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Carbon Nanotubes to Deliver Drug Molecules

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Nanotechnology is rapidly developing field in especially engineering and medical sciences. Carbon nanotubes are one of the most studied nanomaterials in material sciences and physics. Although there are limitted number of studies have been performed with carbon nanotubes in medical sciences and pharmacy, to use carbon nanotubes as drug delivery material is still at beginning and at developing stage. Carbon nanotubes are adsorptive materials and they can actively adsorb drug molecules on the surface. In this study the adsorption properties of carbon nanotubes were investigated for ibuprofen, naproxen, oxaliplatin and paclitaxel. Desorption properties and the posibility of using them as drug delivery systems for mentioned drugs were investigated and determined. Multiwalled carbon nanotubes were also PEGylated and PEGylation was found to be succesfull and effective. Particle sizes and zeta potentials of carbon nanotubes were not altered after PEGylation.

Keywords: Carbon Nanotubes, Drug Delivery, Ibuprofen, Naproxen, Oxaliplatin, Paclitaxel.

CONTENTS

1. INTRODUCTION

Nanoparticles with recent available engineering tools are at the forefront of the rapidly developing field of nanomedicine. Since the first carbon nanotubes were proposed to the market in 1991,[1] they have been widely studied mainly for the development of microelectronic devices. Despite the eminence of carbon nanotubes (CNTs) in nanotechnology, exploration of their pharmaceutical applications still remains at a very early stage.[2]

It has been shown that single-walled carbon nanotubes (SWNTs) and multiwalled carbon nanotubes (MWNTs) can be internalized by living cells and pass across the biological membranes in cell culture studies.[3] The internalization of carbon nanotubes by corneo-cytes has been shown[4] in the literature but their drug carrying properties through the skin have not been fully evaluated. But in our previous study it has been first shown that single-walled carbon nanotubes (SWNTs) and multiwalled carbon nanotubes (MWNTs) can be used to deliver drug molecules through deeper skin layers. The application of iontophoresis using carbon nanotube electrode having adsorb drug molecules on their surface has been shown and molecules successfully transferred through deeper skin layers.[5] Indomethacin was selected as a hydrophobic drug (Log Ko/w = 4.5) and it penetrates through full thickness skin.[6] The penetration of indomethacin through fullthickness of rat skin was enhanced when indomethacin adsorbed CNTs were used Flux values were 0.119 ± 0.037 μg/h for indomethacin alone; 0.330 ± 0.052 μg/h for indomethacin with MWCNTs and 0.347 ± 0.106 indomethacin with double walled carbon nanotubes (DWCNTs, $n = 3$, $\pm$SD).[6] The penetration enhancement was higher with DWCNTs and MWCNTs, however the mechanism was still unknown. The CNTs may act to facilitate presentation of the drug to the lipophillic membrane and/or they facilitate penetration

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through the skin accompanying the drug into the dermal tissue. Similar results were obtained when pig ear skin was used. Penetration of indomethacin was found to be much higher when indometahcin molecules were introduced to the skin surface with CNTs.[6]

All previous studies have shown that carbon nanotubes can be used to deliver drug molecules to the active site. Therefore it was aimed to determine adsorption characteristics of paclitaxel (PAC), oxaliplatin (OX) and other non-steroidal anti-inflammatory drug ibuprofen (IBU) naproxen (NAP) on carbon nanotubes and to determine how they can deliver the drug molecules. Recent studies indicated and showed that cyclooxygenase (COX) can have a role in development of cancer and tumors. Non-steroidal anti-inflammatory drugs (NSAIDs) are therefore have noticible effects on reducing tumor mass and its development.[7,8] Therefore IBU and NAP sodium were selected as model NSAIDs. The drug adsorption capacity of DWCNTs and MWCNTs were found to be 14.25 and 14.70% ($weight_{CNTs}/weight_{Drug}$) respectively.

Sibel Ilbasmiş-Tamer graduated from Gazi University, Faculty of Pharmacy in 2000 in Turkey and has completed Master thesis in 2003 at the Department of Pharmaceutical Technology at Gazi University Faculty of Pharmacy. She is currently a Ph.D. student at same department. She has been working on skin penetration of drugs and iontophoresis.

