20 Oct 2009

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Recommended Citation

P. Zhang and D. B. Henthorn, "Fabrication of High-capacity Biomolecular Carriers from Dispersible Single-walled Carbon Nanotube-polymer Composites," *Langmuir*, vol. 25, no. 20, pp. 12308 - 12314, American Chemical Society, Oct 2009.

The definitive version is available at https://doi.org/10.1021/la9015349

Langmuir
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Fabrication of High-Capacity Biomolecular Carriers from Dispersible Single-Walled Carbon Nanotube−Polymer Composites

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Received April 29, 2009. Revised Manuscript Received September 1, 2009

One of the most interesting applications for carbon nanotubes is as a support material for bioanalytical devices. In this work, we successfully used an ultraviolet light initiated "graft from" polymerization method to fabricate polymer functionalized carbon nanotubes (PFCNTs) with pendant chains of various functionalities, including poly(ethylene glycol) chains to boost dispersibility and pendant epoxy groups for protein conjugate sites. A model enzyme, alkaline phosphatase, was used to study biomolecule loading efficiency as well as the retention of enzyme activity. Samples with various ratios of the two monomers were fabricated to optimize their use in aqueous environments, and an optimal composition was determined. This method allows the enhancement of enzyme loading amount while retaining high enzyme activity. The morphology of the carbon nanotubes were characterized by STEM and AFM before and after functionalization. In addition, the resulting PFCNTs were analyzed by FT-IR, TGA, and XPS.

Introduction

Carbon nanotubes sparked great interest immediately after discovery.[1−8] Their unique mechanical, electrical, optical, and thermal properties have been studied for over a decade and have found use in applications like high-strength nanocomposites,[9−11] sensitive biosensors,[12−15] cancer treatments,[16−18] and fire-retardant materials.[19] Recently, carbon nanotube−polymer composites have drawn much attention.[9−11] While stronger materials have been fabricated through the controlled addition of small quantities of carbon nanotubes as reinforcing agents,[20] it was found that adding surface-functionalized carbon nanotubes, which enhance interaction between the nanomaterial and matrix, resulted in greatly improved mechanical properties.[21]

Polymer-functionalized carbon nanotubes, when conjugated with biomolecules, may also be used as platforms for diagnostic and therapeutic devices. Significant research has been conducted on creating various single-walled carbon nanotube (SWNT)−biomolecule conjugates, incorporating such species as DNA, antibodies, enzymes, and so forth.[22−25] In an example of the simplest manner of conjugation, Karajanagi et al.[26] reported making carbon nanotube−enzyme conjugates through simple physical adsorption of the protein. In this technique, the amount of enzyme loading and retention of activity is highly dependent on the surface characteristics of the target enzyme.

Alternatively, linker molecules may be used to immobilize biomolecules on the CNT surface. In order to improve adsorption of proteins, Chen et al.[27] developed a technique for producing carbon nanotube−enzyme conjugates with the use of a heterobifunctional linker molecule, 1-pyrenebutanoic acid succinimidyl ester. The pyrenyl group interacts strongly with the nanotube surface via $\pi-\pi$ hydrophobic interactions, while the succinimidyl ester groups can react with surface-exposed amine groups on the enzyme through a nucleophilic substitution reaction. Palwai et al.,[28] modifying work done by Graff et al.,[29] used sodium cholate as the linker molecule. In this work, the enzyme loading capability of the carbon nanotubes support was greatly improved

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and enzyme activity was partially retained. However, precipitation of the carbon nanotube−enzyme complex from aqueous media was observed after only 5 days, making this technique difficult to use for long-term applications in the bioanalytical field. Work done earlier by our group[30] evaluated several of these different methods in order to maximize enzyme loading amount and activity retention.

It is of great importance to develop an enzyme−nanotube conjugation technique which can lead to large amounts of immobilized enzyme without sacrificing enzymatic activity. Ideally, these will exhibit good dispersibility in aqueous media and will remain stable for long periods of time. To this end, we have studied the technique of grafting polymer chains with pendant functionalities directly from the carbon nanotube surface. These functionalities act to both disperse the nanotubes in aqueous media and include sites for enzyme conjugation.

The two main methodologies for covalent attachment of polymer chains to surfaces are commonly defined as "graft to" and "graft from" methods. The former relies on the synthesis of a polymer molecule first, and this polymer chain is subsequently attached to the nanotube surface. In the "graft from" method, the polymer chain is usually grown directly from the surface. Typically, an initiator molecule or monomer is immobilized on the surface to act as a reaction site for chain growth. Here, we report a novel ultraviolet light initiated "graft from" polymerization method to fabricate polymer-functionalized carbon nanotubes (PFCNTs) with grafted, copolymerized chains comprising poly(ethylene glycol) methyl ether methacrylate (PEGMA) and glycidyl methacrylate (GMA) repeating units.

