IOP Conf. Series: Materials Science and Engineering **316** (2018) 012013 doi:10.1088/1757-899X/316/1/012013

The effect of hydrochloric acid addition to increase carbon nanotubes dispersibility as drug delivery system by covalent functionalization

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Abstract. This study is to obtain the effect of adding hydrochloric acid (HCl) to the mixture of sulfuric acid (H_2SO_4) and HNO_3 in CNT covalent functionalization. HCl expected to increase the dispersibility of functionalized CNT by improving the dispersion time period done with H_2SO_4 or HNO_3. Functionalization used mixture of H_2SO_4 and HNO_3 with volume ratio of 3:1. Covalent functionalization used 0.5 grams of MWCNT ultra sonicated in 50mL HNO_3 with and mixture of H_2SO_4 and HNO_3. Additions of 200 mL HCl used variation of molarity from 1M, 2M, 3M, 4M, 5M to 6M. CNT were oxidized to form carboxylic and hydroxyl bonds on the surface that increase dispersibility of CNT. FTIR spectrums showed the existences of carboxyl and hydroxyl group on spectra of 2600-3700 cm^{-1} and 900-1400 cm^{-1}. Dispersion tests, which showed functionalized CNT (f-CNT) dispersion capabilities, were done by dissolving f-CNT in water. The study resulted that 6M f-CNT (NSC6) gave the best dispersion with zeta potential value of -37.1mV. NSC6 gave the longest dispersion time which was 20 days until f-CNT settle again. SEM-EDS micrographs showed the surface structure of 6M f-CNT without significant damage and no longer contain impurities of Fe, Ni, and Cl.

1. Introduction

Cancer is one major cause of death in the world with approximately 8.2 million deaths in 2012 and grows up each year. Cancer treatments such as surgery, chemotherapy, radiotherapy and drugs still pose problems such as the content of toxic effects harming healthy cells surrounding the cancer cells called metastasis, hair loss and nausea, and lack of targeted specificity where there is an inability of drugs to reach the point of cell in particular cancers [7]. Instead of the primary cancer cells, most patient died because metastasis effect.

Therefore, we need a new technology in cancer drug which is effective and efficient. Drug delivery must be biocompatible, biodegradable, nontoxic, effective, high drug loading capacity, dispersed well, and have affordable price [11]. Carbon nanotubes (CNT) is being investigated as a drug delivery. CNT has unique physical and chemical properties to be a promising candidate for multifunction drug delivery systems [8]. Not only does CNT pave the way for the development of drug delivery agent, it also can be

equipped with a targeting agent and a molecular disguises [9]. Targeting agents will help CNT to be well targeted and molecular disguises will avoid clearance by the immune system that detects foreign substances in the body.

Applying CNT as a drug delivery requires hydrophilic properties and a high dispersibility. Dispersibility is important since it is the key for CNT biocompatibility as a drug delivery [1]. Compatibility is a material ability to adapt to the environment where the material is placed, and cause no harm and no toxic. Biocompatibility of CNT will produce less side effects to the body. Functionalization is necessary to increase the solubility and dispersibility of CNTs in solution, forming CNT that biocompatible and low toxicity [3]. By functionalization, CNT will reach the cancer cells because it can circulate in the blood and does not lead to blockage of the arteries.

Functionalizing CNT can be done by covalent and non-covalent functionalization. Non-covalent functionalization of CNT is poorly suited to be used as a drug delivery as a result of weaker bond between the carbons and accentuate the electronic properties of CNT [7]. Covalent functionalization is preferable since it can improve the dispersibility of CNT, reduce toxicity, and form active sites on CNT so as to bring the drug to be delivered. Covalent modification of CNT ensures the emergence of the hydrophilic nature of the CNT [3]. Covalent functionalization commonly performed is the oxidation of CNT with strong acids. Strong acids will improve the properties of CNT by forming carboxylic groups on the surface of CNTs which will increase the dispersibility of CNTs in solution [6].