Şükran Yilmaz graduated from Hacettepe University Faculty of Biology in Ankara, Turkey in 1989 and has got Master degree from the same university in 2005. She has been working at the cell bank and laboratory of Mouth and Foot Deseases Institute in Ankara. She has been responsible for animal cell culture collection of the institute since 2006 and she is currently head of Cell and Virus Bank Division of the institute.

Dr. Erden Banoğlu graduated from Gazi University, Faculty of Pharmacy in 1989 in Turkey and has got Ph.D. from Medicinal and Natural Products Chemistry, College of Pharmacy, University of Iowa, USA in 1997. He has been interested in developing dual COX/5-LOX inhibitors which are potential drugs able to block both the COX and the 5-LOX metabolic pathways resulting better tolerated anti-inflammatory agents. The interest of developing such compounds is supported by a large number of pharmacological studies. He is currently working at Pharmaceutical Chemistry department of Gazi University, Faculty of Pharmacy in Ankara in Turkey.

Dr. Ismail Tuncer Değim graduated from Ankara University, Faculty of Pharmacy in 1985 in Turkey and has got Ph.D. from the University of Wales College of Cardiff, the School of Pharmacy in 1996. He has been working on skin or membrane penetration of drug molecules, nano particles etc. and also developed model for prediction of penetration. He has number of publications; US and Russian patents related to skin penetration and iontophoresis (US7,231,242, RU2323015).

The adsorption properties of DWCNTs and MWCNTs were also investigated for oxaliplatin and MWCNTs were found to be adsorbed oxaliplatin higher.

2. MATERIALS

MWCNTs (Nanocyl 3101) and DWCNTs (Nanocyl 2151) were purchased from Nanocyl. Oxalyl chloride, PAC, OX and Diyaliz Membran (M.A < 12000) were purchased from Sigma. Fullaren (Buckminsterfullarene) was from Fluka-Chemica. NAP and IBU were kindly donated by Fargem Drug Co. Düzce, Turkey. All reagents and chemicals were of analytical grade.

3. METHODS

3.1. HPLC Methods

OX and PAC were analyzed by developed HPLC method. HPLC methods were adopted from the literature.[9, 10] Thermo Finnigan, Surveyor (CA, USA) HPLC system was used (Table I).

3.2. UPLC Methods

IBU, NAP and OX were analyzed by developed UPLC methods. Waters Acquity UPLC system (MA, USA) was used. UPLC methods were adopted from the literature (Table II).[9, 11]

3.3. Adsorption of Drugs by CNTs

CNTs (2 mg) were added to drug solution (20 μg/ml, 25 ml for NAP or IBU, 25 μg/ml, 6 ml for OX, 1 ml of propylene glycol (PG) was also used to dissolve whole drug to ensure complete adsorption) and amount of drug remianed in the solution was determined during 24 hours. These experiments were done in sextetle. Experiments were done in acidic medium (pH $\approx$ 5) and in pH 7.4. Similarly, the effect of drug concentration on adsorption was investigated using 2.5 mg CNTs and drug solution with the concentraion of 5, 10, 15, 20, 30, 40 μg/ml NAP solutions and adsorption profile was obtained.

3.4. Particle Size Measurements and Zeta Potentials

Particle size of the CNTs were determined using Malvern Zeta sizer (Worcestershire, UK).

Table I. Details of HPLC systems used for analysis of PAC and OX.

	PAC	OX
Flow rate (ml/min)	0.5	0.6
Mobile phase	ACN (70) 0.2% Phosphoric acid (30)	Methanol (40), ACN (5), 0.2% Phosphoric acid (55)
Λ_{max}	254 nm	254 nm

Table II. HPLC system for IBU, NAP and OX.

	IBU	NAP	OX
Flow rate (ml/min)	0.6	0.25	0.1
Mobile phase	Acetonitrile (46.75), Su (53.25), 0.26 ml 85% Ortho phosphoric acid and 0.234 g KH_2PO_4	Acetonitrile (37.75), Water (64.25), 0.26 ml 85% Ortho phosphoric acid and 0.180 g KH_2PO_4	Methanol (85), Water (15)
Λ_{max}	254 nm	254 nm	254 nm

3.5. DSC Analysis

DSC thermograms of CNTs-Drug mixture were obtained (DSC-60, Shimadzu, Japan). Physical mixtures, dried CNTs-drug mixtures after dissolved in methanol (sonication was used for a minute) were subjected to the DSC analysis. The effect of reheating was also investigated.

