Effect of acid oxidation methods on multi-walled carbon nanotubes (MWCNT) for drug delivery application

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Abstract

Acid oxidation has been widely reported to successfully purify, improve dispersibility profile in biological milieu and increase surface area of carbon nanotubes (CNT). Yet, it also frequently damages the nanotubes structure for an effective drug carrier. This study examined the effect of acid oxidation on multi-walled carbon nanotubes (MWCNT) using a mixture of concentrated nitric and sulphuric acid, aided by mechanical force that includes stirring, and sonication at short (2 h) and longer treatment times (6 h). Stirring of MWCNT in acid mixture overnight successfully introduced carboxylic and other oxygen functional moieties. It was referred as oxidized-MWCNT (ox-MWCNT) that renders solubility in water up to 60 days with generation of cut tubes as observed in Field-emission Scanning Electron Microscopy (FESEM). Sonication method increases the amount of oxygen on the nanotubes in Energy dispersive X-ray (EDX) results, however thermogravimetric analysis (TGA) suggested signs of CNT degradation at both treatment times. The FESEM micrograph also showed its tubular structure that was not preserved. Simple mixing by stirring proved to be the best method for this aim.

Keywords: *Cabon nanotubes (CNT), oxidation, acid, drug delivery, stirring, sonication*

1. Introduction

Carbon nanotubes (CNT) are synthetic nanomaterial that is structurally pictured as rolled sheets of graphene rings. The carbon atoms of CNT are linked together by sp^2-hybridized bonds in honeycomb (hexagonal) pattern [38]. This nanomaterial has received considerable attention among researchers due to its ultrahigh surface area ratio with excellent electrical, optical and mechanical properties. Other appealing feature of CNT is its ability to "pierce" through cellular membranes, which could help avoiding the degradative lysosomal pathways that many other nanocarriers failed to circumvent [18,29].

Research efforts that used CNT as drug delivery system (DDS) has started since the last two decades [40]. Numerous investigations are from the oncology field that reported CNT potential as multimodal DDS [9,23]. Apart from allowing Attachment multiple copies of drug molecules on its surface and/or inner cavity, CNT can also be equipped with targeting agents and stealth molecules to avoid clearance by the immune system.

In utilizing the nanomaterial for medical application, it is very important to note that CNT nature property is hydrophobic [40]. They tend to agglomerate and hinder dispersion in solvents. In addition, metal catalyst residues that embedded in the nanotubes, such as ferum and nickel [37] are harmful to human bodies. They could catalyze the oxidative species in cells and tissues by generating free radicals [6]. However, there are also inconsistent reports indicating no signs of CNT toxicity. This issue has been controversial and remained incomprehensible [16]. One way to render this problem is through surface functionalization to make it less toxic. The functionalized CNT had its heavy

metal residual removed, hence reducing its toxic effects at cellular level [34,10].

The underlying mechanism of CNT functionalization often involves selective breaking of C=C bonds by oxidation. Common methods used in oxidation include wet chemical methods [38], photo-oxidation [8], oxygen plasma [5], or gas phase treatment [39]. Wet chemical oxidation method has been recognized as the most efficient method to purify pristine-CNT. Successful oxidation can be characterized by the presence of oxygen containing groups that was found to decorate its graphitic surface, such as carboxyl (-COOH) and hydroxyl (-OH). These groups could be used as subsidiary sites for addition reaction or further functionalization [34].

Although effective, one major drawback of acid oxidation involves nanotube shortening and additional defect generation of the graphitic network [12]. In delivering therapeutic agents, the high aspect ratio of CNT makes it outstanding from other types of round nanoparticles because it allows large quantities of payloads along the longitude of tubes, without affecting their cell penetration capability. Acid oxidation of CNT usually employs high concentration of reagents and aided by heating (refluxing), mechanical forces such as ultrasonication to produce CNT dispersion. However, they frequently lead to the severe cuts of the nanotubes leading to disintegration [43]. Depending on the purpose of nanotubes development, shortening the CNT could limit its potential for excellent DDS as it may not allow adequate load transfer of drug molecules.

