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APPENDIX

A. SCHOLARSHIPS

- 2007-2009 Thailand Graduate Institute of Science and Technology (TGIST)**
(TG-55-09-50-059D)
National Science and Technology Development Agency
- 2008-2010 The 90th Anniversary of Chulalongkorn University Fund**
(Ratchadaphiseksomphot Endowment Fund)
Graduate School, Chulalongkorn University
- 2010 Conference Grant for Ph.D. Student**
Graduate School, Chulalongkorn University

B. LIST OF CONFERENCES (Oral Presentation)

- 1. 33rd Congress on Science and Technology of Thailand (STT33)**
at Walailuk University, Nakhorn Sri Thammarat, Thailand on 18-24 October 2007.
Title “Noncovalent surface modification of multiwalled carbon nanotubes with chitosan: Effect of degree of deacetylation”
- 2. The first Annual Meeting of Thailand Research Fund Senior Research**
at Faculty of Science, Mahasarakham University, Mahasarakham, Thailand on 22 October 2009.
Title “The effect of degree of deacetylation of chitosan on its dispersion of carbon nanotubes”
- 3. Pure and Applied Chemistry International Conference (PACCON2010)**
at Sunee Grand Hotel, Ubonratchathani, Thailand on 21-23 January 2010.
Title “Layer-by-layer surface modification of multiwall carbon nanotubes”
- 4. Joint Symposium on Advanced Polymers and Nanomaterials by Inha University and Chulalongkorn University**
at Pathumwan Princess Hotel, Bangkok, Thailand on 10 July 2010.
Title “Noncovalent Surface Modification of Carbon Nanotubes”
- 5. X International Conference on Nanostructured Materials - NANO 2010**
at La Sapienza University, Rome, Italy, 13 – 17 September, 2010.
Title “Loading of model drug on layer-by-layer polyelectrolyte coating of carbon nanotubes”

C. LIST OF CONFERENCES (Poster Presentation)

1. The first HOPE Meeting (Advanced courses on Nanoscience and Nanotechnology)

Tsukuba, Ibaraki, Japan organized by Japan Society for the Promotion of Science (JSPS) held on 24-29 February 2008.

Title “Noncovalent Surface Modification of Multiwall Carbon Nanotubes with Chitosan: Effect of Chitosan Concentrations and Degree of Deacetylation”

2. International conference 1st Biannual NanoThailand Symposium (NTS)

Queen Sirikit National Convention Center, Bangkok, Thailand on 6-8 December 2008.

Title “Immobilization of curcumin of modified multiwalled carbon nanotubes with chitosan as novel drug carrier”

D. LIST OF PUBLICATIONS

1. Iamsamai C., Hannongbua S., Ruktanonchai U., Soottitantawat A., Dubas S.T. The effect of the degree of deacetylation of chitosan on its dispersion of carbon nanotubes. **Carbon**. 2010; 48(1): 25-30.

2. Iamsamai C., Hannongbua S., Ruktanonchai U., Soottitantawat A., Dubas S.T. Simple method for the layer-by-layer surface modification of multiwall carbon nanotubes. **Submitted**.

Related Publication

1. Rungrotmongkol T., Arsawang U., **Iamsamai C.**, Vongachariya A., Dubas S.T. and Hannongbua S. How dispersion and separation are the noncovalently modified carbon nanotubes? A case study with a chitosan-polysaccharide biopolymer wrapping on SWCNTs. **In Preparation**.

E. OTHER EXPERIENCES

"The title "HOPE" signifies the promise held for young scientists and the optimism for a bright science and technology future in the Asia-Pacific region....."

Chularat Iamsamai was selected from Office of the National Research Council of Thailand (NCRT) to get the great opportunity from Japan Society for the Promotion of Science (JSPS) to engage in interdisciplinary discussions with five Nobel laureates and other distinguished scientists pioneering the frontiers of knowledge in **The first HOPE Meeting (Advanced courses on Nanoscience and Nanotechnology)** at Tsukuba International Congress Center, Tsukuba, Ibaraki, Japan organized by Japan Society for the Promotion of Science held on 24-29 February 2008.