3.6. Determination of Physical Appearance of CNTs and CNTs-Drug Mixtures

The physical appearances of CNTs were determined by atomic force microscope (AFM, NanoMagnetics Instruments Ltd., Oxford, UK) and transmission electron microscope (TEM). Samples were fixed on mica for investigation with AFM. Briefly, CNTs dispersion in ethanol and sonicated for a minute. Dispersion containing CNTs was placed as a droplet on the surface of mica and dried at 50 °C as described in literature.[12] Noncontact mode was used for investigation. The force constant of aluminium reflex coated cantilever was (All in one-Al, Budget Sensors) 7.4 N/m.

CNTs sample was directly subjected to TEM analysis because of conductive nature of CNTs.

3.7. Franz Cell Diffusion Experiments

A physical mixture of PAC and OX were prepared with Fullerene (C60). The drugs were made adsorbed to the surface of fullerenes after the preparation because of high hydrophobic nature of the PAC and OX. A Franz type of diffusion experiments were performed using free drug and fullerene drug mixtures. Dialysis membranes were used and penetrated amount of drugs were determined by HPLC and penetration profiles were obtained.

3.8. Desorption from CNTs

Following 24 hours drug adsorption on CNTs as mentioned previously, CNTs and drug mixture was placed in a beaker having 25 ml of pH 7.4 phosphate buffer solutions at 37 °C in water bath. Samples were taken by predetermined time intervals and analyzed. The drug desorption profiles were then obtained.

3.9. PEGylation of CNTs

The purchased MWCNTs were already carboxylated (∼4%). The degree of carboxylation of the DWCNTs was much less (∼1%). Accordingly the MWCNTs were selected for PEGylation studies. The PEGylation method was adopted from the literature.[13] Briefly, 50 mg of MWCNTs were dispersed in 26 ml of anhydrous DMF and sonicated for 2 hours. The mixture was cooled with ice to 0 °C under a nitrogen stream, 2 ml of oxalyl chloride was added drop wise and stirred for 2 hours. This mixture was then heated up to room temperature and stirred for 2 hours. The mixture was further heated to 75 °C and stirred overnight to remove all remaining oxalyl chloride. This mixture was then heated to 100 °C and 2.71 g of PEG 4000 was added and stirred for 5 days. This mixture was then cooled, filtered and washed with ethanol and distilled water. A black solid was collected and dried under vacuum overnight.

3.10. Determination of PEGylation Degree of MWCNTs by TGA

The degree of PEGylation degree of the MWCNTs was determined using TGA (Shimadzu model DTG-60, Japan). Samples were dried under vacuum overnight prior to use. They were then placed into the TGA instrument and heated up linearly (15 °C per minute) under a nitrogen gas stream to prevent oxidation or combustion and changes in weight were recorded. The degree of PEGylation of the CNTs was calculated with respect to weight loss.

4. RESULT AND DISCUSSION

NAP was subjected to the adsorption studies and at acidic medium NAP was found to be adsorbed by CNTs (Fig. 1). When neutral pHs (pH 7.4) were used for drug absorption

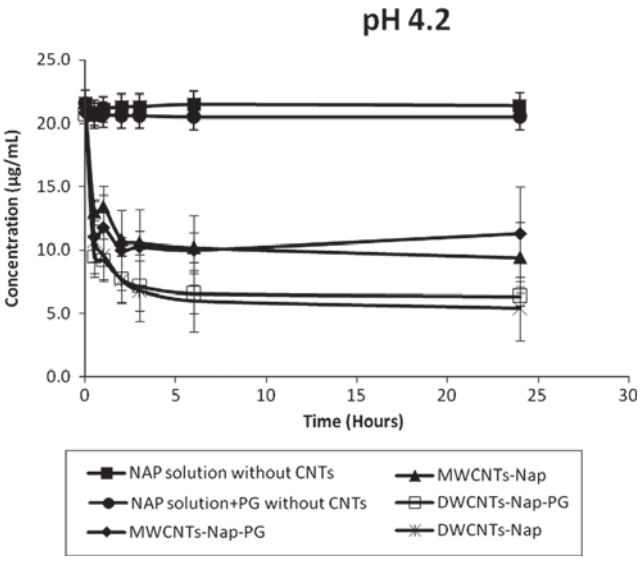

Fig. 1. Adsorption of NAP by CNTs.