Strong acid used is nitric acid (HNO_3), or a mixture of sulfuric acid (H_2SO_4) and HNO_3. Each study of strong acids functionalization successfully produces carboxylic groups on the surface of the CNT. However, the results are not maximized. CNT resulting damage to the surface of CNT wall, and they form aggregates for their salt content makes CNT cannot be directly applied [12]. Other results showed that CNT produced yet stable and perfectly dispersed [10]. Subsequent research began to produce CNT which can be dispersed, but still suffered structural damage to the wall CNT [2].

Chloride acid (HCl) can be another strong acid used to functionalize CNT. HCl is a non-oxidative acid and cannot introduce oxygen-containing groups to CNT wall, but it can enhance the exposure of amorphous carbon. The exposure of amorphous carbon will lead to MWCNT with very low amount of graphitic nanoparticles, bearing higher oxygen existences. The presence of oxygen-containing groups facilitates the exfoliation of CNT bundles, and increases the solubility in polar media. HCl-treated MWCNT provided more available reaction sites, leading to enhanced sidewall functionalization.

This study is to obtain the effect of adding HCl to the mixture of H_2SO_4-HNO_3 which expected to increase the dispersibility of functionalized CNT. The previous literature studies of CNT functionalization have dispersion time less than 20 days. This study will improve the time required for CNT to settle again which demonstrates the CNT's dispersion capabilities. By the high dispersibility, CNT will be easier to circulate, not easily settle, and last longer in blood vessel without causing the blockage.

2. Methodology/Experimental

MWCNTs were functionalized with three different acids.

2.1. Preparation of materials and tools

Pristine MWCNTs were purchased from Chengdu Organic Chemicals Co. Ltd., Sichuan, China. MWCNT has purity >98% with outer diameter 10-20nm and length 10-30μm. MWCNT were placed in 8 different container for each 0.5 grams. Nitric, sulfuric, and chloride acid were purchased from Laboratory of Chemical Engineering, University of Indonesia. In this step, three kinds of solution were made in a room temperature. The solution were HNO_3 6M, 7 mixture solution of H_2SO_4/HNO_3 6M with volume ratio of 3:1 placed in 7 different beaker glasses, and HCl 1M, 2M, 3M, 4M, 5M, and 6M.

2.2. Functionalization of MWCNT

There are eight (8) samples made in the covalent functionalization of MWCNT to get the effect of the addition of different types of acid. First procedure is to immerse MWCNT into H_2SO_4/HNO_3 6M.

MWCNT in acid solution was treated in an ultrasonic bath for 2 hours in 40°C. After being left overnight, HCl 1M was added to the solution. Afterwards, the solution was filtered with 0.45 μm cellulose acetate membrane and neutralized with water until pH 5.5 was reached. MWCNT which have been separated was dried in an oven for 20 minutes in 140°C. The next trials had the same process, however, the addition of HCL were variated into 2M, 3M, 4M, 5M, and 6M. The second procedure had the same process, however, the addition of HCl was eliminated. The last procedure only use HNO_3 6M to functionalize MWCNT in spite of the mixture of acids.

Table 1. Sample number.

Sample Number	Acid Solution	Addition of HCl
NSC1	H_2SO_4/HNO_3	1M
NSC2	H_2SO_4/HNO_3	2M
NSC3	H_2SO_4/HNO_3	3M
NSC4	H_2SO_4/HNO_3	4M
NSC5	H_2SO_4/HNO_3	5M
NSC6	H_2SO_4/HNO_3	6M
NS	H_2SO_4/HNO_3	-
N	HNO_3	-

2.3. Characterization of MWCNT

2.3.1. FTIR spectroscopy. Fourier Transform Infrared spectroscopy (FTIR) data was obtained using Thermo Scientific iS5 Spectrometer. FTIR spectroscopy has been widely used in the determination of functional groups. There are 25 samples, including pristine MWCNT, 24 samples from 8 samples with each of 3 different stages that will be observed that the stages of ultrasonication, filtration and drying. KBr with 0.5% by weight of MWCNT of each samples were prepared for FTIR spectroscopy. MWCNTs were mechanically mixed to the KBr powder and pressed into discs shape. FTIR was used to analyse the changes of surface chemical bonding and structure in the frequency range of 4000–500 cm^{-1}.

