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The covalent attachment of Naltrexone to multi-walled carbon nanotubes byoxalyl chloride agent

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ABSTRACT

This work presents a novel cascade of chemical functionalization of multi-walled carbon nanotubes(MWCNTs) through chemical modification by an opioid antagonist drug of Naltrexone. Naltrexone-conjugated MWCNTs were synthesized involving the sequential steps of carboxylation, acylation,and finally,Naltrexoneconjugation.The active acyl chlorides in MWCNTs were subsequently mixed with opioid antagonist drug of Naltrexone.The modification of MWCNTs with Naltrexone was investigated by Fourier transform-infrared spectroscopy,Raman spectroscopy,Thermo Gravimetric Analysis,Elemental Analysis,High Performance Liquid Chromatography.Size and surface characteristics of chemically modified MWCNTs were monitored by Transmission Electron Microscopy,Scanning Electron Microscopy,Field Emission Scanning Electron Microscopy,Atomic Force Microscopy.

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Introduction

The drugs of choice for the treatment of several pain are the opioids,the activities of which are mediated by their interaction with specific membrane round G-protein coupled receptors(GPCR)[1,2]. The existence of at least three major classes of opioid receptors, viz,μ,k, δ,has been well established [3].These receptors have also been cloned and functionally characterized[4-11].Naltrexone is a potent opioid antagonist with little agonist activity and its along activity opioid antagonist which is used in opiate addiction treatment and rehabilitation.Naltrexone is a mu-opioid receptor antagonist.Narcotic antagonist is used extensively as pharmacologic tools for the investigation of opioid receptors[12].indeed,the recent researchliterature attests to the impact that such antagonist have made in the previous active research area.How over, the reversible nature of conventional narcotic antagonist e.g.,Naltrexone is an inherent limitation to its utility,particularly with regard to the use of such compound in the isolation and purification of opioid receptors.Naltrexone monotherapy was approved for the treatment of opioid addiction in 1984 and alcohol dependence in 1995.Additionally,Naltrexone is approved to treat heroin overdose and to reverse respiratory depression caused by morphine.Naltrexone is used to treat heroin and alcohol abuse.In previous study,were made to formulate a transdermal patch containing Naltrexone which has controlled release.law release is critically significant in drug delivery or minimizing the amount of drug lost before reaching to target. Shell structures and supports can be used or slow delivery of drugs and are usually made from organic materials.For example,liposomes[13-15].Microspheres[16].polymeric shells[17]and polymeric micelles have been well investigated[18-19].In constructing drug delivery system from organic materials,the combinations of shell or support materials,targeting molecules,and drugs are restricted to ensure stability targeting efficiency,and drug effect.Although,many supporting polymers are expensive,this restriction can be reduced by using carbon nanotubes[20].On the other hand,nano medicine,which is an emerging bridge linking nanotechnology and advanced medical technology involves the exploration of nanoscale materials with the aim of developing novel types of drug carriers,imaging agents,sensors,etc[21-22].Nanotubes have several properties that make them suitable for use a nanotube supported drugs.Functionalization CNTs have been shown in many studies to be able to cross cell membranes[23-25].The ability of CNTs to cross cell membranes allowing them to be used as carriers is of particular high interest for drug delivery strategies.The individuals which have used overdoses narcotics,if the opioid antagonist drug of Naltrexone is be given to them of the oral ways,the patient is possible is be caused at first stage respiratory depression,heart attack,intense

convulsive and or the patient can be sometimes caused of the death.Therefore, we have used of solid support in this work for the attachment antagonist drug of Naltrexone which it can be solid phase of carbon nanotube.

2-Experimental

2-1-Materials

Multi-walled carbon nanotube purchased from Chengdu organic chemistry Co.Ltd.Chinese Academy of science.The opioid antagonist drug of Naltrexone was purchased from Tolid Darou Co(Tehran,Iran).Oxalyl chloride,concentrated nitric acid(HNO_3,65%),concentrated sulfuric acid(H_2SO_4, 98%)were obtained from Merk Co,and used as received.Triethyl amine,THF,DMF were poured in Potassium hydroxide(KOH),Na with benzophenone,molecular sieve(0.4nm diameter,2mm length),and were obtained anhydrous triethyl amine,anhydrous THF,anhydrous DMF,respectively.before use.

2-2-Preparation of MWCNT-COOH

0.3g of raw-MWCNTs were placed into a round bottom flask.raw-MWCNTs were treated with a (v/v3:1)mixture of concentrated H_2SO_4(98%) and HNO_3(65%).The suspension solid was refluxed for 12h at 35°C under a magnetic condition,after cooling to room temperature,MWCNT with carboxylic acid groups were added dropwise to 300ml of cold DI.Subsequently,the reaction medium was poured into several centrifugation vials and centrifuged at 350rpm for 20 min.The supernatant liquid was decanted off,and the vials were refilled with DI,and centrifuged again under identical conditions.finally,MWCNT were swept with DI,vacuum-filtered,and thoroughly rinsed with DI and products were dried at 50° C in a vacuum oven for 24h.

