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The attachment opioid antagonist drug of Naltrexone to multi-walled carbon nanotubes by different kinds of reagents carbodiimides

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ABSTRACT

Surface functionalization of multi-walled carbon nanotubes(MWCNTs) by opioid antagonist drug of Naltrexone via chemical modification of carboxyl groups,using of reagents di cyclohexyl carbodiimide(DCC),1-ethyl-3[3-dimethylaminopropyl] carbodiimidehydrochloride(EDC),N-hydroxysuccinimide(NHS),1-hydroxybenzotriazolemonohydrate(HOBT),O-(Benzotriazole-1-yl)-1,1,3,3-tetramethyluraniumtetrafluoroborate(TBTU),were performed. In synthetic organic chemistry,compounds containing the carbodiimide functionality are dehydration agents and are often used to activate carboxylic acids towards amide or ester formation. Carboxylic acids will react with the carbodiimide to produce the key intermediate as O-acylisourea which can be considered as a carboxylic ester with an activated leaving group.We have used of five carbodiimide reagent to synthesis twelve sample in this work.The resulting materials were characterized by different techniques,such as Fourier transform infraredspectroscopy (FT-IR), Thermogravimetricanalysis (TGA),Raman spectroscopy,Scanning Electron Microscopy (SEM),Field Emission Scanning Electron Microscopy(FESEM),Atomic Force Microscopy(AFM),High performance liquid chromatography(HPLC).

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Introduction

The drugs of choice for the treatment of severe pain are the opioid, the activities of which are mediated by their interaction with specific membrane bound 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[4-7].while agonists represents the majority of drugs for the treatment of chronic pain and much consumption and daily doses of antagonists, such as Naltrexone,Nalbuphine,the undesirable effects associate with them, such as respiratory depression[8],development of tolerance and dependence[9],nausea[10],and constipation[11]and etc.,have directed considerable efforts toward the discovery of novel agents with fewer or no adverse side effects.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 an antagonist is used extensively as pharmacologic tools for the investigation of opioid receptors [12]. Indeed, the recent research literature attests to the impact that such antagonist have made in this previous active research area. However, 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. Slow release is critically significant in drug delivery for minimizing the amount of drug lost before reaching the target. Shell structures and supports can be used for slow

delivery of drugs and are usually made from organic materials.Forexample,liposomes [13-15],microspheres [16],polymericshells [17]and polymeric micelles [18-19]have been well investigated in constructing a drug delivery system from organic materials, the combination of shell or support materials, targetingmolecules, 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, nanomedicine, which is an emerging bridge linking nanotechnology and advanced medical technology involves the exploration of nano scaled 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. Functionalized 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 way, the patient is possible is be caused at first stage respiratory depression, heartattack, intense convulsion and 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 by different kinds of carbodiimides reagents, which it can be solid phase of carbon nanotube. The drug carrier of Naltrexone is assuming will be used as skin absorption in this work in the recent future, which the attachment drugs antagonist to carbon nanotubes will desire to absorb by the skin layers and hairs on the skin surface. It will suggest introduce as drug carrier to blood and there it can be transferred to the brain. The absorption opioid antagonist drug on the surface carbon nanotube by the covalent functionalization.it can be promised for reducing dangers of such as; respiratorydepression, heartattack, intenseconvulsive, death and etc.,In the recent future.

2-Experimental Section

2-1-Materials

Multi-walled carbon nanotubes were purchased from Chengdu organic chemistry Co.Ltd.Chinese Academy of Science,were synthesized by catalytic chemical vapor deposition,purity of the as-received MWCNT materials were>95% and their lengths of these MWCNTs were 24-30µm.These specification details were given by the manufacturer.The opioid antagonist drug was purchased from Tolid Daru Co (Tehran,Iran).di cyclohexyl carbodiimide hydrochloride(DCC),1-ethyl-3-[3-dimethylaminopropyl] carbodiimidehydrochloride(EDC),N-hydroxysuccinimide(NHS),1-Hydroxybenzotriazolemonohydrate(HOBT),O- (Benzotriazole-1-yl)-1,1,3,3-tetrafluoroborate(TBTU),dimethylformamide (DMF),Tetrahydrofuran (THF)were obtained from Merk Co.THF and DMF were poured in Na with benzophenone,molecular sieve (0.4nmdiameter,2mm length),and were obtained anhydrous THF,anhydrous DMF,respectively,before use.

