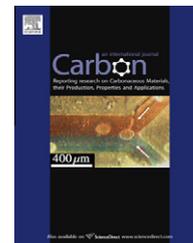


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Detecting and quantifying oxygen functional groups on graphite nanofibers by fluorescence labeling of surface species

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ARTICLE INFO

Article history:

Received 10 May 2010

Accepted 20 July 2010

Available online 27 July 2010

ABSTRACT

The ability to quantify functional groups on graphitic carbon nanofiber (GCNF) surfaces and to covalently attach ligands chemoselectively will aid in the development of functionalized GCNFs and potentially other carbon nanomaterials for applications in nanotechnology. Herein, we report the identification and quantification of functional groups on the surface of GCNFs using fluorescence labeling of surface species (FLOSS). Using reactions that are selective for specific functional groups and fluorescent dyes containing the dansyl group, surface aldehyde/ketone, carboxyl, and hydroxyl groups were identified and quantified in their total and relative amounts by FLOSS. Oxygen-containing functionality that was detected by FLOSS on nitric acid-oxidized GCNFs totaled approximately 2.5% of surface carbon, present as 0.9% aldehyde/ketone, 1.2% carboxyl groups on average, and 0.4% hydroxyl groups. The amounts of oxygen-containing functional groups on as-produced and demineralized GCNFs were much lower, amounting to no more than 0.05% of aldehyde/ketone groups on demineralized GCNFs and 0.3% of these groups on as-produced fibers. The FLOSS method has revealed information about the fiber surface that is not accessible by other methods, while also providing insight into the chemical reactivities of functional groups that can be used as sites for the attachment of ligands to give covalently functionalized materials.

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1. Introduction

Since the discovery of graphite nanofibers (GCNFs) in the mid 1990s there has been an enormous effort to develop these novel nanoscale materials in applications where their unique

structural, electrical, and mechanical properties can be utilized [1–4]. Initial applications of GCNFs focused on their absorption and storage capacities, and led to the development of GCNFs as adsorbents for small organic molecules from aqueous streams [5], electrodes for fuel cells [6], hydrogen

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doi:10.1016/j.carbon.2010.07.035

storage media [7], and heterogeneous catalyst supports [8]. More recently, the well recognized potential of carbon nanomaterials that contain surface functionality for applications in biology, medicine, and energy conversion, has resulted in a broader range of interest in the use of graphite nanofibers. Functionalized GCNFs have been investigated as biosensors [9], biomimetic membranes [10] and neuroelectrochemical electrodes [11]. GCNF composite materials have been evaluated as solid state gas sensors [12].

Surface functionalization and characterization are both critical to the further development of GCNF applications [13]. The presence of functional groups on the nanofiber surface alters their solubility, conductivity, biocompatibility, and other properties. More importantly, the presence of surface functional groups such as hydroxyl, aldehyde/ketone, or carboxylic acid provides the means by which GCNFs can serve as scaffolds for the selective and perhaps orthogonal attachment of varied ligands, such as biomolecules. Current methodology for the covalent attachment of organic ligands to the surfaces of carbon nanotubes (CNTs) [14] and GCNFs [15] involves first treating the fibers with an oxidant such as nitric acid to introduce carboxyl groups. The carboxyl groups are then activated with thionyl chloride and the ligands attached by acyl transfer. Surface carboxyl groups have been quantified [15] while aldehyde and ketone groups, as well as other reactive surface oxides such as hydroxyl groups are neither quantified nor utilized as potential sites of covalent functionalization.

Most of the existing methods used for the analysis of functional groups on the surfaces of carbon-based nanomaterials provide neither the chemical specificity nor sensitivity that are required, and few are based on selective chemical reactions [15,16]. Thus, there is an unmet need for the development of mild, efficient, and selective methods for the surface functionalization of GCNFs that take advantage of the different groups that are present and their associated reactivities. It is important that both the *nature* and *quantity* of functional groups that are accessible for reactions be determined, so that properties of the derivatized nanofibers can be correlated to known structural modifications. The difficulties in determining these two characteristics by direct chemical methods have been noted [13,17]. Several methods for the detection of functional groups on carbon surfaces have been recently reviewed [13,18–20]. FTIR and Raman spectroscopy, TEM, X-ray photoelectron spectroscopy (XPS), and fluorescence-based methods are the most widely used techniques. Selected area electron diffraction (SAED) [21], temperature programmed desorption (TPD) [22], mass spectrometry [15], and titration methods have also been used [23].

Functional groups on the surfaces of polymers have been identified and quantified by fluorescence-based methods that involve the covalent attachment of dyes to these groups [24,25]. The recently developed fluorescence technique known as FLOSS (fluorescence labeling of surface species) has been used to quantify functional groups on the surfaces of alkoxy silane monolayers [26], coated glass surfaces [27], activated carbon [28], and carbon nanotubes (CNTs) [29]. We considered, therefore, that FLOSS might be particularly well-suited to the analysis of graphite nanofiber surfaces, owing to its inherent sensitivity and specificity for functional

groups. Since dyes used in the FLOSS experiment are covalently attached to the surface functional groups, useful information could be gained about the reactivity of such groups within the environment that exists on the graphitic fibers, provided that conditions for FLOSS could be developed for GCNFs. Such information would serve as a valuable guide to aid the design of reaction chemistry for the synthesis of GCNFs that contain complex ligands. Perhaps most significant is that FLOSS could be used not only to identify the surface functional groups, but it could also provide quantitative information about their density on the carbon surface. In this paper, we describe the first application of the FLOSS method for the quantitative determination of functional groups on the surface of graphite nanofibers. We have been able to quantify aldehyde and ketone groups, as well as carboxylic acid and hydroxyl groups on the fiber surface. The chemistry that we have developed for the fluorescent labeling of surface functional groups should be readily adaptable to the covalent attachment of complex organic ligands such as peptides and carbohydrates.

2. Experimental section

2.1. Production of graphite nanofibers (GCNFs)

Ribbon, platelet, and herringbone graphite nanofibers were prepared in a Thermolyne[®] 79400 tube furnace equipped with Omega[®] FMA 5400/5500 mass flow controllers for CO₂, CO, C₂H₄, air, He, and H₂, according to literature procedures: [11–14] 100 mg of iron–nickel catalyst was used to obtain 0.98 g of ribbon GNF; 100 mg of iron-based catalyst was used to obtain 1.31 g of platelet GNF; and 50 mg of copper–nickel catalyst was used to obtain 6.73 g of herringbone GCNFs.

