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Aspirin adsorption on multiwalled carbon nanotubes and its release characteristics in simulated body fluid

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Abstract

Carbon nanotubes (CNTs) have been proposed and actively explored as innovative carriers for drug delivery applications. The functionalization of carbon nanotubes can improve substantially their dispersability and biocompatibility profile, thus offering the potential exploitation of carbon nanotubes in drug administration. In this study, the multiwalled carbon nanotubes (MWCNTs) were functionalized using concentrated nitric acid (68%) and sulfuric acid (98%) in the ratio of 3:1 volume per volume by sonication techniques and were characterized using various characterization instruments. The generation of carboxylic groups was proven by FT-IR spectroscopy. In addition, the loading of aspirin (ASA) drug onto functionalized MWCNTs was done by sonication technique at different concentration of ASA drug solutions and at different sonication time. The presence of ASA attached onto functionalized MWCNTs was determined by FT-IR spectroscopy. The results from UV-Vis Spectrophotometer gave the percentage of ASA loading onto functionalized MWCNTs with the amount of 77.64% (77.64 mg/ 100 mg) at 30 000 ppm for 5 hours sonication. From the release study of ASA in Simulated Body Fluid (SBF) at 37^{o}C (human body temperature) and 39^{o}C (fever temperature), the results show a fast release of ASA from ASA-*f*-MWCNTs composite for the first 5 hours compared to the commercialized aspirin tablets and followed by a controlled release at the following hours. It reveals that MWCNTs have the ability to improve the pharmacokinetics of ASA in the biomedical applications, thus it can be used to improve the drug delivery system.

Keywords: Multiwalled carbon nanotubes, functionalized, drug loading, drug released, aspirin.

1. Introduction

Recently, new approaches are emerging in the field of drug delivery, mainly due to the significant advances in nanotechnology such as liposomes, nanocapsules, nanoemulsions, dendrimers, solid lipid nanoparticles, polymeric nanoparticles, ceramic nanoparticles and carbon nanotubes (CNTs) [1]. CNTs are tubular objects with a high aspect ratio and a diameter in the nanoscale range. It can be classified by their structure into two main types: (i) single-walled carbon nanotubes (SWCNTs), which consist of a single layer of graphene sheet seamlessly rolled into a cylindrical tube, and (ii) multiwalled carbon nanotubes (MWCNTs), which comprise multiple layers of concentric cylinders with the

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space of about 0.34 nm between the adjacent layers [2]. CNTs have very interesting physicochemical properties such as ultralight weight, high mechanical strength, high electrical conductivity, high thermal conductivity, metallic or semi-metallic behavior and high surface area. The combinations of these characteristics make CNTs a unique material with the potential for diverse application, including biomedical [3].

Aspirin or also known as acetylsalicylic acid is one of the most commonly NSAIDs used drugs and billions of tablets are produced every year. Aspirin frequently used in the treatment of mild to moderate pain, including migraine and fever [4]. However, due to administration of free drugs, such as limited solubility, poor bio distribution, lack of selectivity, unfavorable pharmacokinetics, and healthy tissue damage, a drug will be difficult to deliver to the targeted cell. A drug delivery system will be used to overcome this problem and it is generally designed to improve the pharmacological and therapeutic profile of a drug molecule [5].

According to Sekhar *et al.* [6], a drug with poor solubility can be replaced with a drug delivery system where both hydrophilic and hydrophobic environments exist and thereby increase the solubility. A drug may also cause damage to the non-infected tissue. Therefore, with targeted drug delivery, regulated drug release can eliminate the problem. Besides that, when a drug is release fast and not retain in the infected human body even before the body could assimilate it, it can cause the patient to use higher doses to make up the bio-availability. However, with targeted drug delivery system, elimination can be avoided by altering the pharmacokinetics of the drug. Other than that, poor bio-distribution is a problem that can affect normal tissues (non target tissues) adversely through unwanted widespread distribution. The particulates from targeted drug delivery systems reduce the rate of distribution and reduce the effect on non-target tissue thereby drastically reducing the side effects. Therefore, CNTs have been proposed as multipurpose innovative carries for drug delivery applications [7].