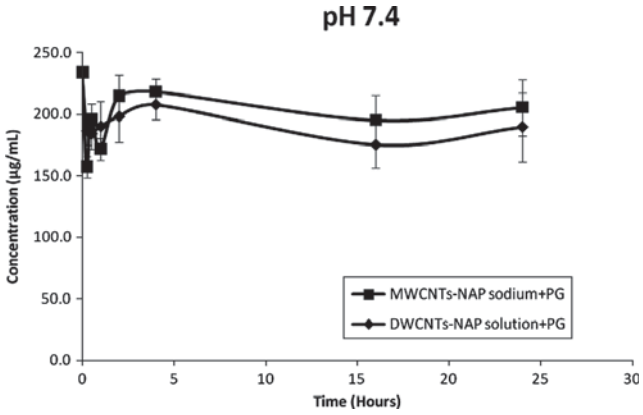

Fig. 2. Adsorption of NAP by CNTs at high pH with high concentration.

few or no adsorption was obtained (data not shown). NAP is a weak acid and it's pKa is 4.2.[14] NAP is mainly in unionized form at acidic pHs,[15] therefore it likes to be present at the hydrophobic surface of the CNTs in aqueous solution. Contrary it prefers to be in the solution when it is in ionized form at neutral or high pHs above then it's pKa.

Amount of NAP was thought to be less when pH 7.4 was used, therefore amount of NAP for adsorption was increased to provide sufficient molecule for adsorption and experiments were repeated but similar results were obtained, in other words NAP did not adsorbed by CNTs much when pH was 7.4 even its concentration is quite high (Fig. 2).

The effect of NAP and IBU concentration on adsorption to the CNTs were also investigated and the adsorbed amount of NAP by CNTs were depicted in Figure 3. The adsorption was found to be high when concentration of the parent drug in the solution is high but this was not found to be very different and statistically significant for NAP. An increasing trend was observed for IBU when high concentration of the drug is provided but again those were not very different from each other. Therefore the concentration range used was found to be suitable for further experiments.

The adsorption of OX on the CNTs were also determined. When distilled water was used (Fig. 4(a)) adsorption was achieved by CNTs but when pH was under control it was appeared that pH has no or little effect for adsorption of OX (Fig. 4(b)). OX is water soluble drug[16] and its solubility does not affected by solution pH therefore the adsorption of OX was found to be in similar range for pHs were studied.

The particle sizes and zeta potentials of CNTs were determined with range of 84–602 nm and −15.2 to 16.8 mV. Particle size was not found to be affected by environmental conditions such as pH and ionic strength and there were no trend obtained with the variation of pH for both. This results show that CNTs studied did not swell or dif not agregate further under experimental conditions.