2.2. Demineralization of GCNFs

Demineralization was carried out to remove the growth catalyst by stirring 300 mg of the herringbone GCNFs in 300 mL of 1 M HCl for 14 days; fibers were collected by filtration through a 0.45 μm nylon membrane each day and resuspended in fresh 1 M HCl. After 14 days, the collected fibers were washed with water until the filtrate was neutral then dried overnight at 80 °C and 3.33 kPa to give 240 mg of demineralized fibers (80% recovery).

2.3. Oxidation of GCNFs

The procedure was modeled after that described by Haddon and Parpura for the oxidation of carbon nanotubes [30]. In a typical run, a sample of herringbone GCNFs (652 mg) was stirred under reflux in 600 mL of 3 M HNO₃ in a 1 L round bottomed flask fitted with a drying tube (CaSO₄) and magnetic stirrer for 24 h. After cooling to room temperature fibers were collected by filtration through a 0.45 μm nylon membrane filter, washed extensively with water until the filtrate was neutral, and dried for 24 h at 80 °C and 3.33 kPa to give 617 mg of oxidized fibers (95% recovery), which were used for characterization without further purification.

2.4. Transmission electron microscopy

Representative sample of GCNFs grown from the various catalyst systems were examined in a Hitachi H-7600 transmission electron microscope (lattice resolution = 0.204 nm). Suitable transmission specimens were prepared by ultrasonic dispersion of carbon nanofibers in isobutyl alcohol from which a drop of suspension was applied to a carbon-coated grid.

2.5. Surface area measurements

The Brunauer–Emmett–Teller (BET) method [31] was used to determine the specific surface area (SSA) of selected samples of GCNFs by measuring the absorption of N₂ at –196 °C with an ASAP 2020 instrument (Micrometrics Instrument Corporation). Results are shown in Table 1.

2.6. Thermogravimetric analysis

TGA and differential TGA (DTG) measurements were carried out using a Perkin-Elmer Pyris 6 analyzer. Samples were placed in ceramic pans and heated from 30 °C to 1000 °C at a rate of 10 °C/min, and an air flow rate of 20 mL/min. The thermal analysis of as-produced and nitric acid-oxidized herringbone GCNFs is presented in Fig. 1.

2.7. X-ray photoelectron spectroscopy

XPS analyses were carried out on a Scienta ESCA 300 instrument equipped with an aluminum Ka monochromatic source. GCNF samples were loosely mounted on double stick tape. XPS survey and deconvoluted C1s spectra are included in the Supplementary data, and results are shown in Table 1.

2.8. Dye synthesis – general

Complete details for the synthesis of compounds 3, 4, 5 and 6 (Fig. 3) are provided in the Supplementary data. Progress of the reactions was monitored by thin-layer chromatography (TLC) using aluminum foil-backed silica gel plates coated with a fluorescent indicator. Plates were developed using cerium molybdate (Hanessian) stain. Solvent mixtures are reported in volume/volume ratios. Flash chromatography [32] was carried out on silica gel. ¹H NMR spectra were recorded at 300 MHz in CDCl₃.

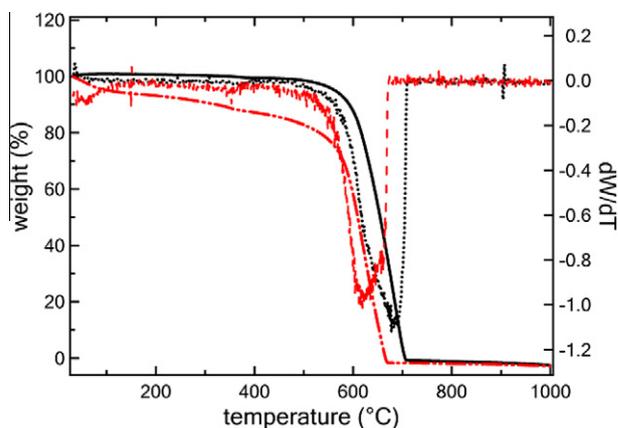


Fig. 1 – Thermogravimetric (TGA) and differential TGA (DTG) curves for as-produced (TGA, black solid line; DTG, black dotted line) and nitric acid treated (TGA, red dashed-dotted line; DTG, red dashed line) GCNFs. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.9. Fluorescence measurements – general

Fluorescence spectra were acquired in 10 mm rectangular quartz cuvettes using a HORIBA Jobin Yvon Fluoromax P fluorimeter with a 5-nm excitation slit width and a 2 nm detection slit width. Scans were taken at one nm per second from 375 nm to 650 nm, with excitation at 350 nm. Fluorescence labeling in this study using the FLOSS method was conducted using dansyl hydrazine 1 (Sigma), dansyl cadaverine 2 (Fluka), 2-(dansylamino)ethanol 4 [33], and 6-(5-(dimethylamino)naphthalene-1-sulfonamido)-hexanoic acid 6 [34] (Fig. 3). Aldehyde and ketone groups were labeled with 1; carboxylic acid groups were labeled with 2 and 4, and hydroxyl groups were labeled with 6. Solutions of the dyes were treated in separate experiments with 20–25 mg samples of GCNFs. After stirring for the times indicated in the following sections (typically 48 h), fibers were allowed to settle and aliquots were taken and treated as described to provide solutions for fluorescence measurements. Depletion was measured against a blank solution of dye that did not contain fibers, and from which the calibration curve was generated for each labeling experiment. Based on the amount of depletion determined from the calibration curve and stoichiometry of the given labeling reaction, the number of groups labeled can be

Table 1 – SSA, surface density (and percentage) of functional groups on herringbone GCNFs determined by FLOSS, and survey XPS analysis.

HB-GCNFs	Specific surface area	–CHO/COR		–COOH	–OH	Survey XPS	
						C1s (%)	O1s (%)
As-produced	37 ± 4 m ² /g	1.0 (±0.1) × 10 ¹³ /cm ² (0.25% ± 0.05%)	<10 ¹² /cm ² (<0.03%)	Not measured	Not measured	99.2	0.8
Demineralized	94 ± 7 m ² /g	1.8 × 10 ¹² /cm ² (0.05%)	<10 ¹² /cm ² (<0.03%)	Not measured	Not measured	99.0	1.0
Oxidized	154 ± 11 m ² /g	3.3 × 10 ¹³ /cm ² (0.9%)	4.5 (±1.1) × 10 ¹³ /cm ² (1.1% ± 0.3%)	9.6 × 10 ¹² /cm ² (0.3%)	1.5 × 10 ¹³ /cm ² (0.4%)	90.0	10.0
Dye	1	1	4	2	6		

calculated. Using the specific surface area (SSA) of the sample, determined from BET analysis, and the mass of the sample, the surface concentration of specific functional groups on the fiber surface was calculated. Control experiments to test for possible physisorption of active dyes were carried out with the hydrazone of dansyl hydrazine and acetone, *N*-(5-(dimethylamino)naphthalene-1-sulfonamido)pentyl)acetamide **3** and 5-(dimethyl-amino)-*N*-(2-methoxyethyl)-naphthalene-1-sulfonamide [35] **5** by protecting the active groups to inhibit chemisorption. Detailed descriptions of the labeling experiments, including control experiments to investigate physisorption and the extent of labeling as a function of time are provided in the [Supplementary data](#).