2.3.2. Dispersion test. Dispersion test was performed to compare the stability of the suspension of MWCNT before and after functionalization. 4 milligrams of each sample MWCNT was dispersed in 10 ml of demineralized water with sonication for 5 minutes. These solutions were observed and recorded the time MWCNT takes to settle again.

2.3.3. Zeta potential. Zeta potential was done to prove the dispersion test results quantitatively. Zeta potential characterization was determining the accumulation of electric charge on CNT surface. Electric charge that shown was representation of dispersibility stabilization of CNT. 8 milligrams of each sample MWCNT was dispersed in 20ml of demineralized water with sonication for 5 minutes. These solution were put on the Malvern Zetasizer 4 to measure zeta value.

2.3.4. Scanning Electron Microscopy- Energy Dispersive Spectroscopy. Scanning Electron Microscope (SEM) with high resolution is a powerful tool for imaging the fine structure of materials and nanoparticles created by nanotechnology. Characterization of the nanotube composition was done using energy dispersive x-ray spectroscopy (EDS). MWCNT observations and their morphological analysis used SEM-EDS JEOL, JSM 6510 LA. Images were recorded by placing a drop of the samples dispersed in isopropanol on copper grids coated with carbon.

3. Results and discussion

3.1. FTIR spectroscopy

The presence of carboxyl and hydroxyl functional groups on the functionalized CNT (f-CNT) surface can be identified by different characterization. FTIR was conducted and analysed on the phase of ultrasonication in which influence the success of functionalization. FTIR spectra can complement the dispersion time data and identify chemical composition of pristine and f-CNT. The comparison of pristine and f-CNT spectra after treatment for each samples were showed on Figure 1 in the range of 500 to 4000 cm^{-1}. FTIR spectra from the pristine MWCNTs show a peak at 2068 and 2346 cm^{-1} which refers to the backbone of pristine MWCNT. Small peak at 1589 cm^{-1} on pristine MWCNT may refers to the C=O stretch of carboxyl group which can be created during purification process by the manufacturer.