The pristine CNTs (non-functionalized) are inherently hydrophobic, therefore the main obstacle in the utilization of CNTs in biology and medical chemistry is their poor dispersion in most solvents. To overcome this problem the surface modification of the CNTs (functionalization) with different molecules is achieved by adsorption, electrostatic interaction or covalent bonding of different molecules and chemistries that render them to be hydrophilic. Through such modification, the water solubility of CNTs is improved and their biocompatibility profile completely transformed [4]. Functional groups including carboxylates, can be further modified with therapeutic agents to create CNTs conjugates to endowed with some kind of pharmacological activities. CNTs are able to carry one or more therapeutic moieties with optical or other probes for imaging. Thus, specific recognition systems for targeting, can offer multimodal options in the treatment of where activity is required only at specific sites in the human body [2].

Potential of aspirin drug loaded onto MWCNTs and release characteristics of aspirin loaded MWCNTs composites has not been much investigated. In this work, we have compared the potential of aspirin drug loaded onto functionalized and non-functionalized MWCNTs. The authors also highlight the release characteristics of aspirin drug from MWCNTs *in vitro* at human body temperature (37^{o}C) and fever temperature (39^{o}C).

2. Experimental

2.1 Materials

Commercialized MWCNTs were purchased from Sun Nanotech Co. Ltd with 10-30 nm diameters and more than 90% purity. Mixture of nitric acid (65%) and sulfuric acids (96%) were used for acid treatment of MWCNTs. While acetylsalicylic acid (ASA) was purchased from Sigma-Aldrich, lnc and aspirin tablets were purchased from Bayer Corp. which contains 500 mg of acetylsalicylic acids as one of the active ingredients.

2.2 Functionalization of MWCNTs using Liquid Phase Oxidation Method

200 mg of pristine MWCNTs were treated with 100 ml of aqueous acid solution H_2SO_4/HNO_3 mixtures in the ratio of 3:l by volume per volume. Mixture of MWCNTs and acid solutions were ultrasonically vibrated in water bath ultrasonic Branson 2000. The suspension was separated by centrifuge process and subsequently filtered through nylon membrane filter (pore size: 0.2 pm). The residue was washed with distilled water during filtration until it is at pH 7. Finally, functionalized MWCNTs were heated in an oven for 24 hours at 60^oC to remove all remains of water.

2.3 Drug Loading onto Functionalized MWCNTs

The functionalized MWCNTs were suspended in a 50 ml solution of aspirin in sodium hydroxide. This mixture was sonicated at 5 hours sonication time with 30 000 ppm of acetyl salicylic acid and left for 24 hours at room temperature (25^oC). Then, the solution was centrifuged for 20 min at 4000 rpm to separate the CNTs. CNTs were subsequently left to dry at ambient temperature.

2.4 Characterizations

Several techniques were used to characterize the non-functionalized and functionalized MWCNTs and ASA-MWCNTs composite (ASA loading onto MWCNTs). FT-IR was used to determine the functional groups generated on the surface of MWCNTs and detection of aspirin absorbed into the MWCNTs. Characterization of MWCNTs was carried out using Perkin Elmer Spectrum^{TM} 400 FT-IR spectrophotometer. Field Emission Scanning Electron Microscope (FESEM), model JSM-6701F, JEOL Company was used to observe the morphology structure of MWCNTs before and after functionalization of MWCNTs and the morphology of MWCNTs after ASA was loaded onto MWCNTs. Energy Dispersive X-Ray Analysis (EDX), model EX-230**BU, JEOL Company Japan was used to observe the elemental analysis of MWCNTs possibly found on the structure of functionalized MWCNTs.

The amount of ASA loaded onto MWCNTs was determined by using UV-Vis Spectrophotometer. Concentrations of ASA were determined by using Beer's Law with the excitation wavelength for ASA of approximately 290 nm [8] and each sample were recorded at the same wavelength.