3. Results and discussion

Gram quantities of GCNFs were produced in three morphologies – ribbon, platelet, and herringbone, from mono- or bimetallic catalysts in a tube furnace as described previously [1–4]. Fibers were examined by TEM to confirm the morphologies expected based on the choice of catalyst. Results of the TGA of as-produced and nitric acid-oxidized herringbone GCNFs are presented in [Fig. 1](#). The TGA analyses indicate that the oxidized GCNFs are less thermally stable than the as-produced GCNFs, which is more likely due to the presence of surface functionalities, for example carboxyl groups, that disrupt the structure of GCNFs and can be lost as carbon dioxide upon heating. TGA also indicates that both samples contain negligibly small amounts of the residual metal (less than 0.4%) which is consistent with the amount of metal used to produce the GCNFs (~0.5%).

This study focused on the herringbone GCNFs, specifically on the surface characterization of fibers that had been treated with nitric acid. We discovered early on in this research that ribbon and platelet GCNFs undergo significant degradation under the conditions used for the oxidation. Loss of structural integrity was evident from TEM images of the fibers obtained after oxidation. However, herringbone GCNFs were not similarly degraded; no significant clipping or other degradation was observed after treatment overnight with 3 M nitric acid under reflux ([Fig. 2](#)).

The degradation observed for ribbon GCNFs was somewhat unexpected due to their lower degree of edge structure

and higher amount of basal plane. Platelet GCNFs, which contain a larger amount of edge structure, were clipped in length by the oxidation, while some samples were found to retain little of the nanofiber structure that can be observed in the as-produced materials. Nitric acid oxidation was chosen because it has been shown that the treatment of CNTs and GCNFs with nitric acid results in the introduction of surface oxides in the form of aldehyde and ketone carbonyl groups, carboxylic acid groups, and hydroxyl groups [15,21,29]. Alternate methods of oxidation, while not pursued in this study, would be desirable as the amount and type of functionalization in the GCNFs may be different than what is produced with nitric acid. In addition, GCNFs with ribbon and platelet morphology may survive a milder oxidation and yet have surface functional groups introduced.

The main purpose of this study was to identify and quantify the surface functional groups that are introduced by nitric acid oxidation of herringbone GCNFs using FLOSS. Owing to the relatively low percentage of surface carbon that undergoes oxidation in CNTs [29], and the diverse nature of oxygen-containing groups that are possible, quantification of surface functionality requires a method that is both sensitive enough to detect functional groups at low concentration and selective for the different types of surface oxides that are likely to be present. The FLOSS method is based on the depletion of a chemically reactive dye from a solution of known concentration. The structures of the dyes used in this study are shown in [Fig. 3](#). Dyes **1** and **2** were commercially available; dyes **3–6** were synthesized using reported procedures or by methods we developed for this study. Dye syntheses are described in the [Supplementary data](#).

One of the reasons we chose dansyl fluorescent labels is that previous studies suggested that compounds with this pattern of substitution of dimethylamino and sulfonyl groups have a low tendency to physisorb to SWCNTs [36]. For the detection of aldehyde or ketone groups, dansyl hydrazine (**1**) was used because it was expected to form a stable hydrazone linkage, as shown in [Fig. 4](#) for oxidized herringbone GCNFs. Emission spectra of dansyl hydrazine at different concentrations in acetone are shown in [Fig. 5a](#), along with a calibration plot (inset) of intensity versus concentration at 514 nm. The fluorescence measurements were carried out in acetone, with which dansyl hydrazine reacts, as this leads to increased

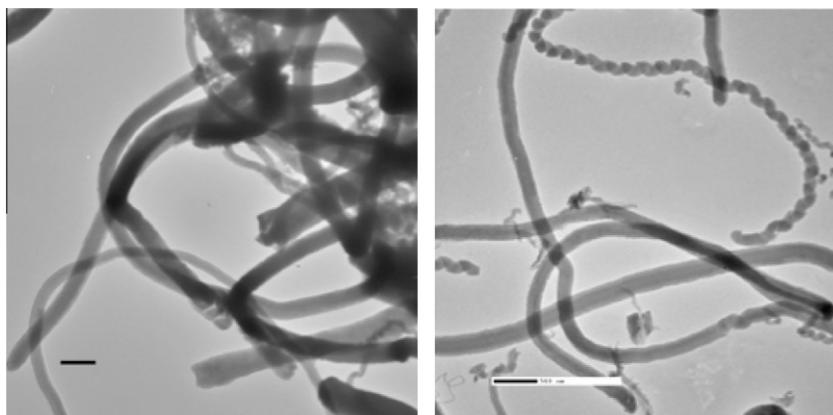


Fig. 2 – TEM images of as-produced (left) and nitric acid-oxidized (right) herringbone GCNFs (scale bars, black, are 500 nm).