The poly(ethylene glycol) (PEG) chains provided by the PEGMA macromonomers were utilized to significantly boost the dispersibility of the carbon nanotubes in aqueous environments. PEG is also featured in biological and biomedical applications because it reduces the nonspecific adsorption of biomolecules on carbon nanotube surfaces.[31] Pendant epoxy groups introduced through the GMA monomer can be directly conjugated with amino groups on enzymes through a ring-opening reaction. This mixture of PEG-rich macromonomers and epoxy-containing GMA monomers allows us to control the level of enzyme conjugation sites as well as aqueous dispersibility. In this work, PFCNTs with different ratios of these two monomers were fabricated to optimize their use as biomolecules carriers in aqueous environments.

Experimental Section

Materials. Pristine HiPCO single-walled carbon nanotubes (SWNT, 1−2 nm) were purchased from Carbon Nanotechnogies Inc. (Houston, TX) and were used as received without any further treatment. Poly(ethylene glycol) methyl ether methacrylate (PEGMA) macromonomer ($M_n \sim 1100$), 1-hydroxycyclohexyl phenyl ketone (HCPK, purity ≥99%), glycidyl methacrylate (97%), ethanolamine (99%), and bovine intestinal mucosa alkaline phosphatase were purchased from Sigma Aldrich (St. Louis, MO) and all used as received. Bradford assay reagents were purchased from Pierce Biotechnology, Inc. (Rockford, IL), and used for determining protein concentrations in solution. Water used in all experiments was purified in aquaMax ultra purification system (labwater.com, van Nuys, CA) and had a resistivity of 18.2 MΩ/cm.

Preparation of Polymer-Functionalized Carbon Nanotubes. SWNTs (30 mg) were dispersed in 75 mL of a 50:50 (v/v) water/ethanol solution and were sonicated (130 W and 40 kHz, Fisher Scientific, Pittsburgh, PA) for 2 h at room temperature in order to cleave the nanotubes into shorter lengths. The free radical photoinitiator HCPK was introduced at 1% by weight of total monomer into the water/ethanol solution. The solution was mildly shaken by a vortex mixer (Vortex Gene 2, Fisher Scientific, Pittsburgh, PA) for another 10 min. The CNT solution was evenly divided among three 75 mm Petri dishes (Fisher Scientific) to maximize exposed surface area. Each dish was stirred at 80 rpm during irradiation to ensure uniform mixing and equal UV dosage. Irradiation of the mixture with ultraviolet light (365 nm, 150 mW/cm^2, IntelliRay 600, UViTron International, West Springfield, MA) was then carried out with directly irradiation of the mixture in a nitrogen atmosphere. After 30 min of UV exposure, the reaction was stopped. Excess unbound HCPK molecules were removed by repeated (three times) centrifugation at 4000 × g (Eppendorf, 5810R, Westbury, NY). PEGMA and GMA monomers with four different ratios—4:1, 1:1, 1:4, and 1:10 (w:w)—were then introduced and polymerization was conducted with another 30 min of UV irradiation in an oxygen-free atmosphere. Unreacted monomer was washed by DI water and then removed by repeated centrifugation at 10000 × g, and the functionalized carbon nanotubes were collected on 0.45 μm nylon membrane filters (Whatman, Maidstone, England) by filtration. The functionalized carbon nanotubes were then rinsed thoroughly with water and dried in a vacuum oven overnight for further characterization and enzyme addition.

Enzyme Attachment onto Functionalized Carbon Nanotubes. A concentration of 1.0 mg/mL PFCNT solution was made for all samples before introduction of the enzyme. In all methods, alkaline phosphatase solution was prepared and diluted to 2 mg/mL. Two hundred microliters of enzyme solution were added to 0.5 mL of PFCNT solution for overnight interaction at room temperature. After incubation, unbound enzyme was removed using centrifugation at 15000 × g for 30 min and the supernatant was discarded. The washing process was repeated three times, with pure water added each time to resuspend the composites. In a control experiment, before the enzyme was introduced into the PFCNT solution, ethanolamine (0.5%, v/v) was added into the PFCNT solution first to quench the epoxy groups. After overnight interaction, the solution was purged by water for 3 times before the addition of enzyme solution.

Enzyme Activity Retention Evaluation. The activity of immobilized alkaline phosphatase was tested using One Step PNPP (Pierce Biotechnology, Inc., Rockford, IL), a widely used substrate for the colorimetric determination of proteins. A small aliquot of PFCNT−enzyme conjugate solution (100 μL) was added to a PNPP solution of the same volume. The entire solution was then immediately transferred to a microplate reader (Beckman Coulter DTX 800/880, Fullerton, CA) where the absorbance at 405 nm was measured after 20 min.