This paper demonstrates optimization of acid treatment conditions that could provide sufficient amount of functional groups on nanotubes with minimal structural damage. To modify the CNT surface, we treated pristine MWCNT (p-MWCNT) with concentrated nitric and sulfuric acid mixture followed by stirring and sonication, respectively. The characterization of all MWCNTs were completed by dispersibility test, thermogravimetry analysis (TGA), Fourier transformed infra-red spectroscopy (FTIR), Field Emission Scanning Electron Microscopy (FESEM) and Energy Dispersive X-ray (EDX). With the experimental results obtained, they are useful to provide information on balance between increasing the oxygen-functional groups and destroying the nanotubes for CNT biocompatibility and drug delivery application.

2. Materials and methods

2.1 Materials

MWCNT (outer diameter <8 nm, length ~30 μm, purity > 95%) fabricated by chemical vapor deposition (CVD) was acquired from Timesnano, Chengdu Organics (China). All chemicals were used without further purification. Nitric acid (HNO_3) 65% was acquired from Emsure and sulfuric acid (H_2SO_4) 98% was from J.T Baker Chemical Co. (Phillipsburg, N.J).

2.2 Oxidation of PVA-MWCNT

The liquid oxidation method was performed to purify and initiate the formation of –COOH functionalities on the MWCNT surface following method by Aviles et al. [2] with slight modification. Typically, p-MWCNT (2 g) was mixed by stirring with 400 ml of H_2SO_4/HNO_3 (98%/68%) acid mixture (v/v:3/1) for 24 hours. The MWCNT produced (ox-MWCNT) was thoroughly washed with deionized water to near neutral and filtered using 0.2 μm nylon filter membrane. The ox-MWCNT was dried in an oven at 70°C for 24 hour and labeled as Treatment A. The procedure for Treatment B and C was carried out as in Treatment A, except that the MWCNT was sonicated (100 W, 42 kHz) in the acid mixture for 2 h and 6 h, respectively. All dried ox-MWCNT obtained were finally collected for characterization.

2.3 Characterization of ox-MWCNT

The MWCNT before and after oxidation was examined for its dispersibility to evaluate their stability to form colloidal suspension. In the study, 5 mg of nanotubes was sonicated (ultrasonic bath) in 10 ml deionized water for 10 min. The time required for the CNT to sediment was monitored. TGA analysis of p-MWCNT and ox-MWCNT were performed using a Mettler TA4000 TG Analyzer (Mettler Toledo, Switzerland) from 40 to 850 °C with a heating rate of 10 °C/min under nitrogen. TGA thermogram indicates the weight loss as a result, which enable us to describe carbon decomposition process in MWCNT. In FTIR, MWCNT samples were analyzed using Perkin Elmer Spectrum One, USA. Approximately 0.5 mg sample was transferred into a mortar crucible and mixed with potassium bromide (KBr) powder at a ratio of 1:100 (sample: KBr). The mixture was ground into a fine homogenous powder and pressed into discs. The discs were mounted in the sample holder and analyzed at spectral range of 4000 to 400 cm^{-1}. FESEM analysis was carried out on a JEOL JSM-6701F equipment (Japan). It was used to observe the morphological structure of MWCNTs before and after functionalization, while EDX evaluated the ratio of the elements in the samples.