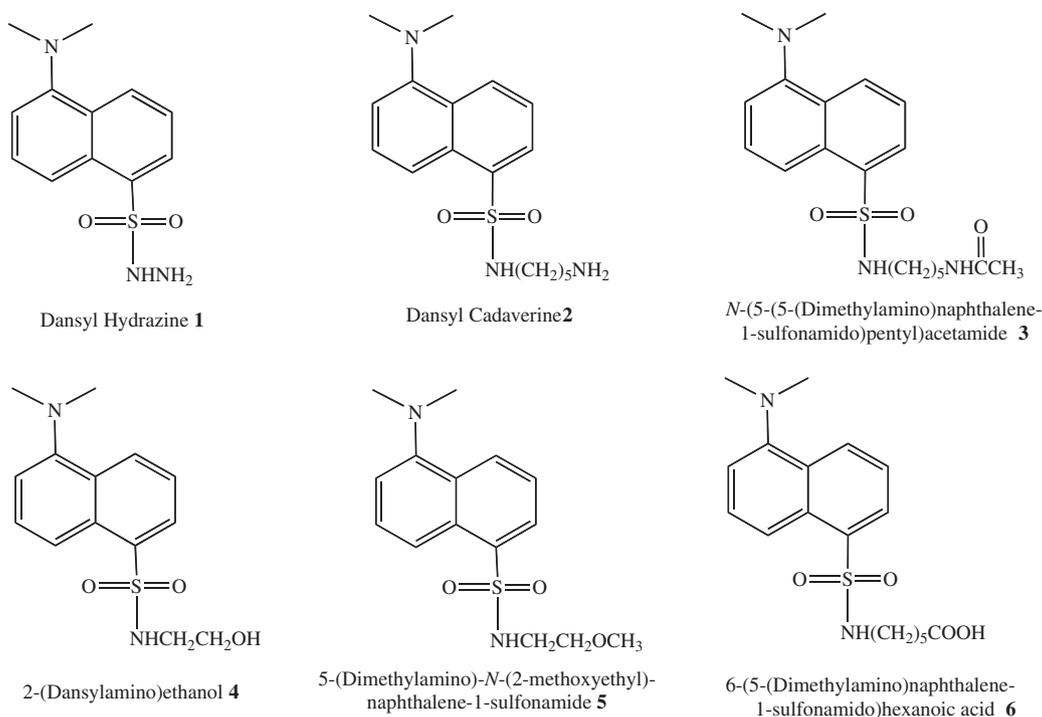


Fig. 3 – Fluorescent dyes used to label GCNFs.

fluorescence. However, there was no acetone in the solvent when GCNFs were reacted with dansyl hydrazine. The emission spectra measured for dansyl hydrazine solutions that were treated with nitric acid-oxidized nanofibers are shown in Fig. 5b. These show a decrease in emission intensity relative to the blank with no fibers that corresponds to a depletion of 1.8 μmol of aldehyde or ketone carbonyl groups, assuming a 1:1 stoichiometry. Converting this concentration to the number of carbonyl groups, and using the value of 154 m^2/g for the specific surface area of nitric acid-oxidized GCNFs, gives an estimate of the number of carbonyl groups per cm^2 . Using the value 3.85×10^{15} carbon atoms per cm^2 for the surface density of carbon atoms in graphene (based on a C–C bond length of 0.142 nm) gives the resulting atomic fraction of 0.9% surface carbon that is present as aldehyde or ketone groups labeled by the FLOSS method. This represents, to our knowledge, the first characterization of surface aldehyde/ketone groups on a graphite nanofiber surface using a selective chemical method.

X-ray photoelectron spectroscopy (XPS) is another analytical technique that might give some quantitative information about surface oxides on GCNFs [19]. XPS provides the elemental composition of the sample as well as the oxidation state of elements it contains. The determination of specific functional groups by XPS alone; however, is not straightforward [18,29,37]. Specifically the choice of background correction and the deconvolution of small peaks lead to uncertainty in the results especially when the functional groups are present in low concentrations. XPS analyses were carried out on as-produced, demineralized, and nitric acid-oxidized GCNFs. Analysis of the survey XPS data (Table 1) based on the comparison of the area under C1s and O1s core electron binding energy peaks shows that as-produced and demineralized

GCNFs contain mostly non-oxidized carbon (~99%), while oxygen content in the oxidized GCNFs is higher (10%). Survey and deconvoluted XPS spectra are included in the Supplementary data. The deconvoluted spectra provide some insight into the possible distribution of oxidation states in the GCNFs. It should be noted that the oxygen content obtained from the XPS data represents total oxygen content, and includes oxygen that is present in functional groups that were not detected by FLOSS in the present study, for example, ether and lactone functionalities. The FLOSS experiment gives an estimate of the number of groups that can be *chemically derivatized*. This information will be extremely valuable in the chemical labeling of GCNF surfaces with more complex ligands.

It was important to verify, through control experiments, that the depletion observed is not compromised by physisorption of the dyes on the fiber surface, which could be expected to take place due to the π – π interactions between aromatic rings of the dyes and the GCNFs. Physisorption was shown to be negligible by the results of control experiments in which nitric acid-oxidized herringbone GCNFs were treated with deactivated dansyl hydrazine under the same conditions as the labeling reaction. The hydrazine moiety of the dye is deactivated by hydrazone formation with acetone prior to exposure to the dye. Emission spectra for two samples of fibers treated with deactivated dye are shown in Fig. 5c. The two emission spectra are superimposable with that of the control that contained no fibers, indicating that no depletion, and therefore no binding of the dye to fiber, took place within the detection limit of the FLOSS experiment. It was also necessary to determine if the reaction time was long enough for the complete labeling of the accessible functional groups. Fig. 5d shows emission spectra for solutions of dansyl hydrazine treated

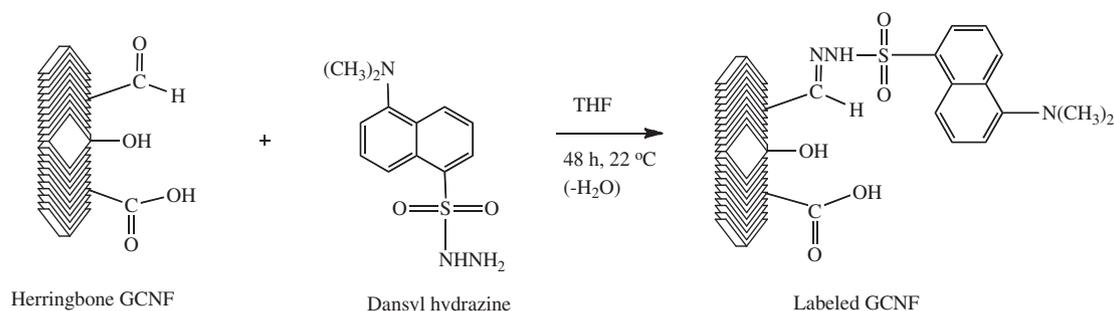


Fig. 4 – Labeling of a surface aldehyde group with dansyl hydrazine.

with oxidized GCNFs for different lengths of time. Greater depletion was observed in the reaction at 48 h compared to 24 h, but no significant change was observed at 72 h.