2-2-Preparation of MWCNT-COOH

0.5g of raw-MWCNTs were placed into a round bottom flask.Raw-MWCNTs were treated with a (v/v 3:1) 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 vacuum-filtered,and thoroughly rinsed with Deionized Water and result product was dried at 50°C in a vacuum oven for 24hr.

2-3-The attachment carbodiimides reagents to MWCNT-COOH

2-3-1-The attachment drug of Naltrexone to MWCNTs-COOH by reagent of DCC

0.2g of the MWCNTs-COOH were added into 250ml round bottom flask,and were stirred in 100ml of anhydrous DMF for 15 min under argon gas.0.3g DCC were added to flask,and were sonicated for 30 min to give a homogeneous suspension,under argon gas,after 30 min,mixture in flask was stirred for 1h,then by adding drug of Naltrexone,Flask was stirred for 6 hr under a magnetic stirring condition.The reaction mixture was then filtered on a PTFE membrane filter.The black solid collected on the filter was washed with excess anhydrous THF and DMF,and then resulting derivative was dried at 50°C in a vacuum oven for 8hr.

2-3-2-The attachment drug of Naltrexone to MWCNTs-COOH by adding mixture of reagents DCC and 4-Dimethylaminopyridine(DCC-Base)

0.2g of the MWCNTs-COOH were added into 250ml round bottom flask,and were stirred in 100ml of anhydrous DMF and 0.1g 4-Dimethylaminopyridine (DMAP) for 40 min under argon gas.after 40 min,0.3g DCC were added to flask,and were sonicated for 30 min to give a homogeneous suspension,under argon gas.mixture into flask was stirred for 1hr,then by adding drug of Naltrexone,Flask was stirred for 24hr under a magnetic stirring condition.The reaction mixture was then filtered on a PTFE membrane filter,the resulting solid on filter was washed with excess anhydrous THF and DMF,and was dried at 50°C in a vacuum oven for 8hr.

2-3-3-The attachment drug of Naltrexone to MWCNTs-COOH by reagent of EDC

0.2g of the MWCNTs-COOH were added into 250ml round bottom flask,and were stirred in 100ml of anhydrous DMF for 15 min under argon gas.0.3g EDC were added to flask,and were sonicated for 30 min to give a homogeneous suspension under argon gas. After 30 min mixture into flask was stirred for 1hr,then by adding drug of Naltrexone flask was stirred for 6hr under a magnetic stirring condition.The reaction mixture was then filtered on a PTFE membrane filter,the resulting solid on filter was washed with excess anhydrous THF and DMF,and was dried at 50°C in a vacuum oven for 8hr.

2-3-4-The attachment drug of Naltrexone to MWCNTs-COOH by adding mixture of reagents EDC and 4-Dimethylaminopyridine(EDC-Base)

0.2g of the MWCNTs-COOH were added into 250ml round bottom flask,and were stirred in 100ml of anhydrous DMF and 0.1g 4-Dimethylaminopyridine(DMAP) for 40 min under argon gas.0.2g EDC were added to flask,and were sonicated for 30 min to give a homogeneous suspension,under argon gas.after 30 min,mixture into flask was stirred for 24hr under a magnetic stirring condition.the reaction mixture was then filtered on a PTFE membrane filter,The resulting solid on filter was washed with excess anhydrous THF and DMF,and was dried at 50°C in a vacuum oven for 8hr.