3. Results and discussion
3.1 Colloidal dispersion

Dispersibility test is among common techniques that provide fast and cheap qualitative information. Fig. 1 depicts the dispersed state of MWCNT samples in water at three different time points: at the moment of dispersion, 30 minutes and 60 days after dispersion. The p-MWCNT sedimented 30 minutes after sonication, while ox-MWCNT in all treatment conditions (Treatment A-C) showed stable colloidal suspension even after 60 days of dispersion.
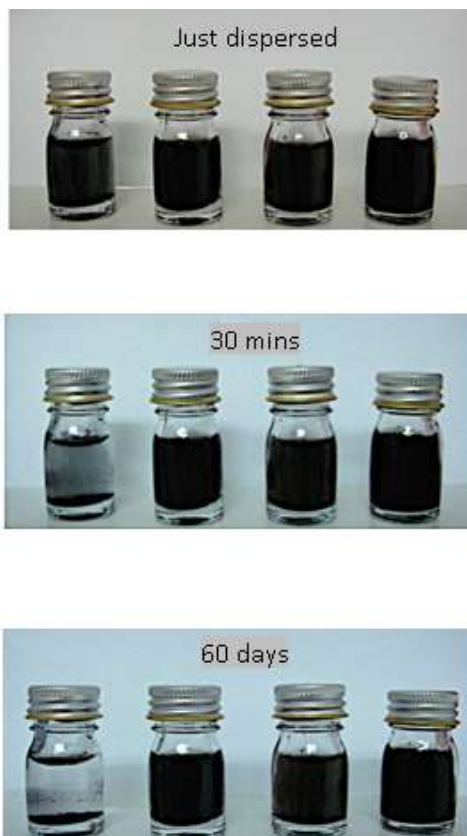

Fig. 1 Colloidal dispersion of pristine-MWCNT and ox-MWCNT (Treatment A-C)

The agglomeration showed by p- MWCNT was due to hydrophobicity of graphene sidewalls and the strong π-π interaction between the individual tubes. Its surface that was unmodified constituted absence or deficiency of functional groups that led to lack of hydrogen bonding with water molecules [31]. As a consequence, the water failed to sustain stable nanotubes suspension.

When the CNT surface suffers oxidation, chemical elements are adsorbed thus forming functional groups. These groups are positive or negatively charged. In this case, hydroxyl (OH-) and carboxylic groups (COO-) are inserted in the nanotubes surface, where they are equally charged. The presence of same-sized charged particles on the surface of CNT enables the tubes to repel to each other to keep the solution dispersed [36, 43, 31]. This showed that both stirring and sonication method are suitable to enhance dispersion of CNT in acid mixture. The results corroborated with a study by Avilés, et al. [2], who used the same acid mixture for dispersion of CNT. Treatment using one type of acid and mild acid concentration can also promote the increase of C=O and OH groups, however it may not be sufficient to completely oxidized the CNT surface.
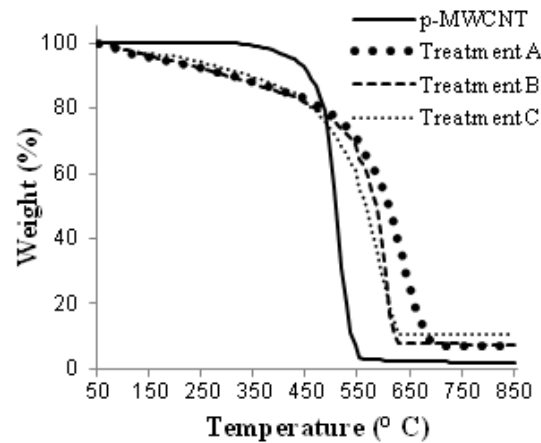

Fig. 2 TGA curves of p-MWCNT and ox-MWCNTs according to treatment method.

3.2 TGA analysis
TGA analysis is very helpful in providing information on its oxidation and possible structural damage [32]. According to Datsyuk and co-workers [4], thermal degradation of MWCNT generally occurs in a multistage process (Table 1). A well graphitized structure as such CNT usually start to oxidize at higher temperature between 600 and 700 °C, depending on type of CNT. It also describes as purer and less defective type of carbon [33,1].