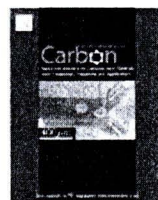
In addition, she was interviewed about her research work in Bangkokbiznews which online in column of "IT-Innovations: Innovations" on 5 December 2008. Available from: http://www.bangkokbiznews.com/2008/12/05news_317969.php.



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The effect of the degree of deacetylation of chitosan on its dispersion of carbon nanotubes

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ABSTRACT

The effect of the degree of deacetylation (DD) of chitosan biopolymer on the noncovalent surface modification of multiwall carbon nanotubes (MWCNTs) is presented. MWCNTs were modified by chitosan having different degree of deacetylation (61%, 71%, 78%, 84%, 90% and 93%) and UV-Visible spectroscopy was used to evaluate their dispersion efficiency as a function of chitosan concentration and degree of deacetylation. Results showed that the dispersion of MWCNTs could be dramatically improved when using chitosan with the lowest degree of deacetylation (61%DD) possibly due to a higher surface coverage of the MWCNTs. Zeta potential measurements were used to confirm that the chitosan surface coverage on the MWCNTs was twice as high when modifying the nanotubes surface with the 61%DD than when using the 93%DD chitosan. These results suggest that the dispersion of MWCNTs with chitosan can be improved when using chitosan having a degree of deacetylation of 61%. These results are of interest in particular for the improved dispersion of MWCNTs in aqueous solutions such as in drug delivery applications.

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1. Introduction

Among all the carbon nanostructures (fullerene, nanotubes, and nanofibers), the carbon nanotubes (CNTs) are probably being the most studied and used in applications ranging from the electronic to the biomedical [1–6]. Recently, carbon nanotubes have been proposed, as carrier for drug delivery applications [7,8]. Also, due to their high specific surface area combined with the proper surface modification, they have been used as carrier for drug delivery in cancer therapy [9–11]. Although CNTs show great potential in the biomedical

area, they can hardly be dispersed in any solvents due to nanotube–nanotube or van der Waals interactions [12] and tend to aggregate. Their poor solubility is a major problem and can lead to thrombosis of blood vessels when injected in living systems [13]. In order to achieve water dispersion, noncovalent and covalent surface modification of the CNTs surface has been developed. Covalent modification of the CNTs can, for example, be achieved with grafting of functional groups directly on the nanotube by reflux in strong acid for several hours [14]. While water dispersible nanotubes can be obtained using this method, the acidic

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treatment often leads to fragmentation of the nanotubes in smaller sections, which might impair their properties. In contrast, the noncovalent modification of CNTs surface is an attractive approach since it only involves the adsorption of a surfactant or biopolymer and preserves the CNTs integrity [15–18]. Surfactants such as sodium dodecyl sulfate and sodium dodecyl benzene sulfonate have been proposed as coating agent to promote the dispersion of the CNTs and provide good stability for several months in aqueous solution [19,20]. However, the use of such surfactants in drug delivery applications is not possible as they are thought to be toxic by inducing denaturation of proteins present in the blood [21]. This fact was demonstrated by Dong et al. who recently reported that individual single wall carbon nanotubes modified with surfactants, were toxic to 1321N1 human astrocytoma cells when compare with unmodified single wall carbon nanotubes [22]. As an alternative to potentially toxic surfactant, biopolymers have been proposed to noncovalently modify the CNTs surface [23,24]. Biopolymers such as gum arabic or gelatin have been used in the surface modification of CNTs for the preparation of conducting microelectrode used in bio-electrochemistry [25,26]. Chitosan, a polysaccharide biopolymer obtained from the deacetylation of chitin, has been widely used in medical applications because it can, not only be economically processed from chitin, but is also nontoxic, biocompatible, and biodegradable [27,28]. Chitosan biopolymer is to be treated as a random copolymer of *D*-glucosamine (deacetylated unit) and *N*-acetyl-*D*-glucosamine (acetylated unit) with a degree of deacetylation (%DD) representing the molar fraction of *D*-glucosamine along the backbone of the polymer [29]. Naturally, chitosan and its derivatives have been reported as polymer of choice for CNTs modification to improve their dispersion [30]. Chitosan structure contains both acetyl hydrophobic groups, which could bind onto the CNTs surface, and the amino groups to provide water dispersion. Furthermore, Peng et al. also demonstrated using computational simulation that chitosan could wrap along the CNTs axis [31]. This was also supported by surface decoration of carbon nanotubes with chitosan, followed by a cross-linking step [32]. In all cited work, chitosan with a high degree of deacetylation was used mainly for the modification of single wall carbon nanotubes probably because high %DD chitosan display a better solubility in aqueous media. Yet the highly hydrophilic character of the high %DD chitosan might be a disadvantage for the surface modification of carbon nanotubes and lead to poor adsorption. In the presented work, our starting hypothesis was that a lower %DD chitosan would be preferable and might allow a better dispersion of the nanotubes. Literature search confirmed that no previous report has been made on the effect of degree of deacetylation of chitosan to the dispersion and stability efficiency of CNTs.