Although little surface oxidation would be expected on as-produced or demineralized herringbone GCNFs, it was of interest to carry out labeling experiments using FLOSS on these materials. Demineralization was conducted with mild acid treatment (1 M HCl). This procedure is typically carried out to remove the growth catalyst for the study of applications of GCNFs as electrodes, in fuel cells, as well as in other areas. We encountered some difficulty in the use of FLOSS on as-produced and demineralized fibers. It appeared that the GCNFs catalyze the decomposition of dansyl hydrazine to the corresponding sulfonic acid, and that at very low concentrations of dye spectra became more difficult to interpret due to interference from other absorbing species, as seen by the change in the shape of the spectra, for example, the appearance of a peak at 425 nm, in Fig. 5b. FLOSS analysis of demineralized herringbone GCNFs indicated the presence of 0.05% surface aldehyde/ketone groups; the results for as-produced fibers suggest 0.3%. The higher value observed for as-produced fibers suggests that partial purification, specifically, removal of non-crystalline graphite, may be occurring during demineralization. Purification of CNTs by a similar procedure has been reported [38]. Emission spectra for as-produced and demineralized fibers are included in the Supplementary data (Fig. S1), along with calibration data that was used for these experiments. These data reveal the presence of a lower level of aldehyde and ketone groups on the fiber surface. Exposure to air/acid during demineralization is likely to be responsible for surface oxidation at this level. Graphite nanofibers contain crystalline and non-crystalline regions, so it is reasonable that higher levels of non-crystalline graphite present in the as-produced fibers would undergo oxidation more readily, and that partial purification to higher levels of crystalline graphite occurs during the demineralization process.

Fluorescent labeling of carboxyl groups was investigated by two methods in this study. One method was based on TBTU-mediated coupling of dansyl cadaverine 2, which would attach the fluorophore to the surface via an amide linkage. The other was based on Fischer esterification using 2-(dansylamino)ethanol 4. TBTU and related uronium salts are reagents used in the synthesis of peptides and in other cases involving the formation of amide bonds in complex substrates [39,40]. Unlike thionyl chloride activation of carboxyl

groups, TBTU activation was expected to be more mild and selective, with fewer side reactions, such as oxidation at benzylic positions [41] or reaction with phenolic hydroxyl groups [42]. We considered that a TBTU-based method for the attachment of groups to the surface of GCNFs via amide linkages would be valuable not only in FLOSS but also as a means to attach complex ligands such as carbohydrates or peptides to the fiber surface. An abbreviated mechanism for TBTU activation on the fiber surface and coupling to dansyl cadaverine is shown in Fig. 6. In the labeling experiment, nitric acid-oxidized herringbone GCNFs were treated for 1.5 h with TBTU in the presence of triethylamine, then dansyl cadaverine for an additional 1.5 h. Emission spectra for the dansyl cadaverine solutions treated with TBTU-activated fibers are shown in Fig. 7. Emission spectra for dansyl cadaverine at different concentrations and the calibration plot are included in the Supplementary data (Fig. S2).

The decrease in emission intensity observed in the solutions of dansyl cadaverine that were treated with oxidized GCNFs, after activation with TBTU, corresponds to a 0.43 μmol depletion of dansyl cadaverine. From the SSA for nitric acid-oxidized herringbone GNFs, the value of 0.3% is obtained for surface carbon that is present as carboxylic acid groups. The small amount of depletion that is observed in the control that contains fiber, but no TBTU (dark green line) is ascribed to imine formation, and was found to increase if reactions were carried out for longer time periods. From the amount of depletion in this control, imine formation is estimated to account for about 25% of the total depletion of dansyl cadaverine by oxidized GCNFs. Time-dependent depletion for TBTU-activated GCNFs is shown in Fig. S3 (Supplementary data). The depletion that is observed when control solutions of dansyl cadaverine are treated with GCNFs for longer periods of time (72 h) in the absence of TBTU also suggests labeling via imine formation with surface aldehyde/ketone groups. Our experiments with TBTU-mediated labeling reveal two aspects of this chemistry that are worth noting here: (1) selectivity of labeling of carboxyl groups can be achieved if the reaction times are kept to a few hours, and (2) labeling of aldehyde and ketone groups can be carried out with dansyl cadaverine by treatment of the fibers for longer periods in the absence of carboxyl activation. This latter observation may provide the basis for the covalent attachment of amines to the GCNF surface through reductive amination.

In order to investigate physisorption of dansyl cadaverine, oxidized GCNFs that had been activated with TBTU were also

treated with the acetamide derivative **3** of dansyl cadaverine, which is deactivated. The emission spectra from these