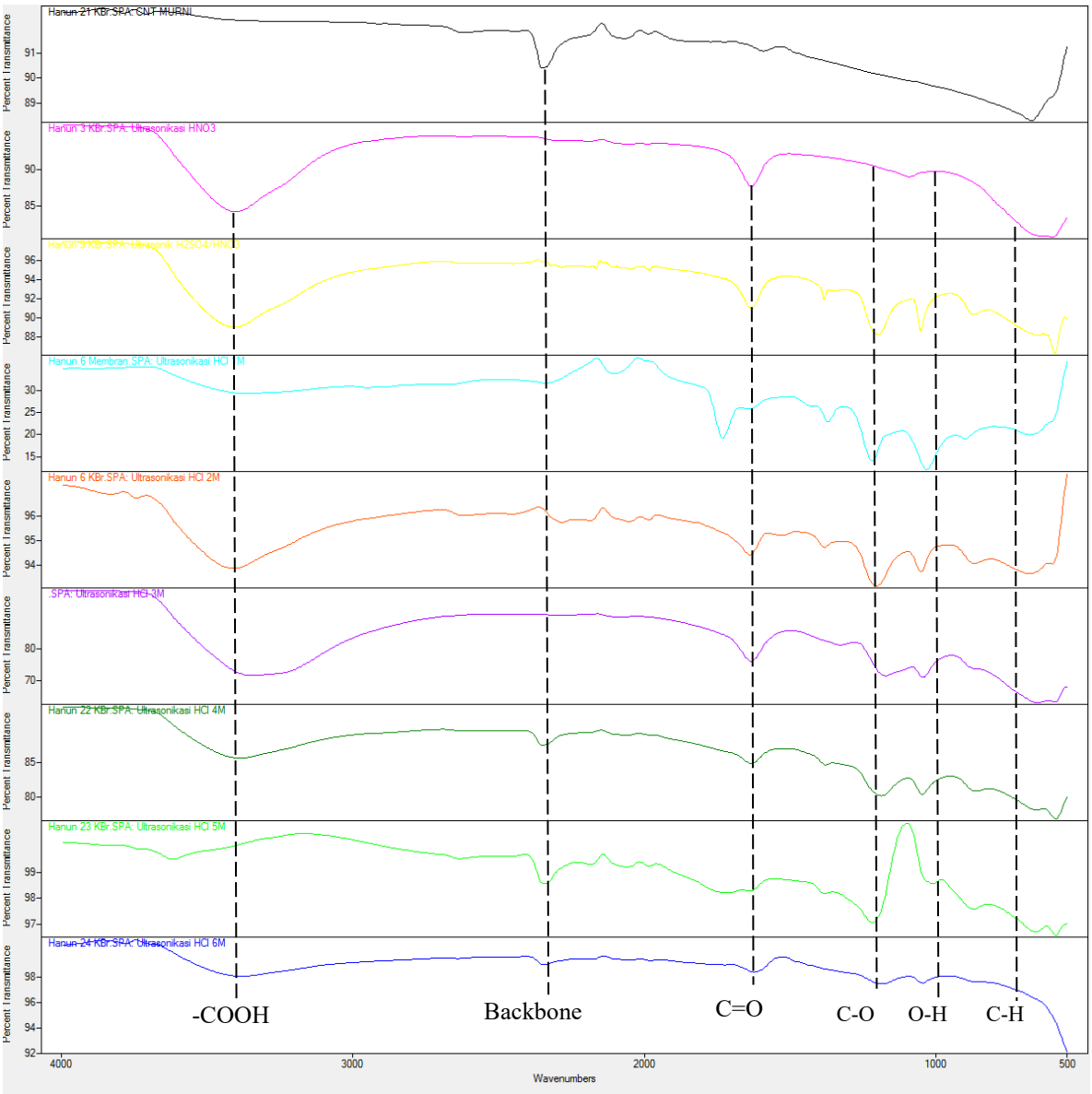

Figure 1. Comparison of FTIR spectra of pristine MWCNT and treated MWCNT.

Most of samples have been inserted carboxyl and hydroxyl groups successfully. Peaks between 2600 and 3700 cm-^{1} are the characteristic of C-H vibrations and O–H bonds. These peaks can be related to carboxylic groups (-COOH). The strong peak of MWCNT backbone in 2346 cm^{-1} reduces in

functionalized MWCNTs spectra. It means, the structure of carbon bonds has been broken and added with new functional groups. However, backbone spectra should be existed to make sure that MWCNT has no fatal damage after functionalization.

Peaks between 1680-1800 cm-1 are the characteristics of C=O bond type from carbonyl and carboxyl groups. Peaks between 1000 and 1250 cm-1 are the characteristics of C-O bond type from alcohol, ether, and carboxylic acids functional groups. Peaks between 900 and 1400 cm^{-1} are the characteristics of O-H bond type from hydroxyl groups. Peaks between 680 and 880 cm^{-1} are the characteristics of C-H bending bond. From FTIR characterization, it can conclude that samples with addition of HCl of 2M, 5M, and 6M are three samples with the highest amount of functional groups.