2-3-Conversion of MWCNT-COOH to MWCNT-COCl(preparation of MWCNT-COCl)

0.25g of the MWCNTs-COOH were added into 250ml three-neck round bottom flask,and were stirred in 100ml of anhydrous DMF for 30min under argon gas.Then,MWCNTs-COOH were sonicated for 30min at 30°C to give a homogeneous suspension,under argon gas.oxalyl chloride was added dropwise to MWCNT suspension at 0°C under argon gas.The mixture was stirred at 0°C for 2h.excess oxalyl chloride was remove with rotary under vacuum.The reaction mixture was then filtered on a 0.25µm pore-sized PTFE membrane filter.The black solid collected on the filter was washed with anhydrous tetrahydrofuran.The resulting acid chloride derivative was dried at 35°C in a vacuum oven for 24h.

2-4-The attachment of opioid antagonist drug of Naltrexone to MWCNT-COCl(MWCNT-OX).

0.1g of MWCNT acyl chloride were placed into a flask,then were added 2ml anhydrous DMF,50µL anhydrous triethyl amine,0.07g drug of Naltrexone under argon gas.The flask was sonicated at 30°C for 30min,then mixture was stirred for 24h.The reaction mixture was then filtered on a 0.25µm pore-sized PTFE membrane filter.The black solid collected on the filter was washed with 120ml anhydrous DMF and anhydrous THF.The resulting material was dried at 35°C in a vacuum oven for 5h.Functional group of hydroxyls on the benzene ring can be more as active as than to functional group of hydroxyls on the cyclosix-member ring(resonance effect).Functional group of hydroxyls on the cyclosix-member ring has steric hinderance with cyclopropyl methyl and axial hydrogens 1,3 on the cyclosix-member ring.strong base triethyl amine catches proton of acid(OH-Phenol) drug of Naltrexone,and have also been reported that group of phenoxide of drug has been done nucleophilic addition reaction.The following picture presentscovalent attachment drug of Naltrexone to MWCNTs.

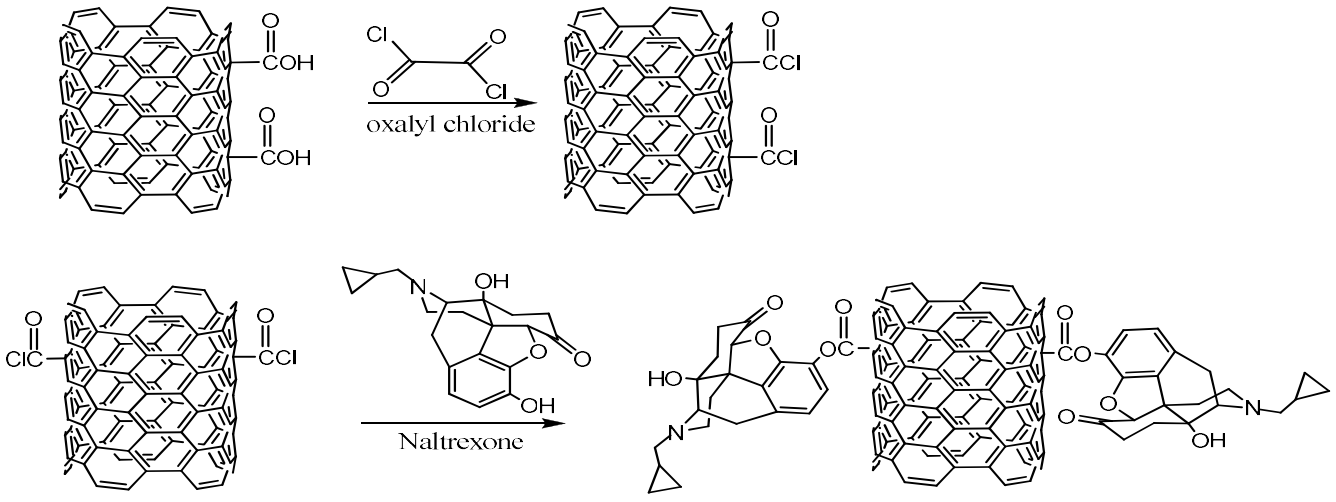

Figure 1: covalent attachment drug of Naltrexone to MWCNTs

2-5-instrument

FTIR spectra ranging from 800 to 3500cm^{-1} were recorded with 4 cm^{-1} resolution using a Thermo Nicolet Nexus 870.Raman spectra were recorded using Nicolet Dispersive Raman