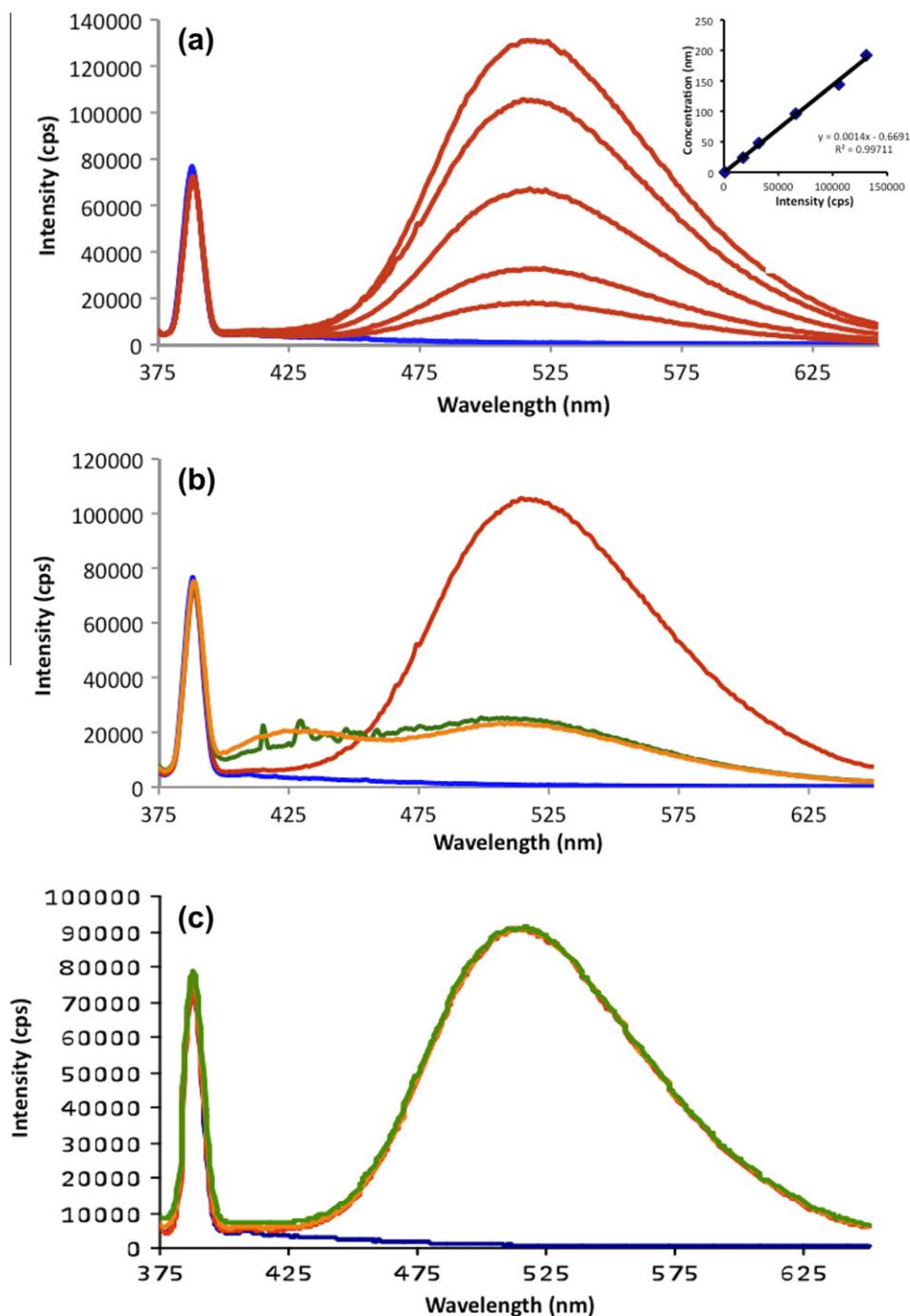


Fig. 5 – Labeling of aldehyde/ketone groups on nitric acid-oxidized GCNFs. (a) Emission spectra of dansyl hydrazine at different concentrations (0, 24, 48, 95, 143, and 190 nM) in acetone and calibration plot (insert) of fluorescence intensity at 514 nm vs. concentration. Excitation at 350 nm. (b) Spectra of dansyl hydrazine treated with oxidized herringbone GCNFs: acetone (blue line), control without fibers (red line), treated with 21.5 mg HNO₃-oxidized GCNFs (green line), treated with 21.6 mg HNO₃-oxidized GCNFs (orange line); (c) Control experiment to determine physisorption of deactivated dansyl hydrazine treated with oxidized herringbone GCNFs: acetone (blue line), control without fibers, treated with 23.5 and 22.4 mg HNO₃-oxidized GCNFs (overlapping red, orange, and green lines); (d) Emission spectra of GCNF-treated solutions of dansyl hydrazine taken at different times: acetone (blue line), control without fibers (red line), treated with 21.4, 20.8, and 20.6 mg HNO₃-oxidized GCNFs for 24 h (orange line), 48 h (green line), and 72 h (black line).

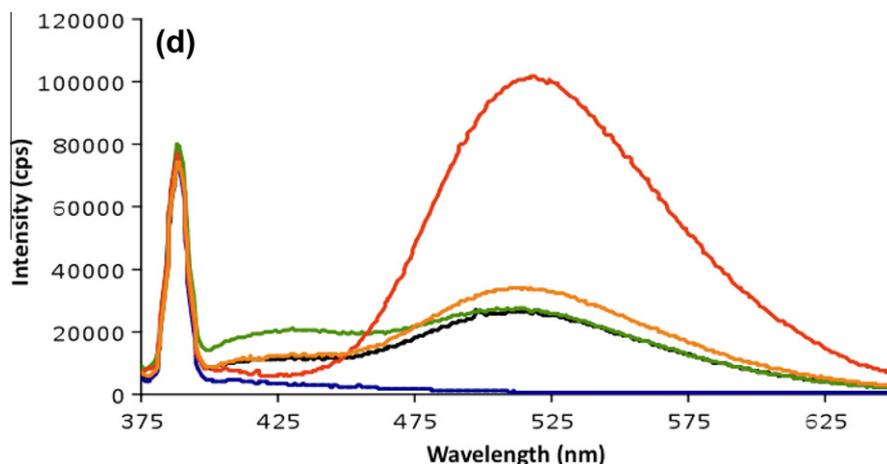


Fig 5. (continued)

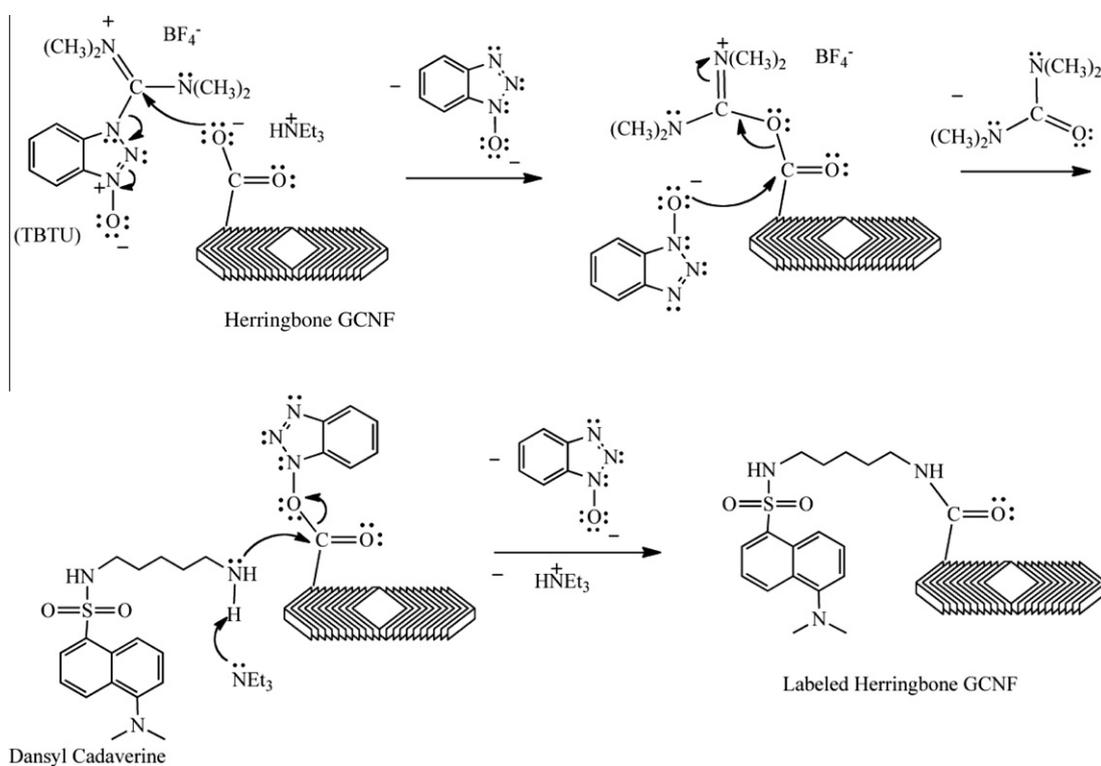


Fig. 6 – Labeling of a surface carboxyl group with TBTU/dansyl cadaverine.

experiments (Fig. S4, Supplementary data) show no depletion of dye relative to the control that does not contain fibers, indicating that binding of dye to the fiber surface through physisorption is negligible. The TBTU-based method for labeling surface carboxylic acid groups on oxidized GCNFs should allow for the functionalization of the fiber surface with a wide range of substrates, since the method involves very mild conditions. Due to the relatively low level of surface carboxyl groups measured using this chemical method in the FLOSS analysis, we decided to examine another technique, Fischer esterification, for labeling these groups.