3.2. Dispersion test

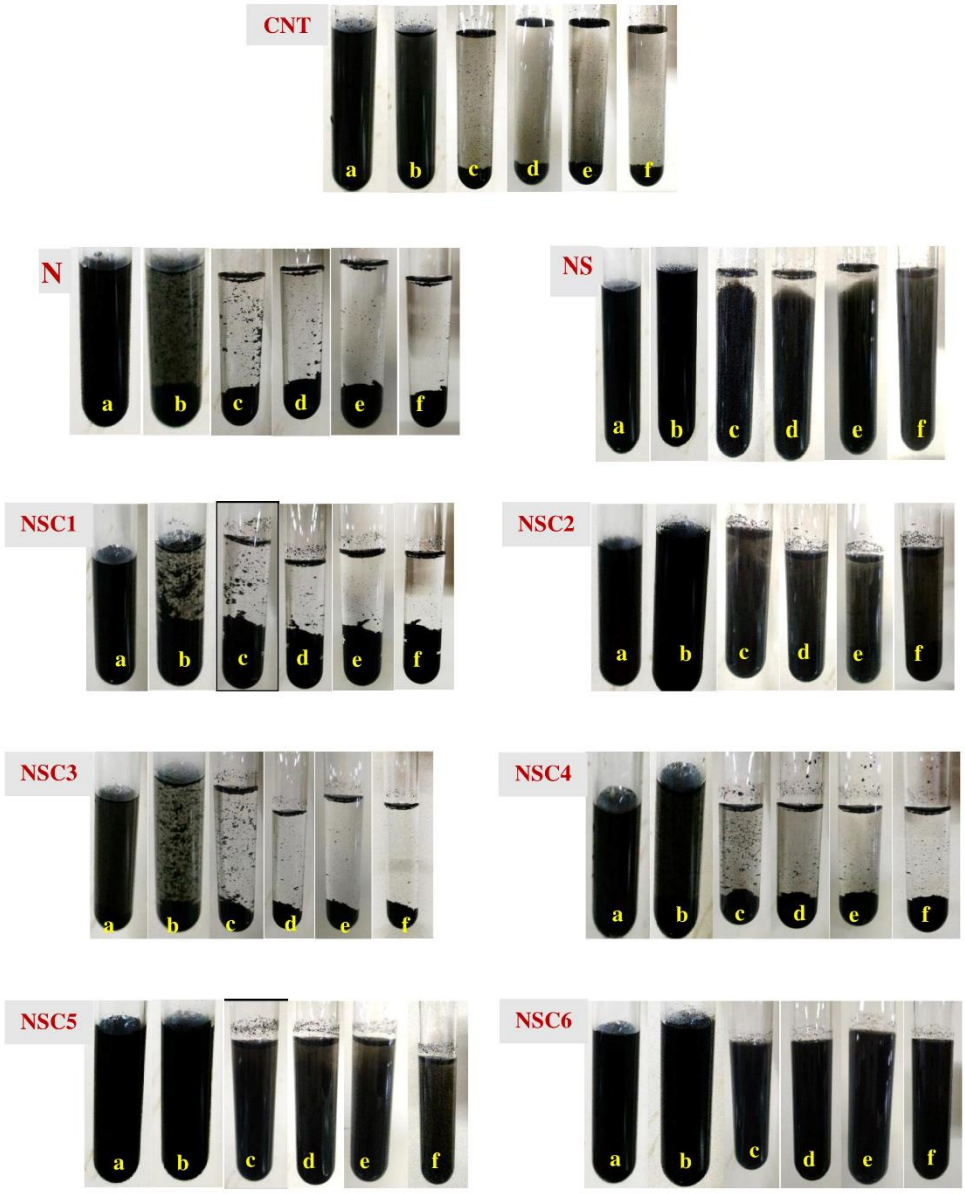

Figure 2. Dispersion test of samples: (a)after sonication, (b)day 1, (c)day 2, (d)day 3, (e)day 4, and (f)day 6

Dispersion test is occurred to identify the presence of hydrophilic functional groups on f-CNT surface and make sure that functionalization is succeed. The time required for CNT to settle in a polar solvent show how effective the functionalization works. This method is fast, cheap, and provide qualitative information. Water is chosen because it represents blood human well since blood contains 95% of water. F-CNT were upheld for several days after being sonicated in water for 5mins. Figure 2 shows photographs of pristine and f-CNT in water 144 hours (6days) after sonicated in water.