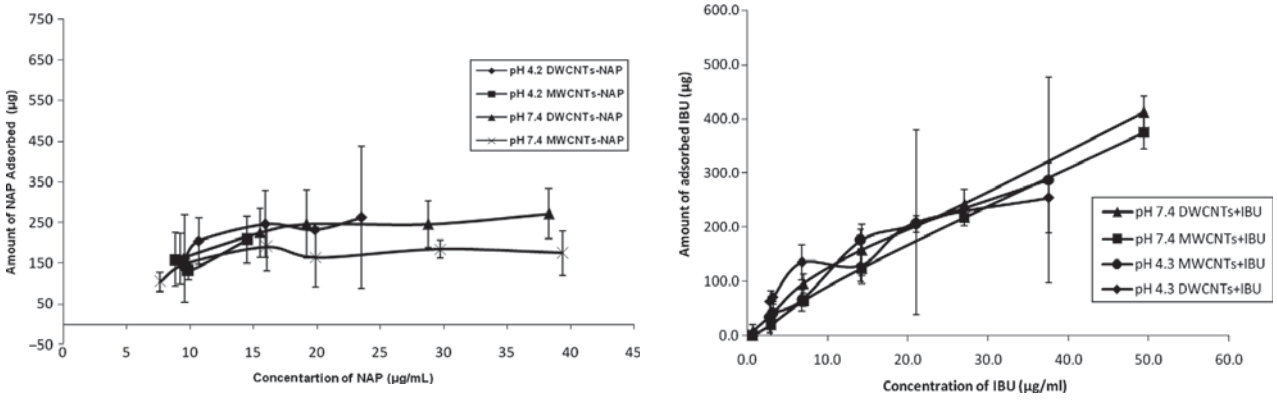

Fig. 3. Effect of drug (NAP and IBU) concentration and pH on adsorption by CNTs.

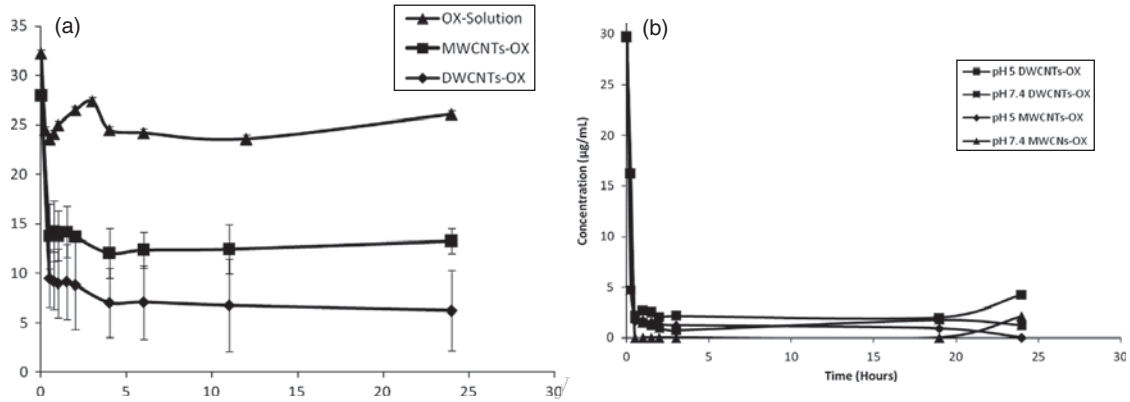

Fig. 4. Effect of pH on adsorption of OX by CNTs.

Any possible interaction between drug molecules and CNTs were investigated by DSC for IBU and NAP (Fig. 5). DSC peak is responsible to crystal structure and broadening or disappearance of the peak indicates impurity or weakening of bonds in the crystal structure.[17] It is indeed an interaction was found between drug molecule and CNTs. The DSC peak responsible to crystal structure was disappeared when prepared with CNTs. This is found to be because of adsorption. When drug molecule dissolved and then adsorb to the surface of the CNTs, it cannot rebuild its crystalline structure, the bonds in the structure possibly weakened due to the interactions with CNTs surface. It was found to be very similar for both MWCNTs and DWCNTs.

The physical appereance of MWCNTs were determined using AFM (Fig. 6). The tubular shapes of CNTs were clearly seen. On the Figure 6 branches of CNTs and carbon particles can be observed. This gives information mainly