2.5 *In Vitro* Released Study

Simulated body fluid (SBF, pH=7.4) was used to investigate drug release process using prepared simulated body fluid. Simulated body fluid (SBF) has a composition very similar to the human plasma (ppm: 142.0/5.0/2.5/1.5/147.8/4.2/1.0/0.5 $Na^+/K^+/Ca^{2+}/Mg^{2+}/Cl^-/HCO_3^-/HPO_4^{2-}/SO_4^{2-}$). The release of drugs was done by soaking 20 mg of ASA-*f*-MWCNTs composites (ASA loading onto functionalized MWCNTs) into 350 ml SBF at 37°C (normal human body temperature) as well as 39°C (fever temperature) before SBF was taken out at a predetermined time interval. The samples were analyzed using UV-Vis spectrophotometer to determine the concentration of aspirin released from ASA-*f*-MWCNTs composites.

3. Results and Discussion

3.1 Functionalization of MWCNTs

The FT-IR spectra (Fig. 1 (a) & (b)) show the comparison of the absorptions bands before and after the functionalization of the MWCNTs. From FT-IR spectrum on non-functionalized MWCNTs, a single peak was observed at 1636 cm^{-1} which is associated to the C=C stretching of carbon nanotubes backbone. After MWCNTs were treated with H_2SO_4/HNO_3 mixture, peaks appeared on functionalized MWCNTs as around 1700 cm^{-1} associated with C=O stretching vibration of carboxyl groups. With the presence of C=O stretching, C-O stretching and O-H bending deformation peaks revealed that carboxylic groups have been introduced onto the functionalized MWCNTs.

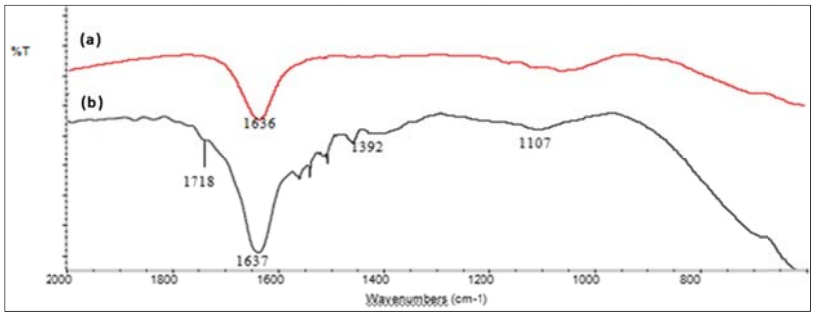

Fig. 1: FT-IR spectra of MWCNTs (a) before functionalization, and (b) after functionalization.

Fig. 2(a) shows the micrograph of non-functionalized MWCNTs before acid treatments. Non-functionalized MWCNTs showed the presence of a smooth surface and loosely packed bundles of MWCNTs arrangement as reported in literature. After the functionalization process, the smooth surface of the MWCNTs was transformed to groovy surface (Fig. 2(b)). The rough and groovy surface of the sidewall of acid treated MWCNTs was possibly associated to the functional groups chemically attached onto the MWCNTs surface [9]. Hence, the MWCNTs floss was loosely entangled due to the functionalization process as reported by Kumar *et al.* [10]. After the acid oxidation, some of the MWCNTs tips were exposed, which indicated that the breaking of the C-C bond along the graphene layers of the co-axial tubes. Thus, allowing the generation of functional groups at the open ends of the MWCNTs [11].

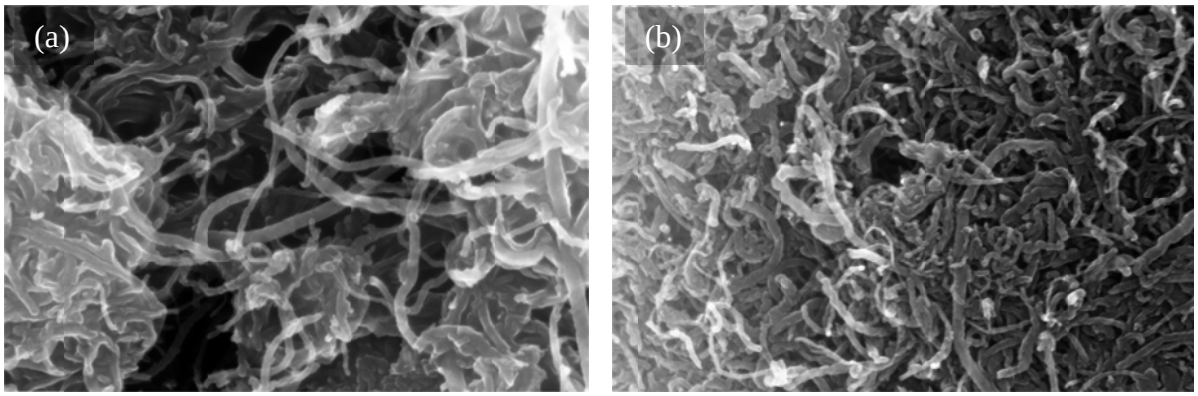

Fig. 2: Micrograph FESEM for (a) non-functionalized MWCNTs before acid treatment, (b) after acid treatment (functionalized MWCNTs).