spectrometer with a Nd:YlF Laser(532nm).Thermo Gravimetric Analysis(TGA) was conducted in argon with a heating rate 10°C/min using a TGA Q50 Build 1890 morphology of MWCNTs were characterized by Scanning Electron Microscopy(SEM,Filips XL30),Field Emission Scanning Electron Microscopy(FESEM,Model Hitachi),Transmission Electron Microscopy (TEM,microscope set at an accelerating voltage of 120KV),Atomic Force Microscopy (AFM,model Hitachi). Elemental Analysis was characterized by the Perkin Elmer 2400 series 2.instrument HPLC is model MerckHitachi.

3-Result and discussion

3-1-FT-IR

Blue spectrum of MWCNT-COOH in figure 2 shows band entered at 3424cm^{-1} is the contribution from the OH stretching mode of COOH groups,at 1711cm^{-1} and 1141cm^{-1} can be indicated stretching vibration C=O in COOH groups,stretching vibration C-O in COOH group,respectively[26].The bands also at 3268cm^{-1} and 1021cm^{-1} can be attributed to stretching vibration OH of phenolic and C-Oof phenolic,respectively,in oxidation process.Also bands at 1554cm^{-1},2833-3054cm^{-1}, 3152 cm^{-1} can be attributed to C=C stretching of benzenoid rings in CNTs,asymmetric and symmetric stretching of C-H in rings CNTs, stretching vibration C-H in rings CNTs,respectively[27,28].Black spectrum of MWCNT-OX in figure 2 confirms the attachment drug of Naltrexone to functionalized MWCNTs.The bands at 1713cm^{-1} and 1140cm^{-1} can be attributed to stretching vibration C=O of esters,C-O esters,respectively[29].The bands at 998cm^{-1} and 1437cm^{-1} can be attributed to out of plane bending vibration C-H in drug of Naltrexone,bending vibration C-H in drug,respectively,and by reducing band OH of carboxyl in nano-ester can be demonstrated covalent.attachment drug of Naltrexone to MWCNTs.