2-3-5-The attachment drug of Naltrexone to MWCNTs-COOH by reagent of NHS

0.2g of the MWCNTs-COOH were added into 250ml round bottom flask,and were stirred in 100ml of anhydrous DMF for 15 min under argon gas.0.3g NHS were added to flask,and we sonicated for 30 min to give a homogeneous suspension,under argon gas.mixture into flask was stirred for 1hr,then by adding drug and 0.1g DMAP,Flask was stirred for 32hr under a magnetic stirring condition.The reaction mixture was then filtered on a PTFE membrane filter,The resulting solid on filter was washed with excess anhydrous THF and DMF,and was dried at 50°C in a vacuum oven for 8hr.

2-3-6-The attachment drug of Naltrexone to MWCNTs-COOH by adding mixture of reagents NHS and DCC(NHS-DCC)

0.2g of the MWCNTs-COOH were added into 250ml round bottom flask,and were stirred in 100ml of anhydrous DMF for 15 min under argon gas.0.25g DCC were added to flask,and were sonicated for 30 min,then 0.15g NHS were added to flask,and were sonicated for another 30 min.Flask was stirred for 1hr,then by adding drug and 0.1g DMAP,Flask was stirred for 24hr under a magnetic stirring condition.The reaction mixture was then filtered on a PTFE membrane filter,The resulting solid was washed with excess anhydrous THF and DMF,and was dried at 50°C in a vacuum oven for 8hr.

2-3-7-The attachment drug of Naltrexone to MWCNTs-COOH by adding mixture of reagents NHS and EDC(NHS-EDC)

0.2g of the MWCNTs-COOH were added into 250ml round bottom flask,and were stirred in 100ml of anhydrous DMF for 15 min under argon gas.0.25gEDC were added to flask,and were sonicated for 30 min,then 0.15g NHS were added to flask,and were sonicated for another 30 min.After 30 min,flask was stirred for 1hr, then by adding drug and 0.1g DMAP, flask was stirred for 24hr under a magnetic stirring condition.The reaction mixture was then filtered on a PTFE membrane filter,The resulting solid was washed with excess anhydrous THF and DMF,and was dried at 50°C in a vacuum oven for 8 hr.

2-3-8-The attachment drug to MWCNTs-COOH by reagent of HOBT

0.2g of the MWCNTs-COOH were added into 250ml round bottom flask,and were stirred in 100ml of anhydrous DMF for 15 min under argon gas.0.3g HOBT were added to flask,and were sonicated for 30 min to give a homogeneous suspension.Mixture into flask was stirred for 1hr,then by adding drug and 0.1g DMAP,flask was stirred for 42hr under a magnetic stirring condition. The reaction mixture was then filtered on a PTFE membrane filter,The resulting solid was washed with excess anhydrous THF and DMF,and was dried at 50°C in a vacuum oven for 8hr.

2-3-9-The attachment drug to MWCNTs-COOH by adding mixture of reagents HOBT and DCC (HOBT-DCC)

0.2g of the MWCNTs-COOH were added into 250ml round bottom flask,and were stirred in 100ml of anhydrous DMF for 15 min under argon gas.0.25g DCC were added to flask,and were sonicated for 30 min,then 0.15g HOBT were added to flask,and were sonicated for another 30 min.Flask was stirred for 1hr,then by adding drug and 0,1g DMAP,Flask was stirred for 24hr under a magnetic stirring condition.The reaction mixture was then filtered on a PTFE membrane filter, The resulting solid was washed with excess anhydrous THF and DMF,and was dried at 50°C in a vacuum oven for 8hr.

2-3-10-the attachment drug to MWCNTs-COOH by adding mixture of reagents HOBT and EDC(HOBT-EDC)

0.2g of the MWCNTs-COOH were added into 250ml round bottom flask,and were stirred in 100ml of anhydrous DMF for 15 min under argon gas.0.25g EDC were added to flask,and were sonicated for 30 min,then 0.15g HOBT were added to flask,and were sonicated for another 30 min.Flask was stirred for 1hr, then by adding drug and 0.1g DMAP,flask was stirred for 24hr under a magnetic stirring condition. The reaction mixture was then filtered on a PTFE membrane filter, The resulting solid was washed with excess anhydrous THF and DMF,and was dried at 50°C in a vacuum oven for 8hr.