EDX results (Table 1) shows changes in the elemental composition of the MWCNTs before and after the acid treatments with mixtures. For the non-functionalized MWCNTs, besides the presence of carbon and oxygen, a small amount of impurities such as aluminum was found in the EDX analyses. After the functionalization with mixture of acids, aluminum was not found in the functionalized MWCNTs. It shows that functionalization can eliminate the impurities such as aluminum from the MWCNTs [l 2].

Table 1: Elemental percentage of non-functionalized and functionalized MWCNTs using EDX technique

MWCNTs	**Weight Elements (%)**		
	Carbon (C)	**Oxygen (O_2)**	**Aluminum (Al)**
Non-functionalized MWCNTs	94.63	5.19	0.17
Functionalized MWCNTs	88.91	11.09	

3.2 Drug Loading Analysis of Aspirin Loaded MWCNTs (ASA-MWCNTs) Composites

The FT-IR spectra (Fig. 3 (a), (b) and (c)) show the comparison of the absorptions bands of ASA, ASA-*nf*-MWCNTs composites and ASA-*f*-MWCNTs composites respectively. The significant peaks observed for ASA before loading onto MWCNTs are at 1658 cm^{-1}, ll88-l248 cm^{-1},1578-1658 cm^{-1}, 964 cm^{-1}, 893 cm^{-1}, 759 cm^{-1} which correspond to the C=O stretch, C-O stretch, C-C, C=C bend, aromatic O-H stretch and *o*-benzene, respectively. From the FT-IR spectra, the appearance of the ASA peaks in the ASA-*f*-MWCNTs composites and ASA-*nf*-MWCNTs composites have proven that the ASA loading was successful.

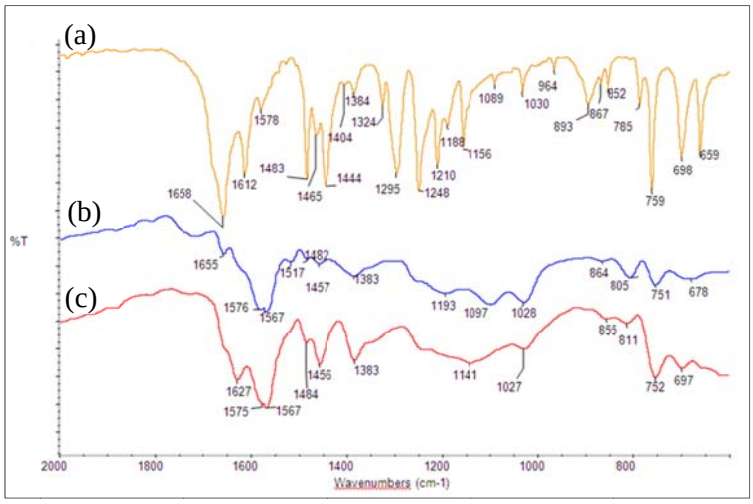

Fig. 3: FT-IR spectra of (a) ASA, (b) ASA-*nf*-MWCNTs composites, and (c) ASA-*f*-MWCNTs composites.

Fig. 4(a) show the micrograph of ASA loaded onto functionalized MWCNTs. There are no differences in the morphology observed for both structures before (Fig. 2(b)) and after drug loading, as they appear the same. The functionalized MWCNTs micrograph after ASA loaded is shown in Fig. 4(b). From the micrograph, it can be observed that the smooth surface of the non-functionalized MWCNTs Fig. 2(a) has been altered into rough and groovy surface of MWCNTs (Fig. 4(b)).