Characterization. Field emission scanning electron microscopy (FESEM) was recorded on a Hitachi S-4700 (Hitachi, Osaka) to investigate the morphology of the pristine carbon nanotubes. The morphology of the pristine carbon nanotubes, the mixture of SWNTs and monomer following sonication, and PFCNTs was studied by a scanning transmission electron microscope (STEM) (Helios Nanolab 600, FEI, Hillsboro, Oregon) operating at 250 kV. Drops of the pristine carbon nanotubes, pristine carbon nanotubes, and monomer (1:1 PEGMA/GMA weight ratio) without reaction, and PFCNT suspensions were sonicated for 20 min before they were put on individual 400-mesh carbon-coated copper TEM grids and allowed to dry in the open atmosphere. The morphology of the PFCNTs after the introduction of enzyme was studied by atomic force microscopy (AFM) in tapping mode on a Nanocsope III (Digital Instruments, Veeco Metrology Group, Santa Barbara, CA). A drop of PFCNT−enzyme solution was placed onto a freshly cleaved mica surface. The specimen was dried in atmosphere for 24 h before imaging.

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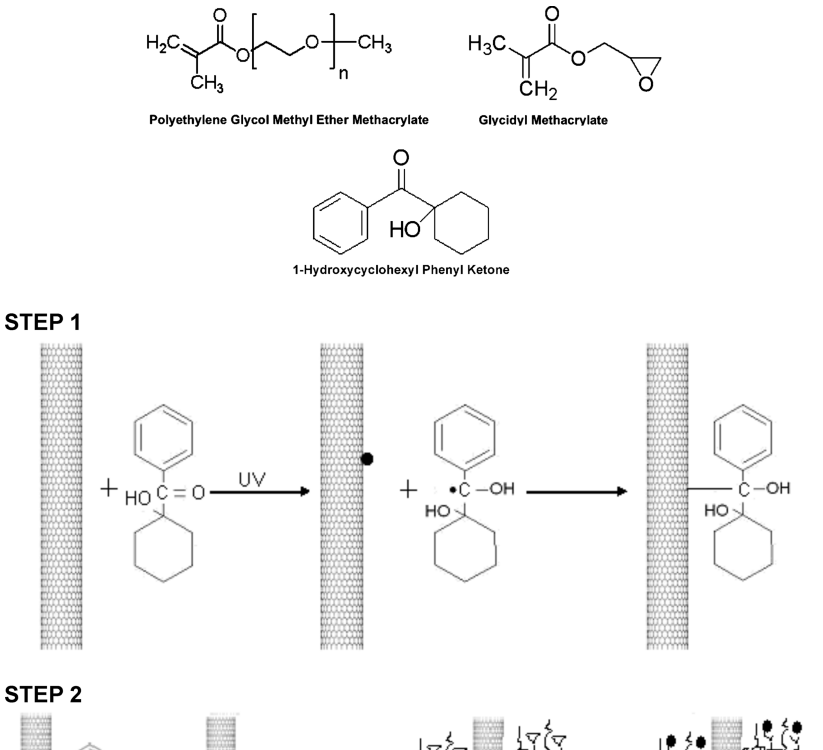
Scheme 1. Illustration of the Process of Fabrication of High-Capacity Biomolecular Carrier from Dispersible Single-Walled Carbon Nanotube−Polymer Composites



Raman spectroscopy (633 nm, Horiba Jobin YVON, Edison, NJ) was utilized to observe pristine CNTs and PFCNTs. Attenuated total reflectance Fourier transform infrared (ATR-FTIR) (Nicolet 6700, Thermoelectron Corporation, Madison, WI) was used to characterize the functional groups associated with the PFCNTs. X-ray photoelectron spectroscopy (XPS, Kratos Axis 165, Kratos Analytical, Chestnut Ridge, NY) analysis was employed to investigate the surface of the PFCNT and PFCNT−enzyme conjugates. Low-resolution survey scans and higher-resolution scans of C, N, and O were taken. Thermogravimetric analysis (TGA, Netzsch, Exton, PA) experiments were carried out under nitrogen at 10 °C/min to 700 °C.

Results and Discussion

Synthesis and Characterizations of PFCNT−Enzyme Conjugate. We have adapted the method proposed by Ma et al.[32] for the modification of poly(propylene) surfaces for use with carbon nanotubes. First, irradiation of dissolved photoinitiator leads to dissociation into radical fragments which subsequently attack the nanotube surface. Unbound photoinitiator is then removed by centrifugation or dialysis. Further irradiation leads to cleavage of this newly formed bond and, in the presence of monomer, the site of a grafted polymer chain. Following reaction and separation, enzyme molecules may be introduced through reaction with pendant epoxy groups. The entire process is shown in Scheme 1.

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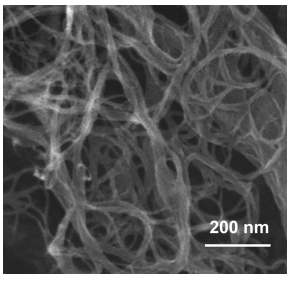

Figure 1. SEM image of pristine SWNTs.