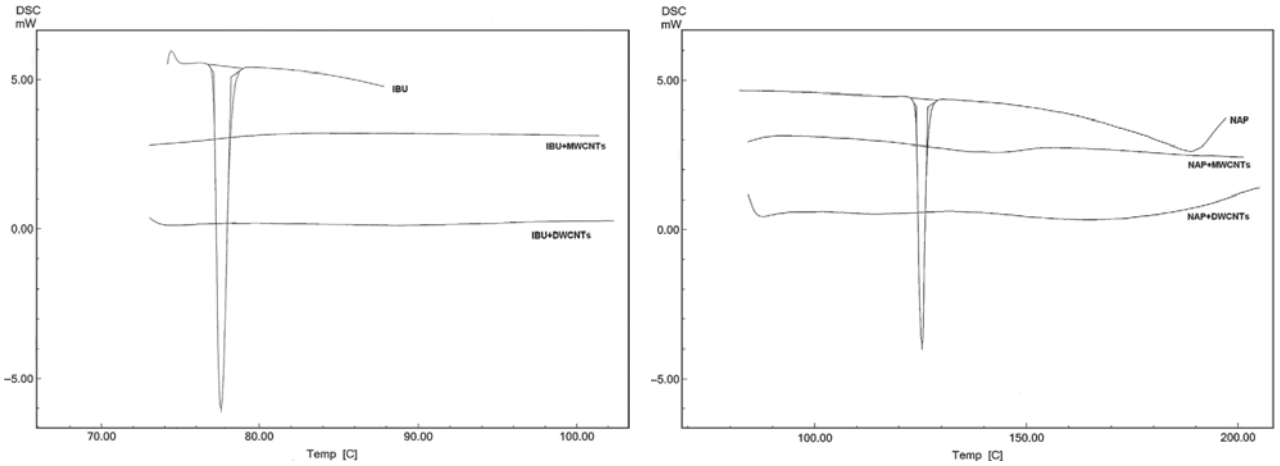

Fig. 5. Molecular interaction of drug and CNTs under DSC.

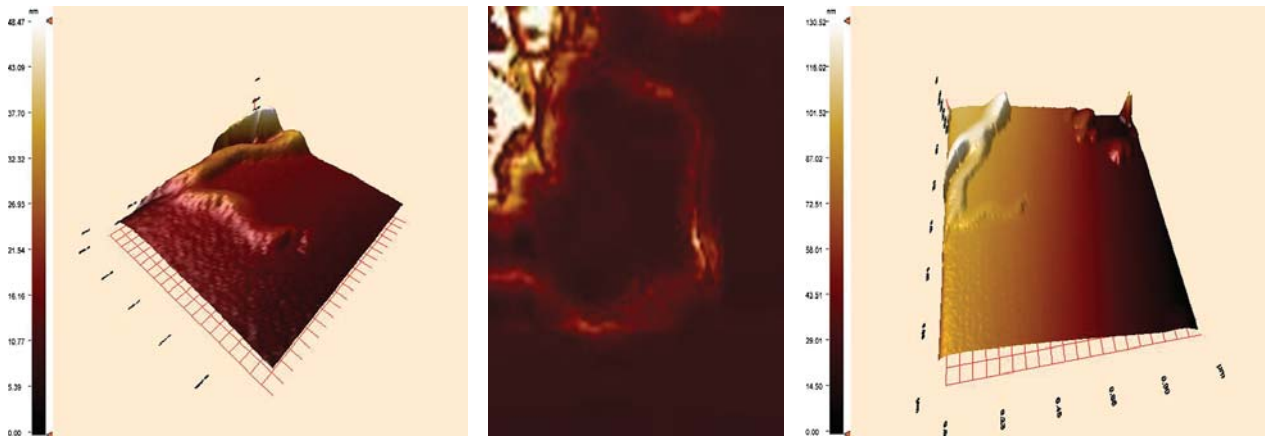

Fig. 6. AFM images of MWCNTs on mica surface.

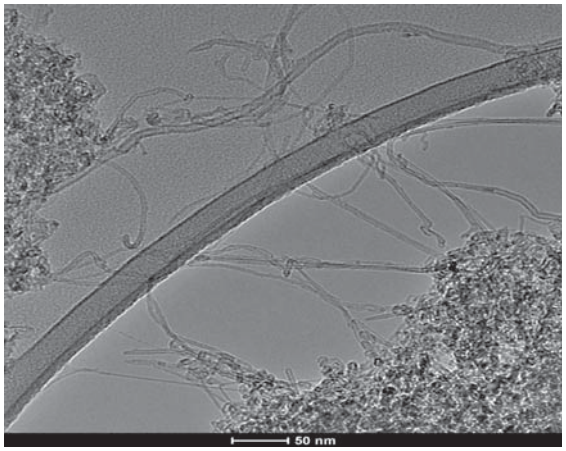

Fig. 7. TEM images of DWCNTs and NAP crystalls.

about the lenth of nanotubes and approximate valuation of the bundles diameter. It was found not to be very accurate to determine exact value of diameter of CNTs as it was reported in the literature[12] because some nanotube does not lie directly on a mica and oscilated under the daze.