In this article, the effect of the degree of deacetylation (%DD) of chitosan on the dispersion of MWCNTs is reported. The dispersion efficiency and stability against sedimentation of the modified MWCNTs was also investigated a function of the chitosan %DD. The degree of deacetylation of chitosan was found to play a critical role in the dispersion efficiency of MWCNTs and their stability based on noncovalent modification. Our results suggest that lower %DD chitosan is more efficient to disperse MWCNTs.

2. Experimental

2.1. Chemicals

MWCNTs with a diameter of 110–170 nm and length of 5–9 μ m were purchased from Aldrich, Thailand. These nanotubes were synthesized by chemical vapor deposition (information provided by the distributor). Chitin extracted from shrimp and used in the synthesis of chitosan was obtained from A.N. (aquatic nutrition lab) Ltd., Thailand. Concentrated sodium hydroxide 50% w/w was purchased from Vittayasom Co., Ltd., Thailand. Analytical grade glacial acetic acid was purchased from Labscan Asia Co., Ltd., Thailand. All chemicals and solvents were used as received without any further purification. Double distilled water was used in all experiments.

2.2. Experimental methods

2.2.1. Synthesis of chitosan with various %DD

Chitosan with various degree of deacetylation (%DD) was prepared by reacting 50 g of chitin in 750 ml of concentrated sodium hydroxide (50% w/w) under constant shaking. Different chitosan batches of increasing %DD were obtained by increasing the reaction time from 2 to 13 days at ambient temperature. The resulting chitosan powder was then filtered and rinsed with water until obtaining neutral pH and finally dried in air. The %DD of each chitosan batches was measured by first derivative spectroscopy using a UV-Visible spectrophotometer (SPECORD S 100, Analytikjena) [33]. In each experiment, the chitosan samples were prepared by appropriate dilution of the stock solutions in order to obtain the needed concentration of chitosan in 20 mM acetic acid.

2.2.2. Effect of chitosan concentration on the dispersion of MWCNTs

UV-Visible spectroscopy was used to determine the efficiency of the dispersion of the carbon nanotubes in solution. As MWCNTs absorb all wavelengths in the visible range, their dispersion can be evaluated by recording the changes in absorbance at fixed wavelength (550 nm) [34,35]. The wavelength of 550 nm was chosen as it represents the midway of the visible range and is often used in turbidity measurements. In each experiments, a fixed amount of MWCNTs (5 mg) was first dispersed in a 100 ml solution of a 0.01 mM chitosan (%DD = 61). The absorbance of the solution was measured after each adjunction of chitosan until the final concentration of 10 mM chitosan was reached. At each step, the mixture was stirred and sonicated for 10 min using an ultrasonic bath (CREST Model 2/5D, USA).

2.2.3. Effect of chitosan %DD on the dispersion of MWCNTs

To evaluate the effect of the %DD on the dispersion efficiency of MWCNTs by chitosan, 12.5 mg of MWCNTs were added to different solutions of chitosan having a fixed volume of 50 ml and a fixed concentration of 5 mM but having increasing %DD (61, 71, 78, 84, 90 or 93%DD). After mixing, the MWCNTs and the chitosan solutions were stirred and sonicated for 30 min. The absorbance at 550 nm of the pitch black solutions was then measured by UV-Visible spectroscopy.