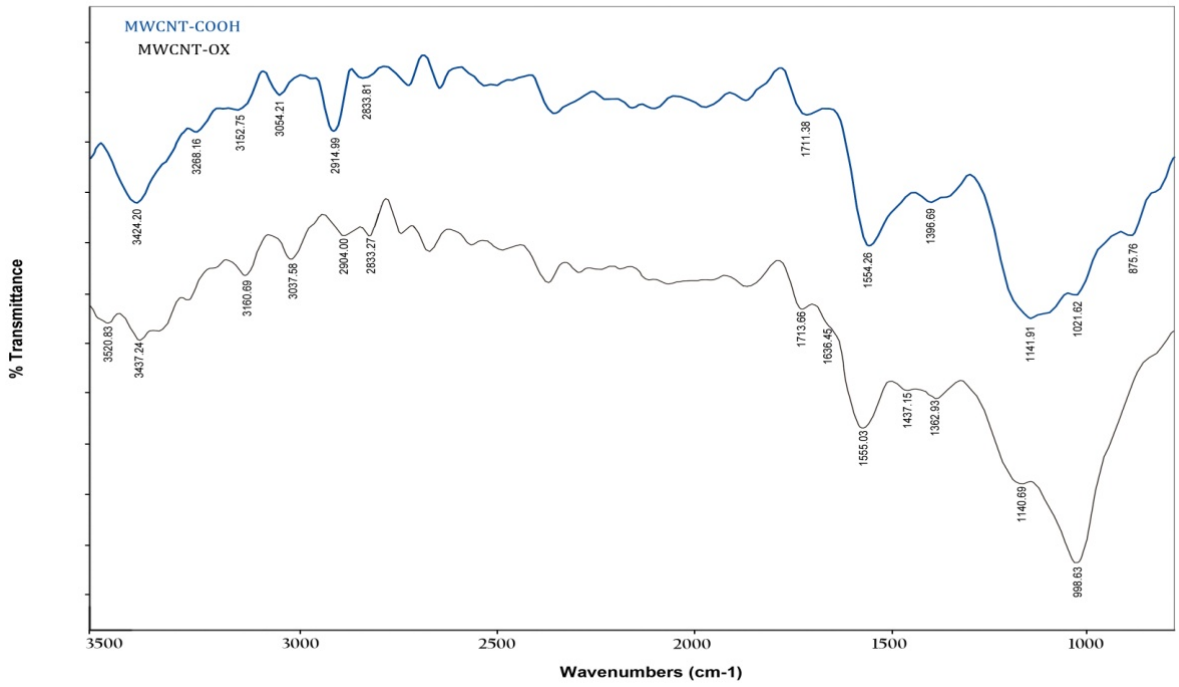

Figure 2: FT-IR MWCNT-COOH and MWCNT-OX

3-2-Raman Spectroscopy

The following Raman spectral analysis of different functionalized MWCNTs was also performed.The MWCNTs that were carboxylated showed bands at 1569cm^{-1} and 1326cm^{-1},whereas acylated MWCNTs presented bands at 1575cm^{-1} and 1334cm^{-1},and nano-ester showed Raman shift at 1572cm^{-1} and 1335cm^{-1}[30].We reported peak intensity of the MWCNT-g-Naltrexone was slightly stronger than to MWCNT-COOH and MWCNT-COCl.Therefore, adherence antagonist drug of Naltrexone to MWCNTs via a covalent interaction can also be seen from Raman spectra.The increase intensity D-band of MWCNT-g-Naltrexone can be to some damage of the graphite sheet caused by the ester functional group on the surface of CNTs or it might also be due to a difference in energy transfer between the MWCNT-COOH and Naltrexone molecular or the influence of the grafted Naltrexone on the electronic properties of the MWCNT-COOH[31,32]. We reported relative degree of functionalization for MWCNT-COOH,MWCNT-COCl,MWCNT-OX,1.35,1.41,1.30,respectively.In this work, we reported that with esterification of functionalized carbon nanotube using oxalyl chloride reagent, peak D-band and G-band of MWCNT-COOH are transposed to the high frequency

region, as shown for synthetic sample in fig 3. This phenomenon can confirm the covalent attachment of Naltrexone to functionalized carbon nanotubes in Raman spectroscopy [33].

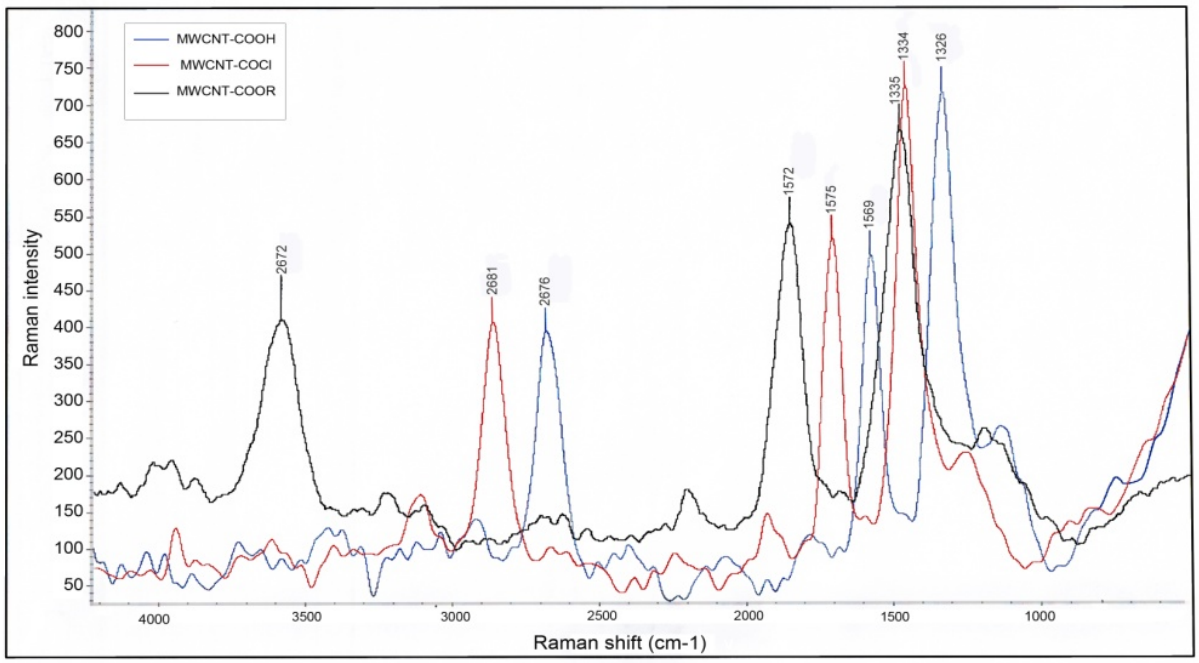

Figure 3: Raman MWCNT-COOH,MWCNT-COCl,MWCNT-OX

3-3-Thermo Gravimetric Analysis(TGA)

As functionalized MWCNT were tested by ThermogravimetricAnalysis(TGA,figure 3).TGA was performed under argon samples were heated from room temperature to 800°C at scan rate of 10°C/min. In TGA graphs of MWCNT-COOH and MWCNT-OX,two distinct decompositions are observable.The first one(bellow 450°C) can be assigned to ester and Naltrexone,while the second one(above 450°C) is related to nanotube as comparing to MWCNT-COOH thermogram.If the mass loss of MWCNT-COOH at 450°C(3.7%) is used as the reference,the mass loss of functionalized MWCNT by Naltrexone at 450°C is about(11.7%).The first decomposition temperature and second decomposition temperature functionalized MWCNT by Naltrexone(black thermogram)are 250°C and 450°C,respectively[34].