The SWNTs used in this work were produced by Carbon Nanotechnologies, Inc., and had an average diameter of 2 nm. SEM images of pristine SWCNTs and PFCNTs (weight ratio of PEGMA/GMA monomer = 1:1) are shown in Figure 1. The average diameter of the pristine SWNTs was determined to be 10−15 nm, indicating that the nanotubes were not individually dispersed and that small nanotube bundles may exist. STEM was employed to identify the presence of a polymer layer on the SWNTs. Images were taken of pristine SWNTs, of a mixture of SWNTs with monomer following sonication (to identify possible adsorption of the macromonomer on the SWNT surface), and finally of the PFCNTs. The morphology of the pristine SWNT sample is clearly shown in Figure 2a and the surface is clean. The polymer-coated SWNTs can be distinguished from pristine SWNTs, as both the polymer and SWNT graphite hollow

structures are clearly visible in the STEM images, as shown in Figure 2b. The polymer shell has a thickness of approximately 5 nm. A simple mixture of SWNTs with the monomers was also characterized and is displayed in Figure 2c. No adsorbed polymer layer was detected on the SWNT surface.

The PFCNT dispersion was also characterized by AFM in tapping mode, as shown in Figure 3. The average height of the tubes was about 6 nm. It was noted that the heights of some nanotubes were as small as 2 nm, indicating that not all CNTs were functionalized with polymer. The distinct globular structures attached on the PFCNT surface represent enzyme molecules.

Raman spectroscopy was utilized to investigate the bonding of the chains to the carbon nanotubes (Figure 4). The Raman spectra of pristine SWNTs show typical breathing modes at 190−250 cm^{-1} and tangential modes at 1590 cm^{-1} (G-band). After functionalization, the increasing intensity at 1301 cm^{-1} (D-band) indicates the formation of sp^3 carbon atom, additional evidence that the polymer chains were covalently grafted to the SWNT surface. Also, the spectrum of a sonicated mixture of SWNTs with monomer is provided. It can be observed that the D-band intensity is almost the same when compared to that of pristine nanotubes, indicating that the sonication technique used in our experimental conditions does not destroy the nanotube structures.

XPS analysis was employed to verify the attack of HCPK molecules on the sidewall of SWNTs, with results shown in Figure 5. The major peak at a binding energy of 285.5 eV is assigned to C1s, the minor peak at a binding energy of about 534 eV is assigned to the O1s. The pristine carbon nanotubes have a 4.06% oxygen atomic concentration, which is likely from the metal oxide catalysts used in manufacture. However, after the attack of the photoinitiator molecules, the atomic concentration increased to 6.5%, which directly indicates the existence of HCPK. We summarize the binding energy and the elemental composition of both pristine nanotubes and HCPK modified nanotubes in Table 1.

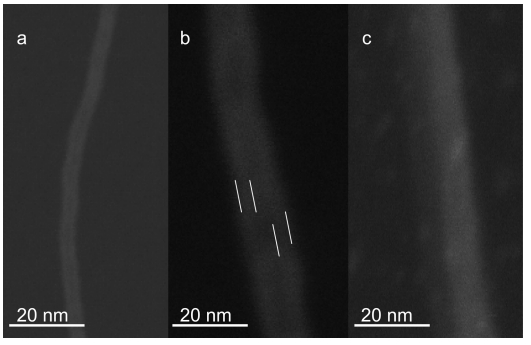

Figure 2. STEM images of (a) pristine SWNT, (b) PFCNT, and (c) mixture of SWNT with monomer.

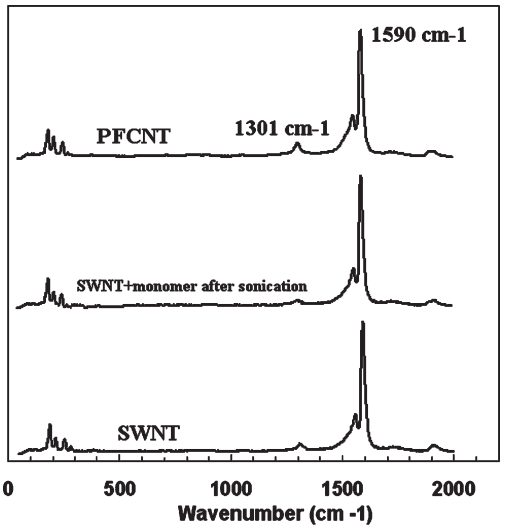

Figure 4. Raman spectra of pristine CNTs, PFCNTs, and mixture of SWNT and monomer with sonication.