The labeling of surface carboxyl groups by Fischer esterification (Fig. 8) involves refluxing the fibers with 2-(dansylamino)ethanol 4 for extended periods of time, using a Dean–Stark trap to remove the water that is produced and drive the reaction to completion. In order to avoid the possibility of complications caused by tendency of fibers to adhere to the glass surface above the liquid level, a constant monitoring was maintained to ensure that fibers were in contact with dye solution. Emission spectra for 2-(dansylamino)ethanol are shown along with a calibration plot in Fig. S5a (Supplementary data). The FLOSS spectra obtained by treating nitric

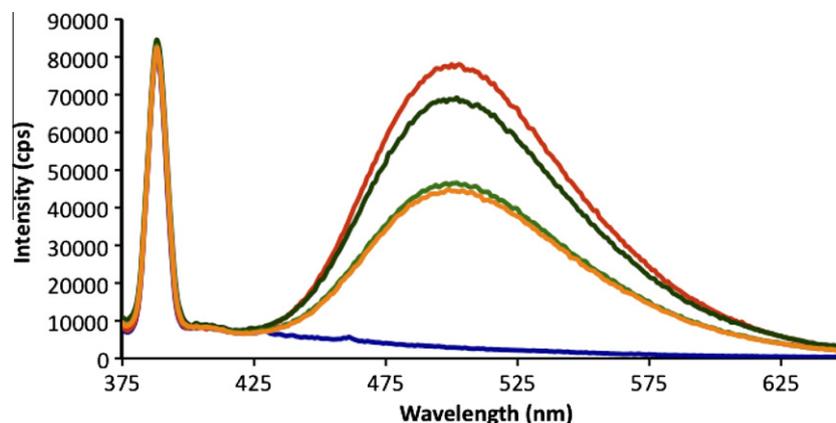


Fig. 7 – Labeling of carboxylic acid groups on nitric acid-oxidized GCNFs with dansyl cadaverine/TBTU: acetone (blue line), control without fibers (red line), control without TBTU (black line), treated with 17.1 mg HNO_3 -oxidized GCNFs (gold line), treated with 17.0 HNO_3 -oxidized GCNFs (green line).

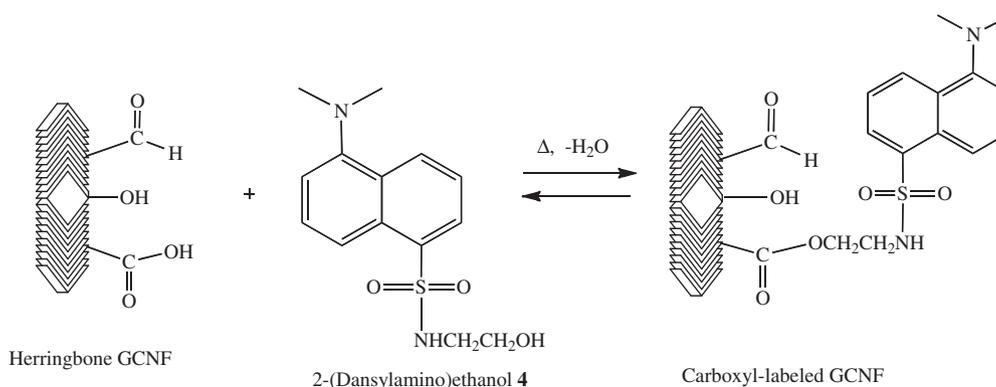


Fig. 8 – Labeling of a surface carboxyl group by Fischer esterification.

acid-oxidized GNFs are shown in Fig. S5b. From these spectra, and additional experiments, an average value of $1.1\% \pm 0.3\%$ carboxylic acid groups was determined via labeling by the Fischer esterification (Table 1).

The higher values of carboxyl group according to the results of Fischer esterification compared to TBTU-activation are not surprising in light of the higher temperatures involved in the esterification and the lack of sterically demanding reagents such as those used in the TBTU-activation. Nitric acid-treated fibers contain enough trace acid so that the addition of an acid catalyst is not necessary. As in previous experiments, we have observed partial degradation of the dansyl fluorophores by hydrolysis to the corresponding sulfonic acids, so additional acid was not used for the esterification. The extent of labeling as a function of time is shown in Fig. S6 (Supplementary data). The extent of depletion observed for reactions run at 48 h and 72 h were identical. In order to test for physisorption of dye during the esterification, compound 5 was synthesized and solutions of it were treated with nitric acid-oxidized herringbone GCNFs under the same esterification conditions. Emission spectra are shown in Fig. S7 (Supplementary data). The absence of a significant decrease in fluorescence intensity of GCNF-treated solutions of 5 is consistent with negligible physisorption of dye on the

fiber surface. The quantification of surface carboxyl groups on nitric acid-oxidized GCNFs by the Fischer esterification indicates a lower presence of these groups than the base uptake method [15]. FLOSS indicates 1.2×10^{-4} mol/g, while the value obtained for the base uptake method is reported as 4.5×10^{-4} mol/g. GCNFs analyzed by the latter procedure were oxidized using concentrated nitric acid instead of 3 M nitric acid. The stronger oxidizing conditions may account for a higher level of carboxyl group functionalization.

The labeling of carboxyl groups by Fischer esterification was attempted on as-produced and demineralized herringbone GCNFs using 2-(dansylamino)ethanol 4. Emission spectra and calibration data for the solutions that were treated with fibers in these FLOSS experiments are shown in Fig. S8 (Supplementary data). As with the attempted labeling of surface aldehyde/ketone groups in demineralized and as-produced fibers with dansyl hydrazine, the decomposition of the dyes has been observed. The absence of the significant depletion suggests that if carboxyl groups are present, they are below the FLOSS detection limit [17] ($\sim 10^{10}$ groups/cm²).