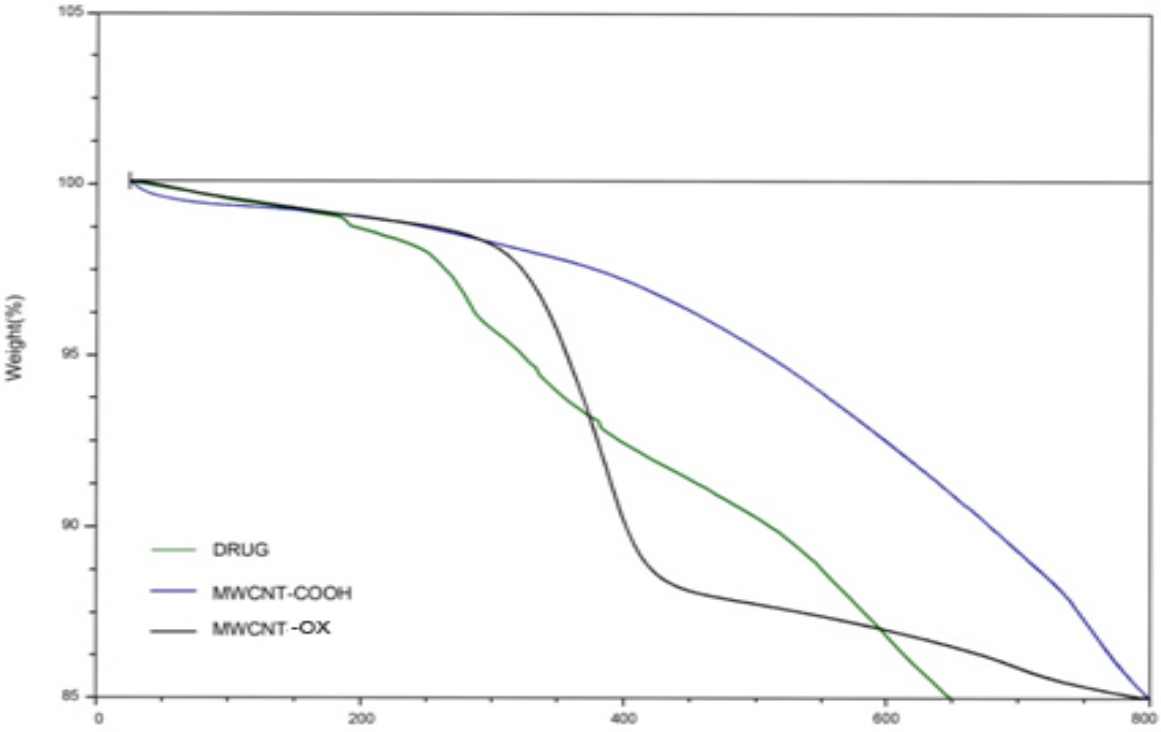

Figure 4: thermogravimetric analysis of Drug,MWCNT-COOH,MWCNT-OX

3-4-SEM,FESEM,AFM

The figures 5,6,7, present Scanning Electron Microscopy(SEM),Field Emission Electron Microscopy (FESEM),Atomic Force Microscopy(AFM)of MWCNT-OX,respectively.According to our SEM,FESEM, AFM results,black strands in the figures show strands of MWCNTs,and white strands show functionalization on surface of CNTs,it can be reported which diameters of CNTs increase with functionalization[35].In addition to,SEM,FESEM,AFM,we reported Transmission Electron Microscope(TEM) of MWCNT-OX in figure 7.

