefact errors that lateral diameter values) of the tubes in smooth areas were 1.06 nm (Figs. 4(a and b), blue line) and 1.70 nm (Figs. 4(a and b), red line), respectively, and can be interpreted as individual SWCNTs. However, the diameter (height) of uneven surfaces increased to 5.44 nm (Figs. 4(a and b), green line). This shift to higher diameters could be attributed to domains of immobilized TMC creating a surface layer rather than being due to bundling. Moreover the length of the nanotubes was found 1340 ± 230 nm ($n = 10$). The AFM results are in good agreement with the Raman spectroscopy data supporting

Fig. 4. Tapping mode AFM images of SWCNTs (0.1 mg/mL) mixed with TMC (0.5% w/v) imaged in air: (a) TMC-modified SWCNTs, (b) height profiles of coated (green line) and uncoated domains (blue and red lines).
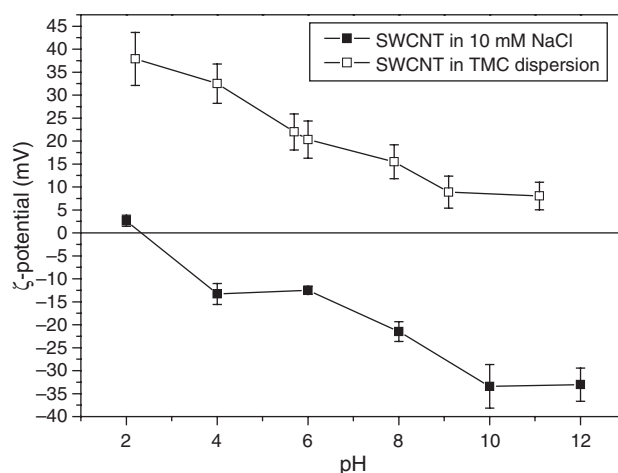
Fig. 5. ζ-potential of raw SWCNTs material and non-centrifuged modified SWCNTs (0.1 mg/mL) in TMC solution (0.5% w/v) as a function of pH, in an aqueous solution containing 10 mM NaCl.

evidence for fractionation of smaller diameter nanotubes in the presence of TMC.

The synergistic effect of ultrasonication and surfactant absorption for the nanotubes isolation from a bundle has been described recently.[22] According to that ultrasonication creates spaces or gaps at the bundle ends, followed by surfactant absorption and finally leading to individual nanotubes. On the other hand the electrostatic interactions between the polymer and the CNT surface will influence the adsorption mechanism. However the results so far on the effect of the head group charge are rather inconclusive since no clear superiority between anionic or cationic surfactants has been demonstrated.[4]

3.5. Zeta Potential Studies

Zeta potential studies of the graphitic material as a function of pH in the presence and absence of TMC are presented at Figure 5. Plain SWCNT's are negatively charged, which is consistent with the previously reported result.[23] On the other hand TMC support a positive charge due to its amino group. TMC coated CNT have a high ζ-potential over a wide range of pH (2–12) exceeding values from 35 mV (pH 2) up to 10 mV (pH 12) respectively. These high values implicate that mainly the amino groups of TMC enable the efficient dispersion of the nanotubes in the medium. In addition the presence of TMC induces electrostatic repulsions which stabilize the CNTs against van der Waals attractions.[24]

4. CONCLUSIONS

We have improved the dispersion of SWCNTs in water by using a biocompatible polymer TMC. Our results are in broad agreement with a previous study where non-covalent solubilisation of SWCNTs was achieved using non-modified chitosan.[11] Smaller diameter carbon

nanotubes dispersed and wrapped by chitosan in the aqueous supernatant in a similar manner like in the present study. Chitosan has a major drawback: its solubility above pH 6 is limited. At physiological pH, chitosan will lose its capacity to enhance drug permeability and absorption, which can only be achieved in its protonated form in acidic environments. In contrast, *N*-trimethyl chitosan chloride (TMC), a quarternized chitosan derivative, shows perfect solubility in water over a wide pH range. From this point of view the potential use of TMC, in neutral and basic environments where normal chitosan salts are ineffective certainly emphasize the superiority of this derivative since it can be manipulated much easier in aqueous solution avoiding acids. In addition, the presence of TMC improves the heterogeneity of the nanotubes leading to an enrichment of small diameter material. Carbon nanotubes can vary in length, diameter and electronic as vectors for biomedical applications.

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