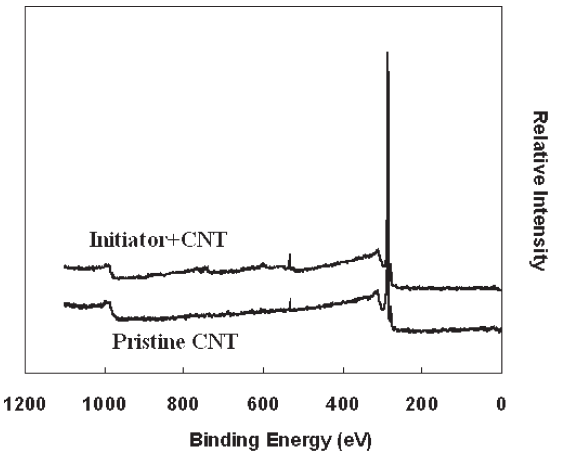

Figure 5. Survey X-ray photoelectron spectra of (a) pristine CNT and (b) HCPK modified CNT.

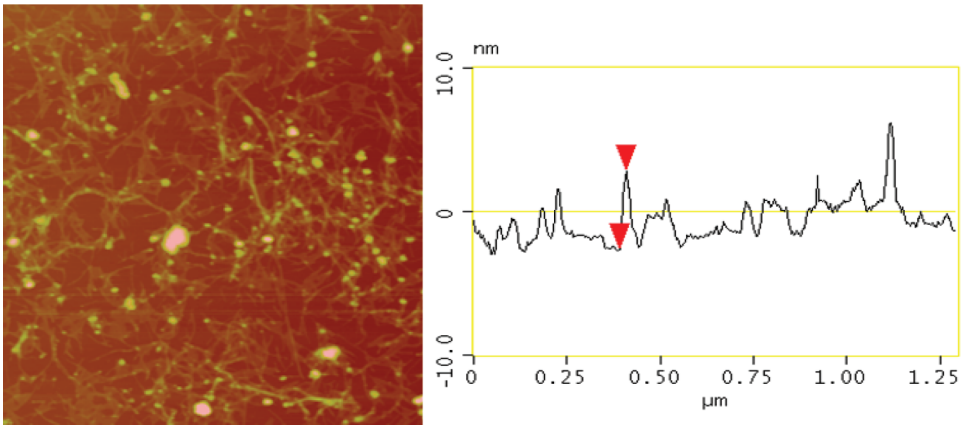

Figure 3. AFM images (1 μm × 1 μm) of individually dispersed PFCNT−enzyme conjugates.

Table 1. Binding Energy and Atomic Composition of Pristine Nanotubes and HCPK Modified Nanotubes As Measured by XPS

	pristine nanotubes		HCPK modified nanotubes	
element	BE	at %	BE	at %
C1s	285.5	94.65	285.5	93.21
O1s	534.0	4.06	534.0	6.50

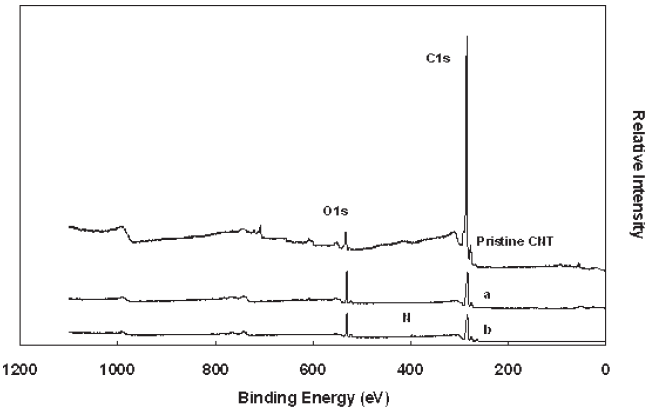

Figure 6. Survey X-ray photoelectron spectra of (a) pristine CNT, PFCNT, and (b) PFCNT-enzyme.

XPS analysis was also employed to investigate the PFCNT surface chemistry, with the results shown in Figure 6. The major peak at a binding energy of about 285.5 eV is assigned to C1s, the minor peak component at the binding energy of 534.0 eV is attributed to O1s of the etheric group of the PEG chain, and a small but clearly discernible peak at the binding energy of 398.5 eV results from the present of N atoms from the enzyme. Compared to pristine carbon nanotubes, where the oxygen atomic concentration was only 4.06%, the enhanced oxygen signal seen with the PFCNTs confirms the presence of oxygen-containing polymer. The oxygen atomic concentration increased to 24.2%, a number higher than reported by researchers using a "graft to" method,[33] illustrating the utility of our "grafted from" technique. In Figure 7, a nitrogen peak is observed in the high-resolution N spectrum, illustrating attachment of enzyme. We summarize the binding energy and the elemental composition of both pristine nanotubes, PFCNTs, and PFCNT−enzyme conjugates in Table 1.