The last oxygen functionalities that we sought to be quantify using FLOSS were hydroxyl groups. Presumably, the nature of the groups is phenolic, and the groups were probably introduced at more reactive edge sites through exfoliation

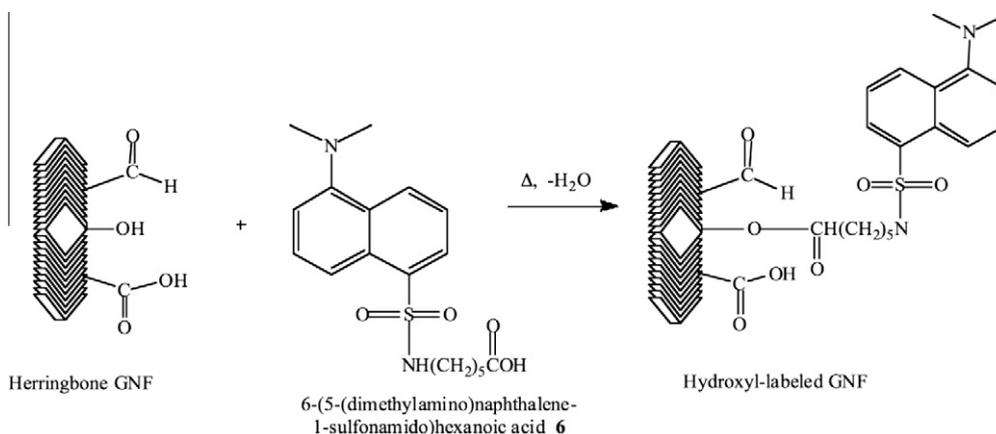


Fig. 9 – Labeling of a surface hydroxyl group by Fischer esterification.

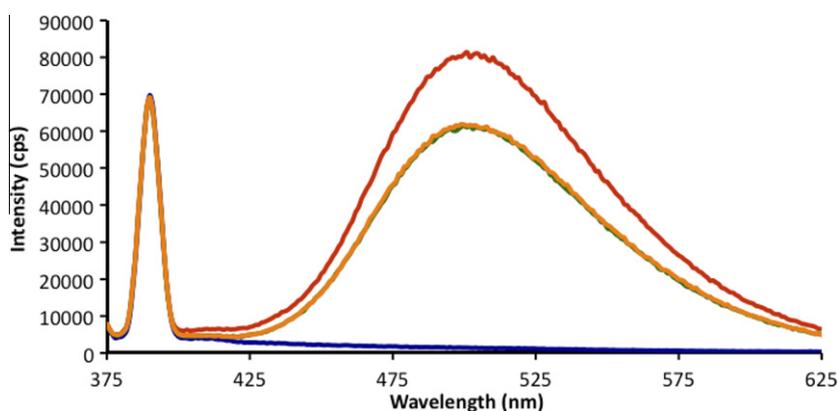


Fig. 10 – Labeling of hydroxyl groups on nitric acid-oxidized GCNFs. Spectra of **6** treated with HNO_3 -oxidized herringbone GCNFs: acetone (blue line), control without fibers (red line), treated with 23.3 mg HNO_3 -oxidized GCNFs (green line), treated with 23.4 mg HNO_3 -oxidized GCNFs (orange line).

during nitric acid oxidation [43]. The results of carboxyl detection by Fischer esterification prompted us to consider a similar approach for the selective labeling of surface hydroxyl groups on GCNFs. Compound **6** was used [34] as a fluorescent probe of glycosidase activity, so we wondered if it would label surface hydroxyl groups on oxidized GCNFs by esterification (Fig. 9).

An advantage of this label is that it is based on the dansyl group, and as mentioned earlier, physisorption would not be expected to be a problem, as has been the case with other dyes used to label hydroxyl groups, for example, the fluorescein derivative 5-DTAF [29]. Emission spectra of **6** at different concentrations and the calibration plot are included in the Supplementary data, Fig. S9. Emission spectra that were obtained by treating solutions of **6** with nitric acid-oxidized GCNFs are shown in Fig. 10. The decrease in emission intensity observed in GCNF-treated solutions of **6** corresponds to a depletion of 0.95 μmol of fluorophore, which corresponds to a surface hydroxyl group concentration of 0.4% observed in each sample.

Results for the fluorescence labeling of functional groups on herringbone GCNFs are summarized in Table 1. The total of the surface oxygen-containing functionality in nitric acid-

oxidized herringbone GCNFs found in this study is on the order of 3%, for aldehyde/ketone, carboxyl, and hydroxyl combined. This is lower than what was observed in the analysis of oxidized SWCNTs. Groups on the surface of CNTs would perhaps be expected to be more reactive toward chemical derivatization in general, due to the sharper curvature of their graphene surfaces, and possible increased steric accessibility, the diameter of GCNFs being much larger. This difference is consistent with the lack of reactivity of GCNFs toward Prato cycloaddition [44], a reaction that has been widely used in the functionalization of CNTs. We attempted this reaction on ribbon GCNFs and found them to be unchanged.

4. Conclusions

We have demonstrated that FLOSS can be used to identify and quantify reactive oxygen-containing functional groups of the surface of oxidized graphitic nanofibers selectively. The detection and quantification of aldehyde/ketone, carboxyl, and hydroxyl groups on GCNFs by a chemoselective method has not been reported previously, and FLOSS has proven to be well-suited to this purpose. FLOSS has also provided

information about the surface functionality of GCNFs that is not available from existing methods, for example, the extent of labeling of carboxyl groups that can be achieved by amide bond formation using TBTU, an activating reagent common in peptide synthesis, and by Fischer esterification with a hydroxyl-functionalized dye.

Covalent functionalization of surface aldehyde/ketone and hydroxyl groups on GCNFs has been demonstrated for the first time using FLOSS. The attachment of the dyes used in the fluorescent labeling occurs by covalent attachment, and not physisorption, as demonstrated by control experiments with deactivated dyes. Different levels of the oxygen functional groups have been observed, with carboxylic acid groups being the most abundant and hydroxyl groups the least. Since both alcohols and aldehydes/ketones may undergo further oxidation to carboxylic acid groups, it is perhaps not surprising that these are the most prevalent groups. The labeling reactions that have been developed and applied successfully to GCNFs in this study provide new methodology for the attachment of complex, chemically labile ligands to the fiber surface. We are particularly interested in the attachment of carbohydrates. Carbohydrate functionalization of CNTs has been used to confer altered solubilities as well as other properties [45,46]. The ability to covalently functionalize GCNFs with carbohydrates, while unexplored, may lead to new applications for GCNFs in biology.

Acknowledgements

The authors thank the Research Corporation, the National Science Foundation (NSF, CHE-0416040) and Villanova University for financial support. This work was supported by NSF while CAB was working at the Foundation. Any opinions, findings, conclusions, or recommendations expressed are those of the authors and do not necessarily reflect the views of NSF.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.carbon.2010.07.035](https://doi.org/10.1016/j.carbon.2010.07.035).

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