The physical appereance of NAP adsorbed DWCNTs were also investigated under TEM. The tubular shapes of CNTs and crystallized NAP molecules are clearly seen in Figure 7. These tinny branches of CNTs lie among the crystallized naproxen particles and in the figure the edge of the plate can be seen. After both visualization technique we used CNTs were observed as very long nanotubes with the value of 200–300 nm and present as branches and bundles; the diameter is found to be around 2–4 nm. There are drug molecules observed as crystalline particle but the main observation was the drug molecule does not present at the surface of the CNTs as crystals. They actually adsorbed to the wall of CNTs but it is in invisible molecular form.

Penetration of drug from dialysis membrane was determined for PAC and OX from Fullarene-drug complexes (Fig. 8). This result show and prove that it is possible to deliver drug substance with CNTs by simply adsorption and subsequent desorption processes.

Desorption characteristics of NAP and IBU from CNTs were investigated and determined (Fig. 9). The effect of pH was also investigated. There was no significant effect observed for desorption with pH variation. The amount of desorption or in other words delivered amount of drug is completely depend on ionisation and attraction of the molecule to the solvent or hydrophobic CNTs wall. The adsorption force was found to be quite strong, because

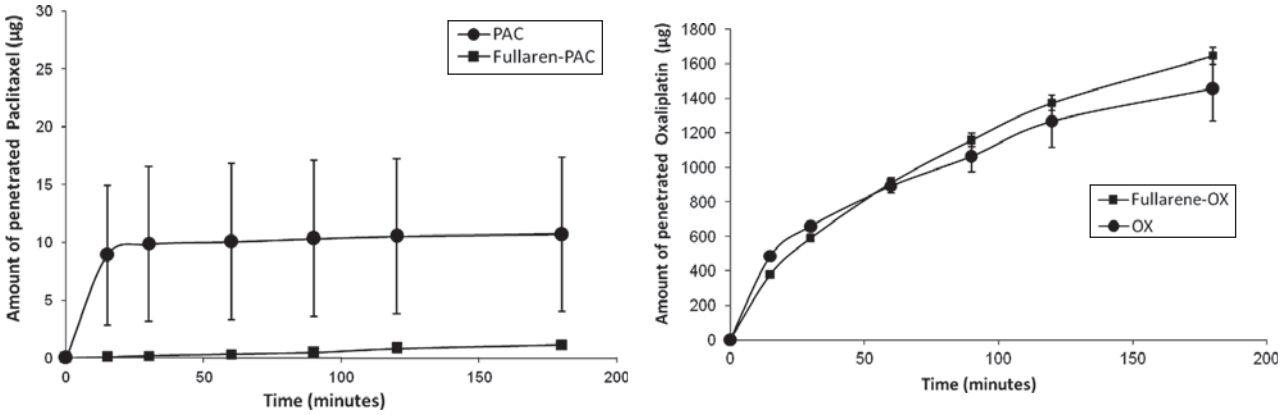

Fig. 8. Penetration of drugs (PAC and OX) through dialysis membrane from Fullarene-drug complexes.