When f-CNT suffers oxidation, chemical elements are adsorbed to form functional groups. The functional groups will positive or negatively charged to keep the solution dispersed in water. From the observation, all samples can be dispersed in water. These show that functionalization are successfully occurs. However, the time f-CNT's need to settle are different for each sample. Some samples with addition of HCl still remained as stable suspension in water for more than 6 days compared to pristine CNT which only can settle for several hours. Acid solution without HCl addition only disperse less than 2 days. The settle of f-CNT may be caused by the agglomeration and lack of hydrogen bonding. In other way, stable dissolved f-CNT proof the presence more functional groups of carboxyl and hydroxyl on MWCNT surface. This fact leads to reduction of Van der Waals interactions among carbon bonds and increase the interactions of carboxylic group and water. The reduction of Van der Waals interactions can lead to increase the viscosity of the solution. Thus, the solution dispersity will last longer.

Variation of HCl molarity added are based on the amount of HCl moles contained on the solution. From dispersion test, best addition of HCl are NSC2 (2M), NSC5 (5M), and NSC6 (6M). Those sample support the results of FTIR spectra data with biggest amount of functional groups adsorbed will more stable in water. Furthermore, only NSC6 (6M) can disperse well for 20 days as shown in Figure 3. This result proof that the more amount of HCl moles possibly add the more hydrophilic functional groups into CNT surface and increase their dispersibility.

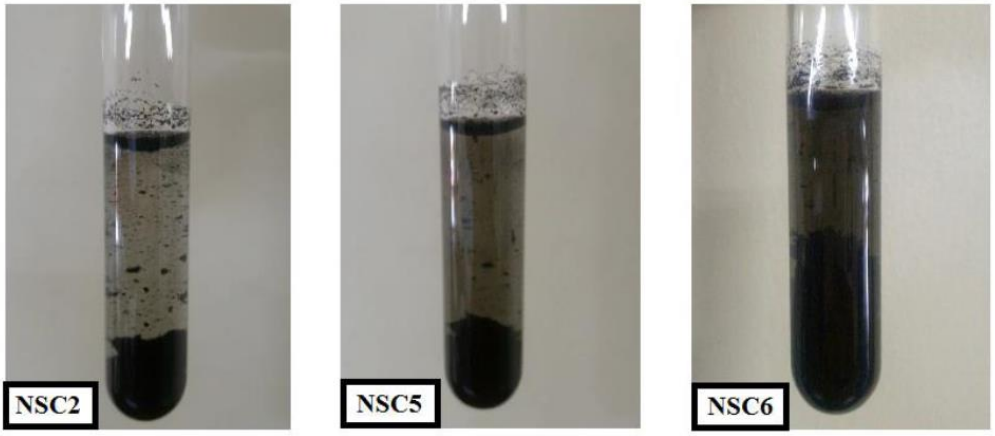

Figure 3. Dispersion test result of NSC2, NSC5, and NSC6 after 20 days

3.3. Dispersion test

High zeta potential values (negative or positive) prevent agglomeration of nanoparticles due to counteracting forces and electrical stabilization of nanoparticle dispersions. The potential zeta value and CNT dispersibility capability are considered as stable when the zeta value is above + 30mV or below -30 mV. If the zeta value is get near to zero, there will be a greater attraction than the repulsive force that causing agglomeration.

Table 2. Zeta potential result.

Sample Name	Zeta Potential (mV)
Pristine CNT	-16.8
NSC5	-21.9
NSC6	-37.1

Based on Table 2, NSC5 zeta value is -21.9mV and considered as unstable state since it is above -30mV. NSC6 shows zeta value -37.1mV. NSC6 value is considered as stable state. This results proofed that NSC6 has greater repulsive force, prevent agglomeration of particle, and gives the best dispersibility.