TGA analysis was used to further evaluate the extent of sidewall functionalization. Figure 8 shows TGA loss of weight data for pristine carbon nanotubes and four PFCNT samples with different starting monomer composition. The percentage weight loss curves indicate that the overall weight loss during the process was 66.2%, 70.1%, 87.5%, and 91.3% for PEGMA/GMA monomer ratios of 4:1, 1:1, 1:4, and 1:10, respectively. The pristine carbon nanotubes have good thermal stability since no obvious weight loss was observed below temperatures of 500 °C. The copolymer attached on the surface of the PFCNTs started to decompose when the temperature reached 200 °C, and the weight of PFCNT decreased drastically between 300 and 400 °C. As the pristine carbon nanotubes showed little decomposition below 500 °C, the PFCNT weight loss at 500 °C was used to estimate the relative weight of grafted copolymer on the surface of carbon nanotubes. The differences of weight loss at 500 °C, between the pristine and the four PFCNT samples, were 54.2%, 58.1%, 75.5%, and 79.3%, respectively. Such high weight loss further

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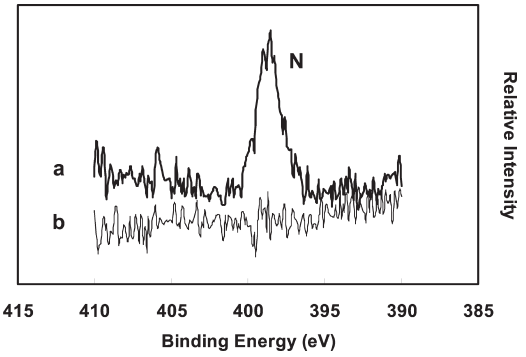

Figure 7. N narrow scan X-ray photoelectron spectra of (a) PFCNT-enzyme, (b) PFCNT.

confirms the previous XPS results and demonstrates that this "graft from" polymerization route was effective and efficient and yielded nanomaterials with high levels of polymer grafting.

Figure 9 shows the ATR-FTIR spectra of PFCNT−enzyme conjugates with four different starting PEGMA/GMA monomer ratios (a) 4:1, (b) 1:1, (c) 1:4, and (d) 1:10 along with pristine carbon nanotubes (e). The pristine carbon nanotube curve is featureless. The peak at ~2890 cm^{-1} corresponding to the stretch mode of C−H bond vibration associated with $-CH_2-$ can be observed in all four samples. As the PEGMA monomer concentration decreased, the intensity of this peak also decreased. However, the band at 1160 cm^{-1}, attributed to C−O stretching in GMA repeating units, increases its intensity as the GMA monomer concentration increases. Clear twin peaks can be observed when the PEGMA/GMA monomer ratio equals 1:1. An absorption peak for C═O group stretching vibration at ~1731 cm^{-1} was recorded as a result of grafted GMA and PEGMA repeating units. The peaks at ~1110 cm^{-1} were attributed to the C−O symmetric stretch of the PEG side chain. The characteristic peaks of epoxide groups appeared at ~910 cm^{-1}, which indicated there were still some epoxy groups that were not reacted with enzymes.

Three control experiments were conducted to elucidate the role of the initiator molecules and to establish that the polymer chains were covalently bonded to the nanotubes. In the first experiment, nanotubes were mixed with the PEGMA and GMA monomers, but no reaction was conducted in order to establish whether any adsorption occurs between monomer (especially the PEG-containing PEGMA monomer) and the CNTs. After conducting the washing and filtration protocol outlined previously, complete removal of the free monomer from the nanotube surface was indicated by the absence of the relevant FT-IR peaks (Figure 10). To confirm that the photoinitiator molecules were essential to the grafting procedure, a second control experiment was conducted where HCPK was omitted entirely. All other steps remained the same. After conducting the same UV polymerization, washing, and filtration procedures, no polymer was detectable with the nanotubes, as indicated from the FT-IR spectra shown in Figure 10. Finally, a control experiment was conducted to establish that the initiator molecules require activation (Scheme 1, Step 1) before polymerization can be achieved. In the first step of the reaction, nanotubes were mixed with the HCPK photoinitiator but no UV irradiation was employed. The same washing and filtration procedures as mentioned previously were applied before introduction of monomers and photopolymerization. The carbon nanotubes exposed to this process showed no indication of having grafted polymer chains, as shown in Figure 10, confirming the need to activate the photoinitiator.