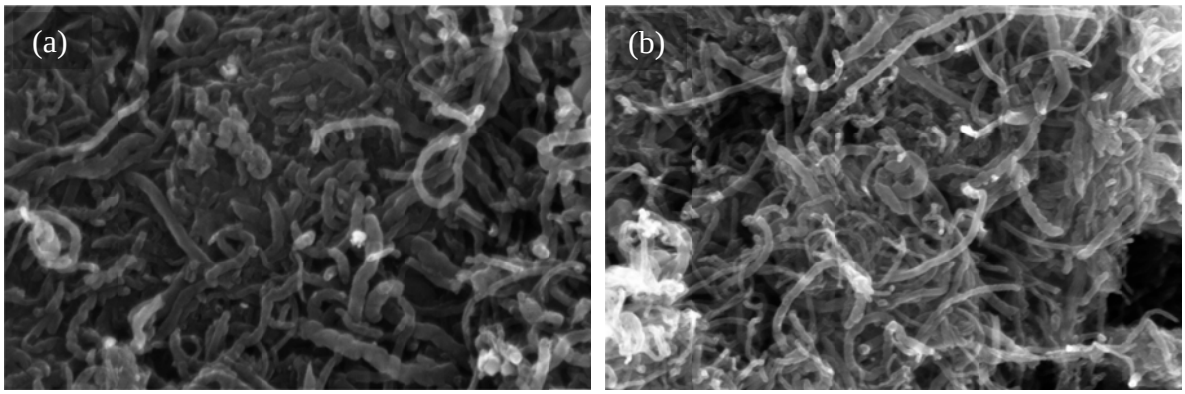

Fig. 4: Micrograph FESEM for (a) loading of ASA onto functionalized MWCNTs, (b) loading of ASA onto non-functionalized MWCNTs.

Fig. 5 shows that the amount of ASA loading onto non-functionalized and functionalized MWCNTs when sonicated and stirred with 30 000 ppm of ASA. ASA can also be loaded onto the non-functionalized MWCNTs with results show that the lower percentage compared to the functionalized MWCNTs. This is because drug loading efficiency depends on the presence of the functional group on the surface of MWCNTs [13]. With the presence of functional group, the ASA molecules may effectively bonded with the carboxylic group on the surface of *f*-MWCNTs.

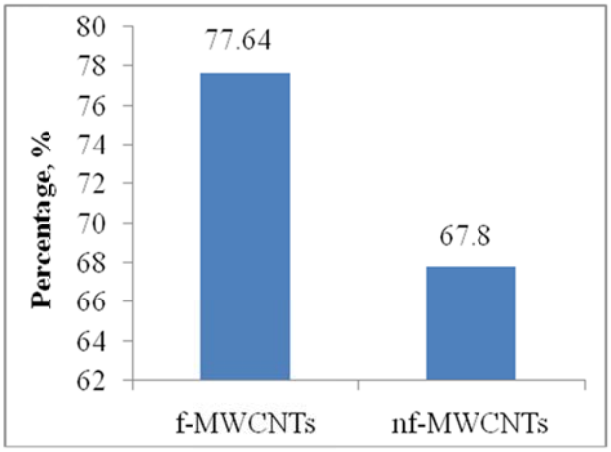

Fig. 5: Percentage of ASA loaded onto *f*-MWCNTs and *nf*-MWCNTs.

3.3 Dispersion Analysis

Fig. 6 shows the dispersed states of ASA-*nf*-MWCNTs and ASA-*f*-MWCNTs composites in water at the moment of dispersion and at approximately one hour, 24 hours and 7 days later. Fig. 6 shows that the ASA-*nf*-MWCNTs composites have poor suspension stability after 7 days in water compared to the ASA-*f*-MWCNTs composites which still remains stable after 7 days. According to Foldvari, *et al.*, [14], it is essential that MWCNTs be dispersed before they are used in therapeutic formulations. This is because increasing water dispersibility through chemical modification will reduce the cytotoxic effects of MWCNTs while increasing their biocompatibility [2].