3.4. Scanning electron microscopy-energy dispersive spectroscopy

SEM was conducted to detect surface morphological changes shown on Figure 4a is pristine CNT and Figure 4b and 4c is chloride-sulphate-nitrate functionalized MWCNT. Figure 4a shows almost typical SEM images of the pristine CNT as well as those treated by HCl, HNO_3, and H_2SO_4. It means, the functionalization with 3 kinds of acid as oxidizing agents do not cause structural damage of the MWCNT. The etching of surface is due to the disordered sites which bonding of carbon and carbon have been replaced with others functional groups, carboxyl and hydroxyl groups (O=C-OH and C-OH). It is possibly because of the mild condition of acid which have been diluted before being immersed with CNT. Based on Figure 4, NSC6 shows clearer image than NSC5 that indicate few impurities (also showed with arrows). This result also supported by high zeta value of NSC6 has high dispersibility, less defects and impurities on surface.

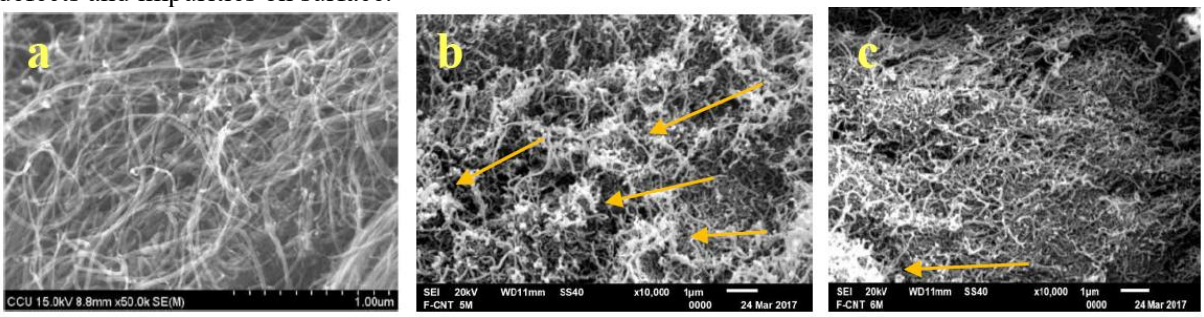

Figure 4. SEM images of CNTs: (a) pristine CNT, (b) NSC5 f-CNT, and (c) NSC6 f-CNT; with arrows show impurities

Table 3 supports that NSC5 have more impurities of Cu and Au than NSC6. Cu is impurity may come from catalyst used on manufacturing or impurity from tube used in EDS characterization. Au detected as the coating of CNT while doing EDS characterization. Both NSC5 and NSC6 have no Fe detected that can be said that both samples are considered as safe [4]. NSC6 gives more functional groups and few impurities because NSC6 have more moles in the solution of HCl. This fact in line with the result of FTIR and dispersion test that show NSC6 have the best dispersibility among others.

Table 3. EDS result of pristine CNT and f-CNT

Sample	Contents (%)						
	C	Cu	Au	Fe	Ni	Cl	O
Pristine	98.39	-	-	0.23	0.93	0.45	-
NSC5	95.06	1.83	3.11	-	-	-	-
NSC6	91.31	1.31	2.61	-	-	-	2.4

4. Conclusion

Additional of HCl to HNO_3 and H_2SO_4 solution successfully increase the dispersibility of CNT. HCl addition of 6M (NSC6) has the best dispersion in water with dispersion time improved from 1 day to 20 days. Sample NSC6 has the best dispersion with the least surface damage and no impurities. This proofed that the more amount of HCl moles clean more impurities and add more hydrophilic functional groups into CNT surface. Sample NSC6 can be implemented further to be drug delivery because it has

qualified the requirement of drug delivery to have high dispersibility in water. The next requirement to be fulfil is the biocompatibility and toxicity of CNT in human body.

Acknowledgement

The author would like to thank The Directorate General of Higher Education, Ministry of National Education Indonesia by HIBAH Fundamental 2017 and Ministry of Research, Technology and Higher Education Republic of Indonesia by PKMP 2017. Thanks to Sustainable Energy Laboratory, Chemical Engineering Department, and Research Center for Biomedical Engineering University of Indonesia for the valuable cooperation.

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