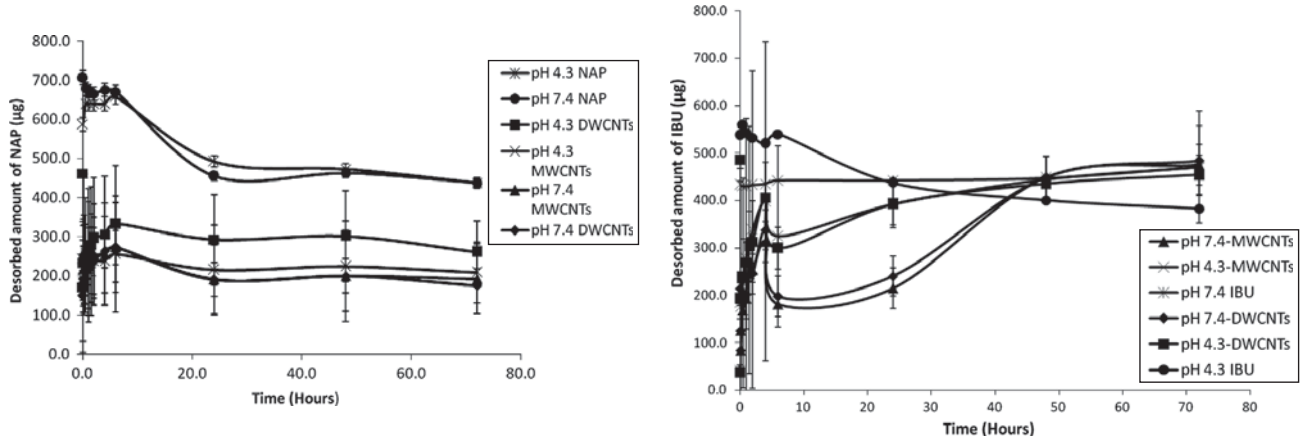

Fig. 9. Desorption of NAP and IBU from CNTs.

Fig. 10. The appearance of the dispersions of PEGylated MWCNTs and MWCNTs in water after 0, 15 minutes and 54 days of preparation.

the CXNTs did not desorp all drug molecules in experimental conditions.

PEGylated MWCNTs were prepared and dispersed in water. Because of hydrophobic nature of MWCNTs they were not easily produce a good dispersion but when they were PEGylated, they produce a homogenous dispersion, the dispersion was still looking very good even after 54 days (Fig. 10).

The changes in particle size and also in zeta potentials were also investigated (Fig. 11). There was no significant alteration observed.

The extent of PEGylation of the MWCNTs was investigated via TGA analysis. The combustion point of carbon has been reported as 420 °C under atmospheric conditions[18] and only burns in the presence of oxygen. It has been reported in the literature that carbon nanotubes can be heated up to 700 °C under nitrogen and that any change in weight can be used to calculate the degree of functionalization of CNTs.[19] PEGylated MWCNTs were subjected to TGA analysis and PEGylation percentages of MWCNTs were found to be 10.625 ± 1.085 according to weight loss. PEGylation was found to be effective and reproducible.

The biocompatibility and solubility of CNTs can be increased by functionalization to add hydrophilic moieties. Specifically, carbon naontubes have been functionalized with biotin and subsequently complexed with a fluorescent streptavidin.[20] These functionalized CNTs were taken up by endosomes following incubation in CHO and 3T3 cells.[20] The mechanism of penetration is not yet completely explained but two possible routes of internalization have been proposed. It has been shown that functionalized CNTs can penetrate cells following passive diffusion across the lipid bilayer. This has been attributed to their "nanoneedle" shape which allows them to perforate the cell membrane without causing cell death.[21–23] It has been shown that both SWNTs and MWNTs can be internalized by cells and pass across biological membranes.[24] The internalization of carbon nanotubes by skin cells namely

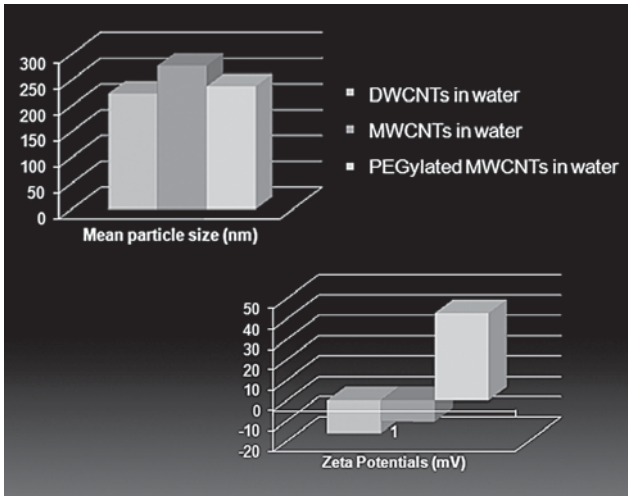

Fig. 11. The effect of PEGylation on particle size and zeta potentials.

corneocytes has been shown.[4] Therefore CNTs can be also used for several different application and to deliver the drug in many different ways.

5. CONCLUSION

To prepare CNTs-drug mixture is possible and it is resulted in drug adsorption. CNTs can adsorp drug molecule on their surface and it can deliver the drug and even they can release the drug by subsequent desorption. Adsorption takes place when drug is nonionized or even if it is ionized. Nonionized form is adsorbed more than ionized form. CNTs were found to be suitable material for drug transport having higher surface area and being an adsorptive material. PEGylation process was found to be effective and useful technique for further studies.

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