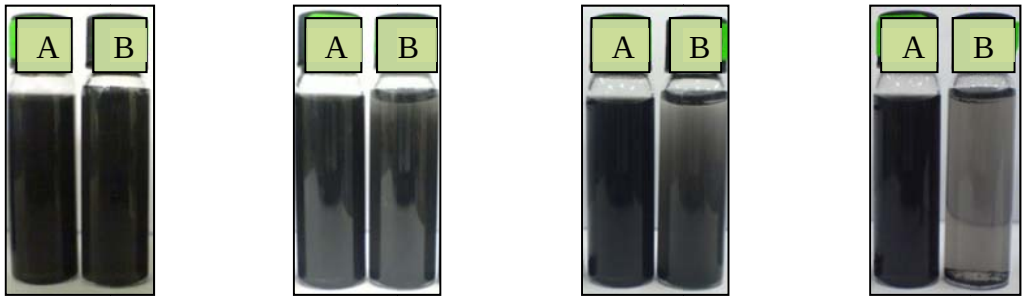

Fig. 6: Dispersion test of ASA-*nf*-MWCNTs and ASA-f-MWCNTs composite in water according to time (A: ASA-*f*-MWCNTs composite in water; B: ASA-*nf*-MWCNTs in water).

3.4 Drug Released Analysis

Fig. 7 and 8 show the comparison of the percentage released of ASA in the prepared Simulated Body Fluid (SBF) from ASA-*f*-MWCNTs composite and from commercialized ASA tablets in the market at normal human body temperature (37^{o}C) and fever temperature (39^{o}C), respectively. Fig. 7 shows the fast release of ASA from the ASA-*f*-MWCNTs within 5 hours compared to the commercialized ASA tablets. However, after 5 hours, percentage of ASA released from ASA-*f*-MWCNTs composite was nearly the same with ASA released from commercialized ASA tablets and after 6 hours onwards, the percentage of ASA released from ASA-*f*-MWCNTs composite was lower compared to the commercialized ASA tablets.

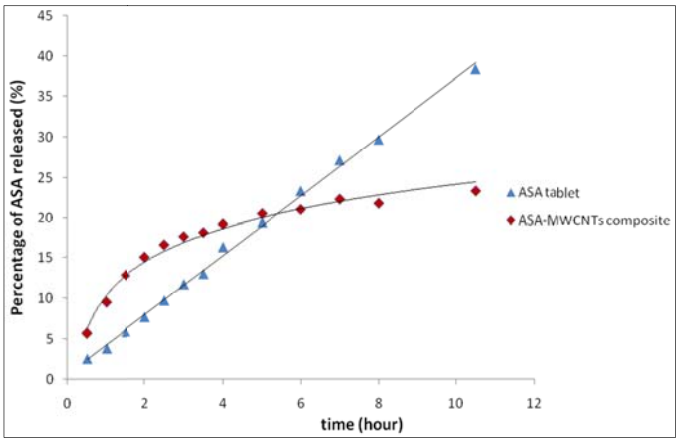

Fig. 7: Comparison on the percentage of ASA released from ASA-*f*-MWCNTs composites and commercialized ASA tablets in SBF solutions at 37°C (normal body temperature).

As shown in Fig. 8, the ASA release trends are quite similar to the release of ASA in SBF at 39°C. However, the ASA release in SBF at 39°C is higher in percentage compared to the drug release in SBF at 37°C. For example, at 37°C, 5.62% of ASA was released from ASA-*f*-MWCNTs composites in 30 minutes and 19.19% was released after 4 hours, whereas 10.23% of ASA was released in 30 minutes and 23.79% was released after 4 hours in SBF at 39°C.

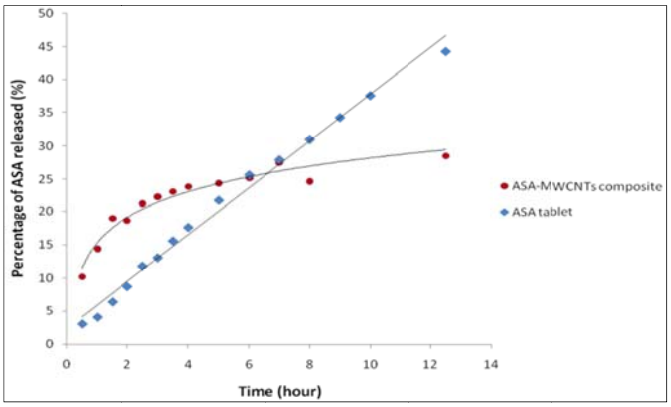

Fig. 8: Comparison on percentage of ASA released from ASA-*f*-MWCNTs composite and commercialized ASA tablets in SBF solutions at 39°C.