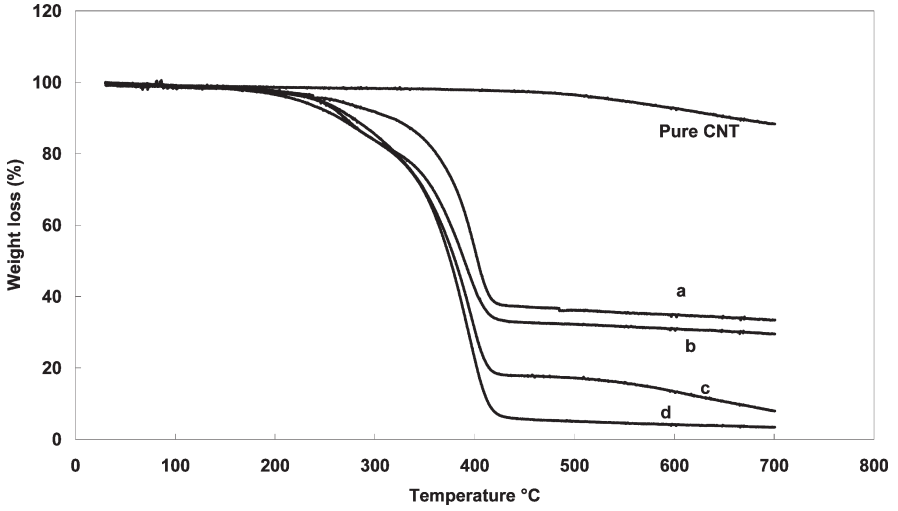

Figure 8. TGA curves of PFCNTs with different starting monomer compositions. PEGMA/GMA with weight ratios of (a) 4:1, (b) 1:1, (c) 1:4, (d) 1:10.

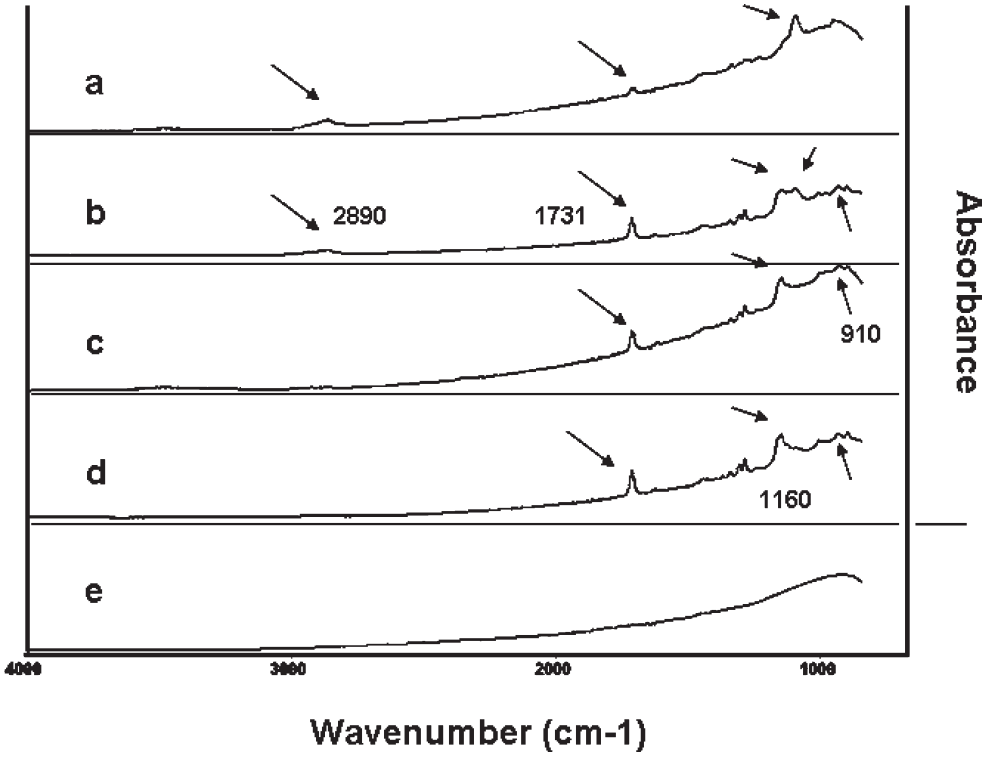

Figure 9. FTIR spectra of PFCNT−enzyme conjugates with different starting monomer compositions. PEGMA/GMA with weight ratios of (a) 4:1, (b) 1:1, (c) 1:4, and (d) 1:10; (e) pristine carbon nanotube.

This evidence, in addition to the XPS data showing an increase in oxygen concentration after the photoinitiator activation (Table 1), leads us to believe that the photoinitiator was covalently bound to the nanotube surface during the activation step.

Quantification of Enzyme Loading Amount and Activity Retention. The enzyme loading amount was quantified by Bradford assay and the retained activity of immobilized alkaline phosphatase on PFCNT was tested using the PNPP substrate. Enzyme loading on CNT is reported as micrograms of enzyme per milligram of dry material. The results for the four PEGMA/GMA formulations are shown in Table 3. The enzyme loading amount increased considerably to more than 409 μg/mg when the GMA/PEGMA monomer ratio was equal to or greater than one. A control experiment was undertaken to establish the importance of the pendant epoxy groups in the immobilization of enzyme on the nanotube surface. Ethanolamine was added to the PFCNT solution to quench the epoxy groups prior to addition of enzyme. Following removal of unreacted ethanolamine, an analysis of enzyme loading was done using the same procedures as outlined previously. The amount of enzyme loading on the quenched PFCNTs decreased to only 25 μg/mg, confirming the importance of the pendant epoxy groups for efficient enzyme immobilization.