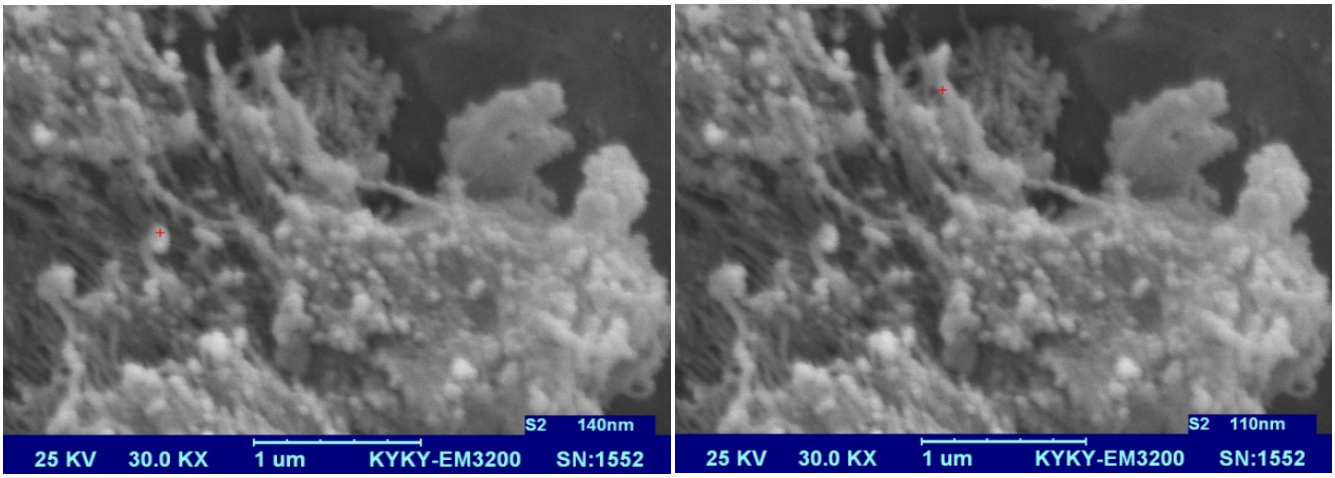

Figure 5: Scanning Electron Microscopy of MWCNTs-OX

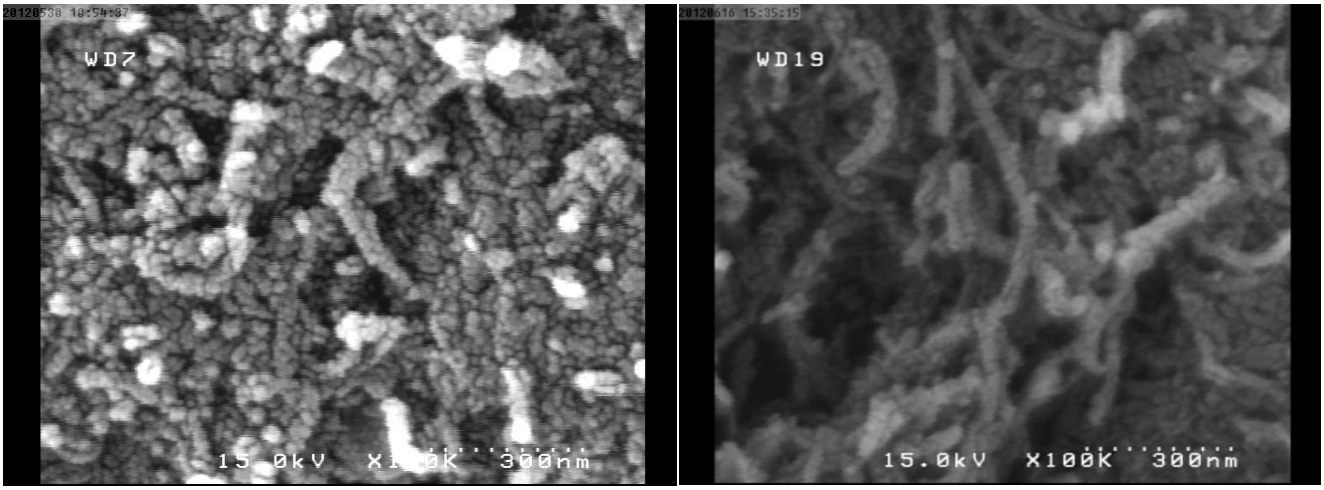

Figure 6: Field Emission Scanning Electron Microscopy of (A)MWCNT-COOH,(B)MWCNT-OX