2.2.4. Stability of modified MWCNTs

To assess the stability against sedimentation of the CNT modified with various types of chitosan (61, 71, 78, 84, 90 or 93%DD), each prepared solution was centrifuged 10 min at a rotation rate of 2000 rpm. The final absorbance at 550 nm of the supernatant was recorded and plotted as a function of the %DD.

2.2.5. Surface charge of the modified MWCNTs

The zetasizer (NanoZS4700 nanoseries, Malvern Instruments, UK) was used to measure the surface zeta potential of the MWCNTs modified with solutions of chitosan having 61, 71, 78, 84 and 93%DD. Each solution contained 20 mM of acetic acid, which remained from the preparation of the chitosan solutions and provided a final pH value of 3.4. The acidic medium is needed to insuring total ionization of the amino groups present in the chitosan to their NH_3^+ form. Measurements in neutral or basic conditions would lead to low zeta potential values as well as poor chitosan solubility due to the de-protonation of the NH_3^+ to NH_2 . The modified MWCNTs were then centrifuged at 4000 rpm for 15 min in order to remove the excess of chitosan. The precipitant was re-dispersed by vortex in 25 ml of 20 mM acetic acid and sonicated. The precipitation and re-dispersion steps were repeated three times prior to zeta potential measurements.

3. Results and discussion

In these experiments, chitosan was used to disperse the MWCNTs by noncovalent surface modification. If one tries to disperse carbon nanotube in aqueous solution, it is well known that prior to the adjunction of any dispersing agent, the solution will appear clear with the MWCNTs aggregated at the air/water interface. Weak van der Waals attraction and π - π stacking between the abundant double bonds found in MWCNTs are thought to be responsible for their aggregation and poor solubility in aqueous solution. Shown in Fig. 1 is a plot of the changes in absorbance of the solution as a function of the added chitosan concentration. From the initial solution of aggregated carbon nanotubes, as chitosan concentration is increased up to 1 mM, the adsorption of the polymer onto the carbon nanotubes leads to dispersion, which in turn

leads to a sharp increase the absorbance at 550 nm. This increase in absorbance quickly levels off suggesting that all the carbon nanotubes, present in the solution, have been dispersed. Further increase of the chitosan solution to 9 mM does not induce any increase in absorbance. When chitosan is added to the solution, noncovalent adsorption of chitosan on the nanotube surface is thought to take place, which initiate the dispersion by repulsion of the nanotubes. Since it has been reported that the acetyl groups represent the most hydrophobic part of the chitosan, the authors suggest that these functional groups could adsorb preferentially on the surface of the MWCNTs. In the mean time, the hydrophilic parts of chitosan (NH_3^+) induce a positive charge at the vicinity of the nanotubes surface, which allow for their stabilization in aqueous solution by electrostatic repulsion. This dispersion process can be studied using UV-Visible spectroscopy by recording the changes in absorbance of the solution at 550 nm, and is therefore equivalent to a turbidity measurement. As carbon nanotubes absorb all the wavelengths in the visible part of the electromagnetic spectrum, increases in dispersion of the carbon nanotubes render the solution more and more pitch black.

Since our hypothesis was that the more hydrophobic acetyl groups present in chitosan might allow a better interaction with the MWCNTs, our interest turned toward the preparation of chitosan having a higher molar fraction of acetyl groups thus a lower %DD. Our hypothesis was that since the hydrophobic character of chitosan is controlled by the fraction of acetylated functional groups [35], chitosan with a lower %DD should be a better molecule for the dispersion of CNTs. Chitosan with a lower %DD can simply be prepared by a shorter reaction time of the chitin biopolymer in the 50% w/w sodium hydroxide solution. In our work we chose to use 61%DD as the lowest degree of deacetylation because the resulting chitosan molecules were not sufficiently soluble and led to very scattered results. Shown in Fig. 2 (squares), is the absorbance of different solutions containing MWCNTs dispersed with chitosan having different degree of deacetylation (61, 71, 78, 84, 90 or 93%DD). Although it was shown in Fig. 1 that a 1 mM chitosan concentration is sufficient to disperse carbon nanotubes, a concentration of 5 mM chitosan was used to

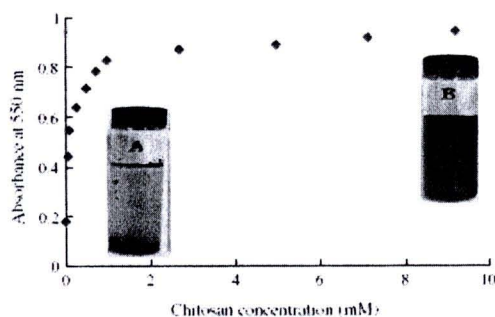


Fig. 1 – Plot of the changes in absorbance of a MWCNTs solution as function of the 61%DD chitosan concentration.