Table 1: Four main stages of MWCNT weight loss according to temperature

Temperature	Stage description on MWCNT
< 150 °C	Evaporation of adsorbed water
150-350 °C	Decarboxylation of carboxylic groups
350-500 °C	Elimination of hydroxyl functional groups
400-500 °C	Degradation of disordered /amorphous carbons, metal impurities

In this study, p-MWCNT showed onset of significant weight loss at around 500 °C (Fig. 2). Irrespective of the treatment, the acid treated samples showed weight losses that were not as severe as p-MWCNT. The onset of the significant weight losses has shifted towards higher temperature (600 °C). Among the three samples, Treatment C
 (sonicated 6 h) showed remarkable different behaviour with a distinctive early weight loss, which indeed represent the harsher treatment examined in this work. The early weight loss indicated the CNT structural damage, and decreased according to Treatment C > Treatment B > Treatment A. The decomposition of p-MWCNT in this experiment that was around 500 °C suggesting the samples may be contaminated with other carbonaceous compounds and impurities during its production. Disordered or amorphous carbon tend to oxidize at around 500 °C due to their lower activation energies for oxidation [4,11]. The p-MWCNT continue to degrade at temperature higher than 500 °C, which was due to thermal oxidation of the remaining disordered carbon [11]. Other observation includes a small weight loss at temperature below 150 °C. This corresponds to the evaporation of moisture contained in the p-MWCNT, as seen in those ox-MWCNT.

Acid treatment purified the nanotubes and substantially introduced oxygenated-functional groups on its surface. This result is in accordance to the dispersion examination results where all of them showed excellent dispersion behavior. TGA curve indicated possible of structural damage in MWCNT Treatment C and B that is greater than that of Treatment A. Both treatments also showed higher percentage of decarboxylation (Table 2), which may

Table 2: Significant increase in oxygen functionalities (%) in all oxidized MWCNT at 151-350 °C and 351-400 °C, which denotes loss of carboxyl and hydroxyl, respectively.

	Weight loss (%)	
	151-350 (°C)	351-400 (°C)
p-MWCNT	1	1
ox-MWCNT (Treatment A)	7.6	2.3
ox-MWCNT (Treatment B)	8.5	2.4
ox-MWCNT (Treatment B)	8.4	2.8

implies to the presence of the small-sized carboxylated carbon with high O/C ratio [3,27]. This

situation is possible as the method could generate considerable amount of cut tubes with increasing –COOH groups.

Consistently, the stirring method (Treatment A) had the highest stability compared to those that were sonicated. It also exhibited absence of disordered carbon. The sonicated MWCNT experienced greater weight loss compared to stirred MWCNT, which occurred during handling (washing, filtering and drying), suggesting CNT degradation. The experimental procedure was repeated at least twice for recovery prior to characterization.

3.3 FTIR characterization

The FTIR analysis was conducted in the spectral range of 4000 -400 cm^{-1} to examine the functional group present on MWCNT before and after acid oxidation. Fig. 3 shows that all ox- MWCNT (Treatment A-C) have four major absorption bands appeared at around 3400, 1720, 1600 and 1200 cm^{-1}. Those spectra are assigned to O-H, C=O, C=C and C-O stretching vibrations [42,17,14].p-MWCNT usually contain intact CNT sidewalls with sp^2-hybridized carbon atoms in hexagonal arrangement [7]. Its conjugated C=C stretching was detected at 1623 cm^{-1} (Fig. 3: p-MWCNT), which explains the condition of nanotubes backbone that remained intact. In addition, stretching bands at 2920 and 2850 cm^{-1} are also detected. They are assigned to asymmetric/symmetric methylene stretching. The results corroborated with findings by [35]. According to the authors, both bands are located at defect sites on the CNT sidewalls. Other distinct detection includes hydroxyl stretching at 3390 cm^{-1}. The OH groups on the p-MWCNT could result from the proprietary production or purification during the manufacturing processes. The existing –OH groups on p-MWCNT could also results from its introduction to ambient atmospheric moisture [2].