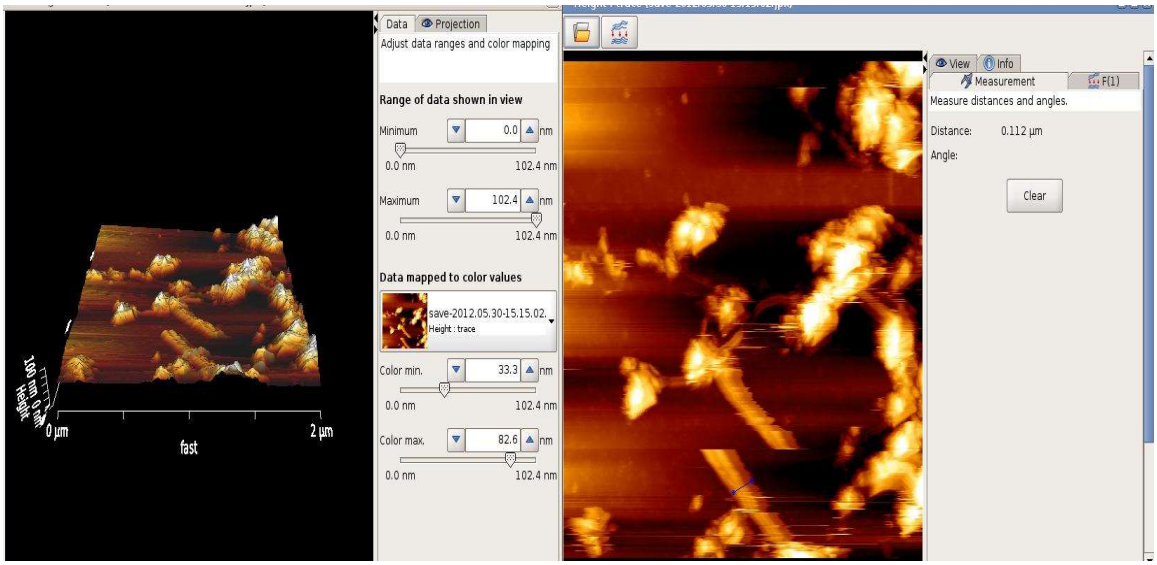

Figure 7: Atomic Force Microscopy of MWCNT-OX

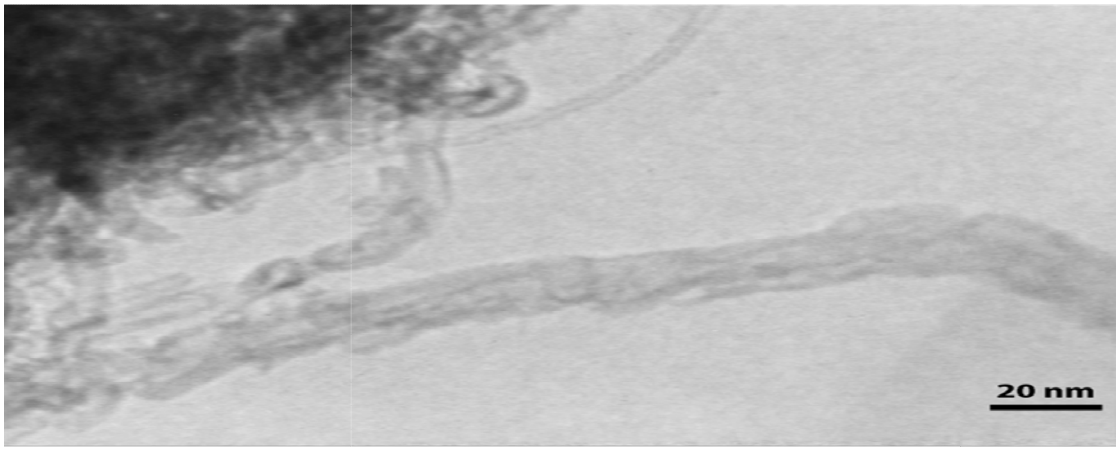

Figure 8: Transmission Electron Microscopy of MWCNT-OX

3-5-Elemental Analysis

Elemental Analysis of the modified-MWCNT 1-2 is shown in table 1.Apart from the carbon values,the atomic percentages of H, 1.76 and N 2.54%, 2(as compared to 1) indicated that 1 is functionalized with Naltrexone. On the other hand,increasing of percentage of H from 0.04 to 1.76% and N from 0.35 to 2.54% confirms covalent attachment Naltrexone to MWCNT-COCl.

Table 1: Elemental Analysis of the modified-MWCNT 1-2

MWCNT	C%	H%	N%
1	95.26	0.04	0.35
2	83.02	1.76	2.54

3-6-HighPerformance Liquid Chromatography(HPLC)

200µg/ml neat drug of Naltrexone injected to instrument HPLC,then 50mg of Nano drug were poured into flask and was added 20ml acidic buffer and then was stirred for 2 hour until is hydrolyzed nano drug.Drug releasing from grafted MWCNT-drug is indeed a hydrolysis reaction,which involves breaking of ester band in acidic buffer.after 2 hrs.,is segregated nanotube and acidic buffer of each other by filter.Then 20µL drug of hydrolysis injected to instrument HPLC.The attachment quantitative drug of Naltrexone to MWCNTs is resulted by considering under surface peak of neat Naltrexone and nano drug of hydrolysis.We reported the attachment quantitative drug of Naltrexone to MWCNTs by oxalyl chloride reagent in this work. The Table 2 shows percent quantitative attachment drug of Naltrexone to MWCNT-COCl.also, the covalent attachment drug to functionalized carbon nanotubes can be confirmed by HPLC in Fig 9.