The fast release of ASA-*f*-MWCNTs composite at below 5 hours at 37°C and 39°C shows the improvement of time release of the drug molecules compared to the commercialized ASA tablets. In the following of drug release of ASA-*f*-MWCNTs, the control release of drug behavior was observed. Therefore, it reveals that the MWCNTs also have the ability to improve the pharmacokinetics, which resulted in the improvement of the drug efficiency [2]. Slow release of the drugs actually has high potential to the pharmaceutical therapy. It is because, during current clinical therapy, patients are still mainly treated by repetitive drug administration and these drugs are eliminated quickly. This resulted in a high dosage uptake, low efficiency and side effects. The controlled release including the release time and site of ASA drugs from ASA-*f*-MWCNTs composites, has been thought to be necessary to improve therapy [14].

4. Conclusion

The results obtained show the generation of carboxylic groups on the surface of MWCNTs. The carboxylic groups will increase the dispersibility of MWCNTs in various solvents and help to anchor aspirin molecules by hydrogen bonding with the carboxylic groups. Analysis of loaded CNTs by FESEM-EDX, FT-IR Spectroscopy and UV-Vis Spectroscopy confirmed the loading of the drug onto the functionalized and non-functionalized MWCNTs. However, ASA-*nf*-MWCNTs composites have poor suspension stability after 7 days in water compared to the ASA-*f*-MWCNTs composites which still remains stable. Therefore, functionalized MWCNTs have the potential application in drug administration. The fast release of ASA-*f*-MWCNTs composite at below 5 hours at 37^{o}C (human body temperature) and 39^{o}C (fever temperature) shows that ASA drug molecules are better released during this time compared to commercialized ASA tablets. The fast release of ASA drug at the early hours (initially) and controlled release for the following hours (finally) by the ASA-*f*-MWCNTs at temperature of 39^{o}C was proven higher than at 37^{o}C. Therefore, it reveals that the MWCNTs have the ability to improve the pharmacokinetics of aspirin drug in the biomedical applications, thus offering the potential exploitation of carbon nanotubes in the drug development of drug delivery system.

References

[1] N. A. Ochekpe, P. O. Olorunfemi, N. C. Ngwuluka, Trop J Pharm Res., **8** (2009) 275
[2] M. Prato, K. Kostarelos, M. Bianco, Accounts of Chemical Research, **4l** (2008) 60
[3] S. K. Vashist, Z. Dan, G. Pastorin, K. Al-Rubeaan, J. H. T. Luong, F. S. Sheu, Carbon, **49** (2011) 4077
[4] R. Benamouzig, Digestion, **79** (2009) 40
[5] A. Bianco, K. Kostarelos, C. D. Partidos, M. Prato, Chem. Commun. (2005) 571
[6] M. S. Sekhar, P. Visakh, T. P. Aneesh, M. Sabitha, The Internet Journal of Nanotechnology **2** (2008)
[7] Lacerda L., Bianco. A., Prato, M,. and Kostarelos, K., Advanced Drug Delivery Reviews **58** (2006) 1460
[8] Quinn, M., Determination of acetylsalicylic acid in aspirin using Total Fluorescence, UV Instruments at Work (1994)
[9] Rizwan Wahab, S. G. Ansari, Young-Soon Kim, T. R. Mohanty, I. H. Hwang and Hyung-Shik Shin, Synthetic Metals **159** (2009) 238
[10] N. J. Kumar, H. S. Ganapathy, J. S. Kim, S. J. Yeong, T. J. Yeon, European Polymer Journal **44** (2008) 579
[11] P. C. Ma, J. K. Kim, B. Z. Tang, Carbon **44** (2006) 3232
[12] H. S. Shui, W. T. Chiang, C. C. Lin, M. Yokoyama, Physica E. **40** (2008) 2322
[13] R. Singh, J. W. Lillard, Experimental and Molecular Pathology **86** (2009) 215
[14] Foldvari, M. and Bagunlori, M., Nanomedicine: Nanotechnology, Biology and Medicine **4** (2008) 183