The CH$_2$ and CH$_3$ bands were found disappeared in ox-MWCNT (Treatment A), except that the bands are in greater intensity
in sonicated samples. The disappearance of CH$_2$ and CH$_3$ group in Treatment A suggested that the oxidation method had successfully created functional groups through the mechanism of defect-consuming or defect-generation steps [42] This is proven through the presence of carbonyl group at 1714 cm^{-1} at a small intensity. The C=O peak was also observed in Treatment B but at higher intensity, indicating more carbonyl groups decorating the nanotubes surfaces. This observation corroborated by our TGA analysis that the decarboxylation of carboxylic groups was highest in Treatment B. We
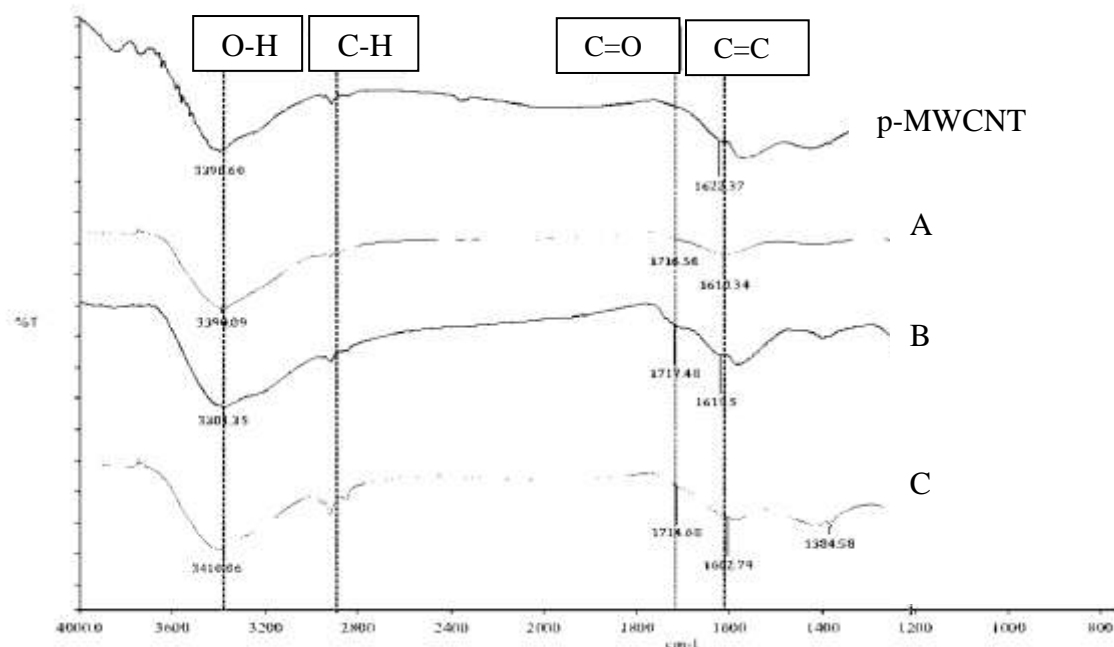
Fig. 3 FTIR spectra of pristine MWCNT and functionalized MWCNT obtained under Treatment A (stirring for 24 h), B (sonicate for 2 h) and C (sonicate for 6 h)

suggested that oxidation process in Treatment B is more successful than stirring, however since the TGA results indicated signs of structural damage, stirring technique has become more favorable for drug delivery vehicle development.

3.4 FESEM and EDX analysis

FESEM analysis was carried out to examine morphological structure of carbon nanotubes before and after functionalization. Fig. 4 shows typical images of ox-MWCNT samples of Treatment A-C in comparison to p-MWCNT. Common observation of p-MWCNT displayed long, fluffy tubular morphology [24]. Several clumps (arrow) that were detected in this study (Fig. 4a) may reflect to the amorphous carbon [13]. Some studies indicated presence of metal catalyst in the CNT bundles too. The catalyst that was employed for the CNT production is mainly metal or metal oxide can be dissolved and oxidized by acids during acid oxidation. However, our EDX result did not find strong detection of any metal impurities Following excessive treatment of the MWCNT with acid, majority of the nanotubes tubular structure were cut and their ends were modified into groovy or twisted surface structure with exposed tips (box) as indicated in Fig. 4b. These tips suggest the rupture of carbon-carbon bond in the CNT graphene, which consequently allowing the generation of functional groups at its open ends [15]. The adequate amount of oxygen-containing group that were introduced on the nanotubes assists in the support of colloidal stability in polar media [26].