In order to quantify retention of activity, control experiments were conducted with known concentrations of enzyme in free solution. These values were then compared to conjugated enzyme activity. Selection of enzyme concentration in the free solution

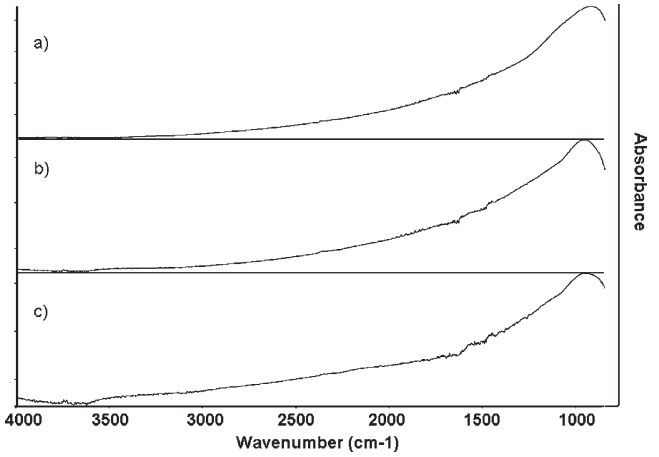

Figure 10. FTIR spectra of control experiments: (a) pristine carbon nanotubes, (b) no HCPK molecules were used, (c) inactivated HCPK molecules were used.

Table 2. Binding Energy and Atomic Composition of Pristine Nanotubes, PFCNTs and PFCNT−Enzyme Conjugates As Measured by XPS

	pristine nanotubes		PFCNT		PFCNT-enzyme	
element	BE	at %	BE	at %	BE	at %
C1s	285.5	94.65	284.0	76.36	284.0	74.45
O1s	534.0	4.06	531.5	23.64	531.5	24.18
N	−	−	406.0	0	398.5	2.40

experiments was done to match the values determined in the enzyme loading experiments. Enzyme activity retention is therefore reported as percentage of the activity measured by the same amount of enzyme in solution. It is noteworthy that monomer composition had no noticeable effect on retention of enzyme activity.

The results improved considerably compared to the previous work done in our lab. In that work,[30] we studied the functionalization of CNTs with the same model enzyme using either simple physical adsorption or through the use of linker molecule chemistry. The optimal method—use of linker chemistry of Chen et al.[27]—produced enzyme loading amounts of 140 μg/mg and a retained activity of 57%. However, the enzyme loading amount in this work increased significantly to more than 409 μg/mg when the GMA/PEGMA monomer ratio was equal to or greater than 1, a 190% improvement, and the retention of enzyme activity is kept still over 50%.

Finally, the dispersibility and stability of the PFCNTs with four different monomer formulations were tested. Ten milligram samples of each different PFCNT sample were dispersed into 10 mL of water with 30 min sonication. All four samples formed homogeneous solutions readily upon sonication. However, small chunks of nanotubes were observed in samples with PEGMA and GMA monomer ratios of 1:4 and 1:10 1 h after sonication, and significant nanotube precipitation ensued within 24 h. No obvious aggregation or precipitation was observed for the PFCNT samples formed with the monomer ratios of 4:1 and 1:1 over a week.

Table 3. Comparison of Enzyme Loading Amounts and Activities for PFCNTs of Different Monomer Ratios

SWNT with PEGMA/GMA mass ratio	enzyme loading μg/mg	percent activity of enzyme in free solution
4:1	228 ± 12	47%
1:1	409 ± 7	51%
1:4	448 ± 9	53%
1:10	470 ± 18	51%

Considering the potential application of this synthesized nanocomposite is in the bioanalytical field, the dispersibility in aqueous environment is of great importance, which prevents the monomer composition with PEGMA and GMA ratio of 1:4 and 1:10 from optimal formulation. In addition, for a potential application as a biological molecule carrier, the PEGMA/GMA composition of 1:1 appears to be optimal because it has a enzyme loading capacity much larger than that of 4:1 sample.

Conclusions

In this study, we successfully developed a simple and effective ultraviolet initiated "graft from" polymerization method to synthesize polymer-functionalized carbon nanotube biomolecular carriers. The attached polymer molecules significantly enhance both aqueous dispersibility and biomolecule immobilization capacity. This technique allows for carbon nanotube modification without harsh oxidative treatment. Also, the proposed technique features grafted structures secured by stable C−C bonds, a significant advantage over potentially hydrolyzable linkages introduced in other treatments.

For use as biomolecular carriers, composites formed in the 1:1 PEGMA/GMA ratio appear to be optimal. Materials formed with this monomer composition exhibited a maximum value of enzyme loading while maintaining excellent aqueous dispersibility. Furthermore, no enzyme precipitation or loss of enzyme activity was measured from the carbon nanotube−enzyme complex even after a week in solution at room temperature.

Acknowledgment. Thanks to Missouri S&T's Materials Research Center for material characterization support.