Table 2: quantitative attachment drug of Naltrexone to MWCNT-COCl

Sample	Mean area(n=3)	Drug concentration in solution of nano drug based on (Microgram/ml)	The amount of drug in the sample based on)mg/ml)	Percent of drug in the sample
Standard (200microgram/ml)	568150			
MWCNT-OX	549184	193.3235941	.193323594	7.435522851

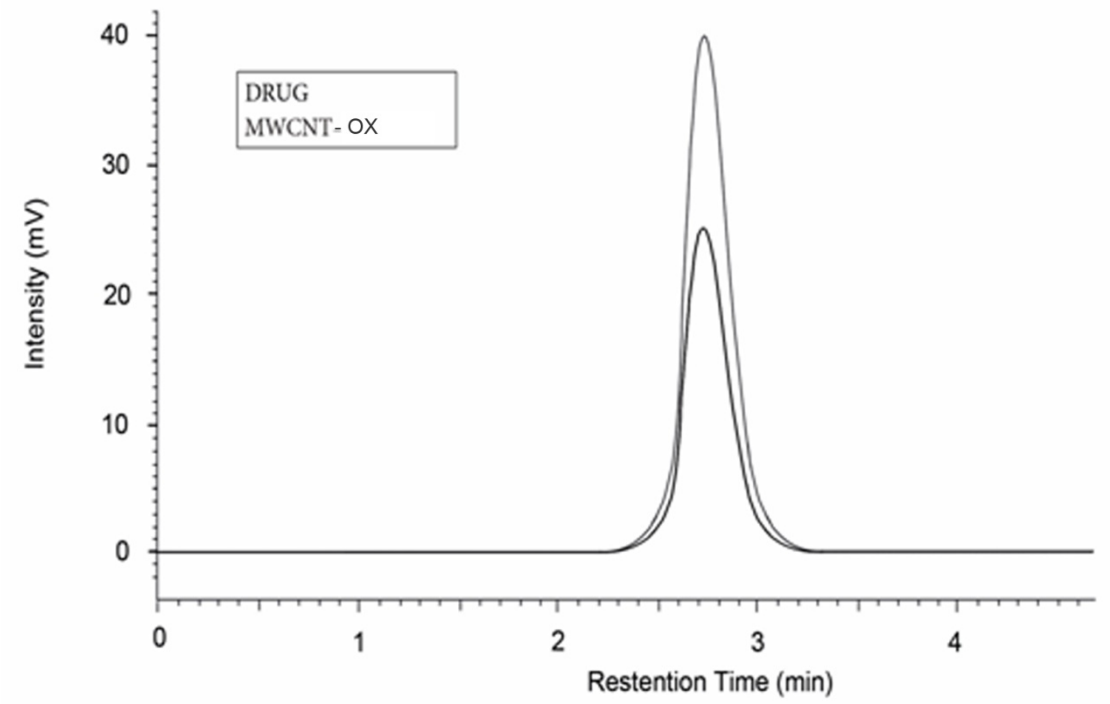

Figure 9: High Performance Liquid Chromatography of Drug,MWCNT-OX

4- Conclusion

Drug of Naltrexone have been attached to MWCNTs through covalent attachment to MWCNTs-COCl.The MWCNTs have been prepared by chemical vaporization deposition method and then functionalized with acyl chloride group.The process follows three steps,(1)the MWCNTs were functionalized with carboxylic groups using acid oxidation treatments,(2)these carboxylic groups were further treated with oxalyl chloride to introduce acyl chloride groups via acyl formation,and(3) acyl chloridewas treated with antagonist drug of Naltrexone.The SEM,FESEM,AFM,TEM, observations clearly confirm the covalent attachment of Naltrexone to the acyl chloride-MWCNTs.The chemical linking Naltrexone to acyl chloride-MWCNTs have been confirmed by FTIR spectroscopy.The FT-IR results show the presence of bands at 998cm^{-1} and 1437cm^{-1} can be attributed to out of plane bending vibration C-H,bending vibration C-H in drug of Naltrexone,respectively,and reducing band OH of carboxyl in nano ester have confirmed covalent attachment drug to MWCNTs. In Raman spectroscopy,we reported peak intensity of the MWCNT-g-Naltrexone was slightly stronger than to MWCNT-COOH and MWCNT-COCl,and the covalent attachment drug to MWCNTs to high frequency regions have been confirmed of Raman spectroscopy. Increasing of percentage of H from 0.04% to 1.76% and N from 0.35% to 2.54%,and first decomposition bellow 250°C,and releasing of drug have been confirmed by elemental analysis,TGA,HPLC,respectively.

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