Higher extend of damage was observed after MWCNT was oxidized by sonication for 2 and 6 h (Fig. 4c and 4d). The tubular structure of both ox-MWCNT were not preserved and heavily altered, although several other images from different zones were viewed. According to [20,2], sonication and refluxing process are the contributing factors that lead to the CNT destruction. The collapse of cavitation bubbles during sonication produces microscopic domains of high temperature that attacks the surface of CNT, leaving an open hole to the tube side [22].

Although prolonged sonication generates an increase amount of COOH groups on MWCNT, it also causes disintegration into shorter and thinner nanotubes, which ultimately transformed them into amorphous carbon [25]. Such damage could render disruption of the π electron system of the CNT, hence resulting in severe degradation of their charge-carrier mobility as well as their mechanical properties. This hampered the potential of such
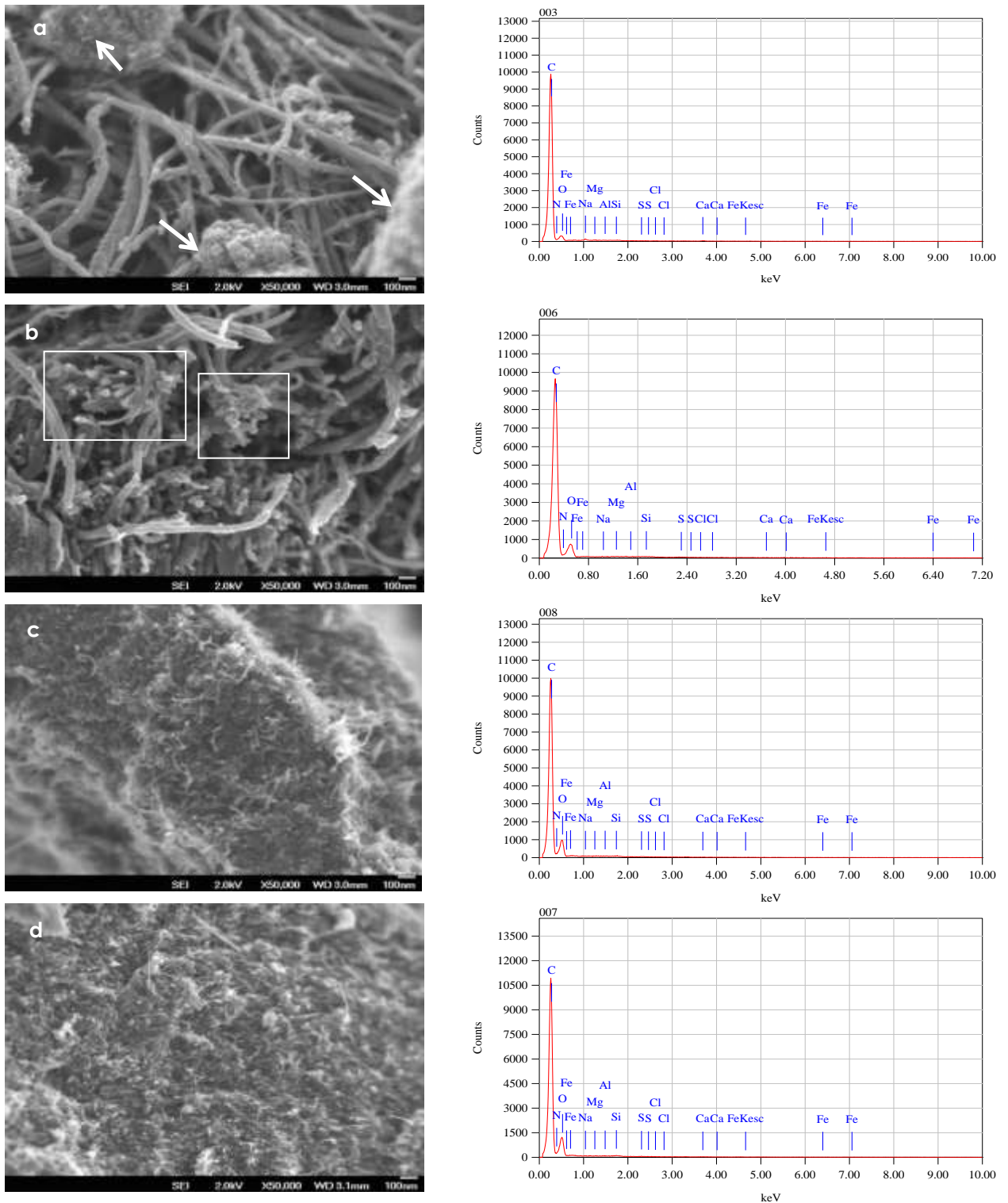
Fig. 4 FESEM and EDX analysis of MWCNT of pristine MWCNT (a), ox-MWCNT Treatment A (b), Treatment B (c) and Treatment C (d)