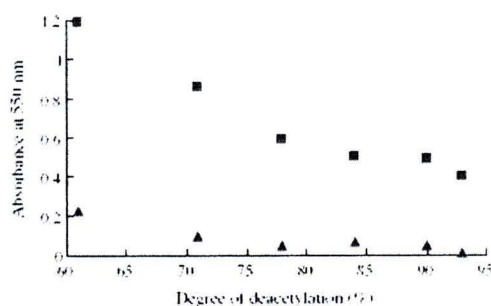


Fig. 2 – Plots of the changes in absorbance of a dispersion of MWCNTs in a 5 mM chitosan solution of various degree of deacetylation before (squares) and after (triangles) centrifugation at 2000 rpm for 10 min.

insure total dispersion of the MWCNTs in an excess solution of chitosan. From the absorbance measurements of the solutions, it can be seen that the efficiency of the dispersion of the carbon nanotubes, decrease when increasing the chitosan %DD. The final absorbance of the solution when using 61%DD is double than when using the 93%DD chitosan. These results suggest that, as expected, more hydrophobic chitosan segment found in the lower 61%DD are more efficient to adsorb onto the MWCNTs leading to a better dispersion of the carbon nanotube in solution. The mechanism through which CNT interact with chitosan is thought to be due to hydrophobic interaction from hydrocarbon backbones and acetyl groups, and π system of the MWCNTs. CH- π interaction which is a weak hydrogen bonding attraction between soft acid C-H bond and soft base π system are also thought to take part in the adsorption of chitosan onto the MWCNTs [36]. At low degree of deacetylation 61%DD, the bonding force between chitosan and MWCNTs is based on hydrophobic interaction due to the acetyl groups that can interact with the surface of the nanotubes. For higher degree of deacetylation from 71%DD to 93%DD, the dispersion efficiency of the MWCNTs decreases due to the more hydrophilic character of the high %DD chitosan which is more soluble.

The efficiency of the surface modification of MWCNTs is often evaluated in term of stability against aggregation and sedimentation. Sedimentation occurs when the repulsion between MWCNTs is not strong enough to prevent the aggregation of the nanotube, leading to their precipitation. After surface modification of the MWCNTs by chitosan, the excess surface charges provided by the amino groups on the chitosan induce nanotube-nanotube repulsion and therefore prevent sedimentation. In our experiment, the sedimentation of the MWCNTs was accelerated with a centrifuge having a rotation rate of 2000 rpm for 10 min. In Fig. 2, is shown the absorbance of each MWCNTs solutions after centrifugation for each %DD (triangles). When compared to the initial absorbance (Fig. 2, square), a much lower absorbance as a result of the accelerated sedimentation by centrifugation was measured. Yet, it is interesting to observe that the lower 61%DD perform again better than the higher 93%DD although the former present a lower charge density when compare with the later. This would suggest that the adsorption of the lower %DD chitosan is greater than for higher %DD. In term of resistance to sedimentation, this improved stability suggests that the MWCNTs modified with the lower %DD have a higher surface charge density, which provides a better stability against sedimentation. In order to access the value of the surface charge, we further characterized the MWCNTs surface by zeta potential measurements.