MWCNT for suitable drug delivery carrier. The above observation is in full agreement with the TGA result where distinctive early weight loss was observed in ox-MWCNT Treatment B and C.

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Energy dispersive X-ray analysis (EDX) is useful to gain information on the elemental composition or chemical characterization of a sample. By far, carbon and oxygen are the main elements that are usually found in MWCNT [41,13,2]. Carbon is the most abundant element, where it could be either graphitic or amorphous. The high carbon content obtained in other studies displayed atomic percentage exceeding 90%, followed by oxygen and traces of metal elements that were incorporated during CNT synthesis. The results obtained by these authors show high similarity with our findings where carbon also appeared at the highest ratio (Table 3).

However, significant amount of nitrogen in p-MWCNT (atomic %) was detected in this study, at a total content of 42%. The nitrogen possibly derived from the CNT

growth using CVD method. During the process, the CNT were produced by passing a hydrocarbon vapor through a tubular reactor, in the presence of a catalyst at sufficiently high temperature (600-1200 °C). CNT grew on the catalyst in the reactor, and they were collected upon cooling the system to room temperature [19]. The pick of hydrocarbon as the CNT precursor is an important factor because its molecular structure has detrimental effect on CNT morphology. Linear hydrocarbons such as methane, ethylene and acetylene thermally decompose into atomic carbon or linear dimers/trimers of carbon, and generally produce straight hollow CNT. Cyclic hydrocarbons such as benzene, xylene and cyclohexane produced curved CNT with tube walls often bridged inside [30,28]. Li et al. [21] for instance, employed imidazole ($C_3H_4N_2$) as hydrocarbon precursor and generate carbon nanotubes with high nitrogen content, which was similar with our findings. Other chemical elements found in p-MWCNT include oxygen content that is less than 0.5% as expected.

Subsequent to the oxidation treatment, the amount of oxygen increased at about 9% in ox-MWCNT Treatment A in comparison to p-MWCNT. A marked increased in oxygen content up to 14 and 15 at. % was observed in Treatment B and C sample respectively, suggesting that oxidation of MWCNT has been promoted more effectively. CNT oxidation is assumed to localize at the tip ends. The use of harsher chemical condition will create an extent of MWCNT surface modification.

4. Conclusion

Many research have been conducted to modify the existing oxidation methods of CNT including the use of harsh chemicals such as H_2SO_4/HNO_3 mixture, hydrogen peroxide and concentrated nitric acid in combination with refluxing and prolonged sonication up to 24 hours. Although these methods were proven to increase biocompatibility of CNT, they often damage its tubular structure. This study purifies and oxidizes MWCNT using common H_2SO_4/HNO_3 mixture under three different techniques that includes stirring and sonication at short and long treatment times. It was found that treatment based on stirring technique (Treatment A) efficiently promote an increased of –OH and C=O groups, as well as facilitated in the removal of disordered carbons. The treatment also preserved most of CNT tubular structure compared to those that were sonicated. With considerable amount of functional groups on ox-MWCNT, this method may be beneficial for anchoring of chemical functionalities such as drug molecules, linkers or polymers on both CNT sidewalls and inner part of the tube for an efficient drug delivery system.

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