The surface charge density of colloidal particles dispersed in solution can be estimated by measuring the zeta potential, which represents the difference in potential between the slip plane of the double layer near the particles surface and the bulk solution. The zeta potential of the pristine carbon nanotube is expected to be initially nearly neutral but should become largely positive after adsorption of chitosan due to the presence of cationic amino groups. In our experiments, the modified MWCNTs were found to have zeta potential values ranging from 34 to 42 mV, which confirm the successful immobilization of chitosan on the MWCNTs. Shown in Fig. 3

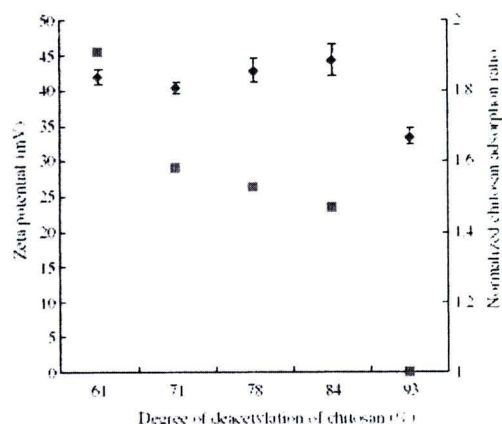


Fig. 3 – Zeta potential of modified MWCNTs (diamonds) and normalized chitosan ratio adsorbed onto the MWCNTs (squares) as a function of the %DD of chitosan.

(diamonds) are plotted the zeta potential values as a function of the %DD, which range from 42 mV (61%DD) to 34 mV (93%DD). These values decrease with increasing %DD and suggest that all MWCNTs dispersed in solutions have similar surface charged. Yet, because the 61%DD chitosan has a lower linear charge density when compared to 93%DD, these values need to be corrected if we want to compare the amount of polymer adsorbed at the surface of the MWCNTs. This lower linear charge density is due to the fact that the 61%DD contain only 61 NH_3^+ groups for 100 monomers while the 93%DD contain 93 NH_3^+ per 100 monomer. Since the zeta potential is proportional to the density of charges, equal zeta potential for two nanotubes would require 1.5 times more 61%DD than 93%DD chitosan. Therefore the 93%DD has a linear charge density 1.5 times higher than the 61%DD. In Fig. 3 (squares) is plotted the corrected normalized chitosan monomer ratio adsorbed onto the MWCNTs for each %DD. This plot is obtained by dividing the measured zeta potential by the corresponding %DD of the chitosan used and normalized. From the plot it can be seen that 1.9 times more 61%DD chitosan adsorb onto the MWCNTs when compared with the 93%DD. Several factors can justify the much lower adsorption of the 93%DD when compared with the 61%DD. The 93%DD has a better solubility, which means it will tend to remain in solution and will be more thermodynamically stable in solution. The higher charge density on the 93%DD chitosan can induce electrostatic repulsion of the NH_3^+ groups, which in turn would lead to lower adsorption density of the chitosan while the 61%DD could adsorb in a more packed fashion.

4. Conclusion

Multiwall carbon nanotubes have been noncovalently modified with chitosan having different %DD. Using chitosan having different %DD had a strong effect on the quality of the nanotubes dispersion. UV-Visible spectroscopy results suggest that the nanotubes dispersion was improved when using

chitosan with a lower degree of deacetylation (61%DD) when compared with higher degree of deacetylation (93%DD). The MWCNT modified with the lower %DD also displayed the best stability against centrifugation. Zeta potential measurements finally confirmed that the amount of chitosan adsorbed onto the nanotubes surface was twice as high with the lower %DD as with the high %DD. These modified MWCNTs with chitosan biopolymer could have the potential immobilization of hydrophobic and hydrophilic drug in drug delivery application.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carbon.2009.06.060.

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PUBLICATIONS IN THIS DISSERTATION

1. **Iamsamai C.**, Hannongbua S., Ruktanonchai U., Soottitantawat A., Dubas S.T. The effect of the degree of deacetylation of chitosan on its dispersion of carbon nanotubes. Carbon. 2010; 48(1): 25-30.
2. **Iamsamai C.**, Hannongbua S., Ruktanonchai U., Soottitantawat A., Dubas S.T. Simple method for the layer-by-layer surface modification of multiwall carbon nanotubes. **Submitted**.

Related Publication

1. Rungrotmongkol T., Arsawang U., **Iamsamai C.**, Vongachariya A., Dubas S.T. and Hannongbua S. How dispersion and separation are the noncovalently modified carbon nanotubes? A case study with a chitosan-polysaccharide biopolymer wrapping on SWCNTs. **In preparation**.