2-3-11-The attachment drug of Naltrexone to MWCNTs-COOH by adding mixture of reagents HOBT and TBTU(HOBT-TBTU)

0.2g of the MWCNTs-COOH were added into 250ml round bottom flask,and were stirred in 100ml of anhydrous DMF for 15 min under argon gas.0.25g TBTU were added to flask,and were sonicated for 30 min,then 0.15g HOBT were added to flask,and were sonicated for another 30 min.Flask was stirred for 2hr, then by adding drug and 0.1g DMAP,flask was stirred for 24hr under a magnetic stirring condition.The reaction mixture was then filtered on a PTFE membrane filter, The resulting solid was washed with excess anhydrous THF and DMF,and was dried at 50°C in a vacuum oven for 8hr.

2-3-12-The attachment drug of Naltrexone to MWCNTs-COOH by reagent of TBTU

0.2g of the MWCNTs-COOH were added into 250ml round bottom flask,and were stirred in 100ml of anhydrous DMF for 20 min under argon gas.0.3g TBTU were added to flask,and were sonicated for 30 min to give a homogeneous

suspension,under argon gas.Then mixture into flask was stirred for 2hr,then by adding drug and 0.1g DMAP,flask was stirred for 48hr under a magnetic stirring condition.The reaction mixture was then filtered on a PTFE membrane filter, The resulting solid was washed with excess anhydrous THF and DMF,and was dried at 50°C in a vacuum oven for 8hr.

2-3-13-Instrument

FT-IR spectra were recorded using a thermo Nicolet, Raman spectra were recorded using Nicolet Dispersive Raman spectrometer with a Nd:YLF laser(532nm).Thermogravimetric analysis (TGA) was conducted in Nitrogen with a heating rate of 20°C/min.morphology of MWCNTs were characterized by Field Emission Scanning Electron Microscopy (FESEM). Instrument HPLC is model MerckHitachi and detector is used model L-7400, stationary phase C_{18} with groups octane.

Result and Discussion

3-1-FT-IR

Fig1 shows FT-IR spectrum of sample MWCNT-COOH in range 800-3500cm^{-1}.The bands around 1711cm^{-1},3424cm^{-1},1141cm^{-1} are due to stretching mode C=O of COOH,stretching mode OH of COOH groups,stretching mode C-O of COOH groups,respectively[26].The bands in 3268cm^{-1},1021cm^{-1}are due to stretching mode OH,stretching mode C-O alcohol side wall carbon nanotube, and also peaks at 875cm^{-1},1396cm^{-1},2833cm^{-1},2914cm^{-1},3054cm^{-1},3152cm^{-1} can be attributed to out of plane deformation of C-H in rings CNTs,stretching sulphonic acid group,symmetric and asymmetric stretching mode C-H,stretching mode C-H in rings CNTs respectively. In synthetic organic chemistry,compounds containing the carbodiimide functionality are dehydration agents and are often used to active carboxylic acids towards amide or ester formation. Carboxylic acids will react with the carbodiimide to produce the key intermediate as O-acylisourea which can be considered as a carboxylic ester with an activated leaving group. In other words,carbodiimide reagents DCC,EDC,NHS,HOBT,TBTU, suitable dehydration agents are for esterification and amination of synthetic reactions,alsothe result in synthetic process,The production are water molecules in mix. Also peaks about 3400-3500cm^{-1} in spectrums can be considered to stretching mode OH in water molecular. In peaks FT-IR,bands at about 1618-1680cm^{-1} can be attributed to C=O ketone drug of Naltrexone in ester-nanodrug,at about 1700-1730cm^{-1} to C=O ketone of formation ester with CNTs.because interaction H-bonded ketone groups by water molecular,are transposed peak ketone group drug of Naltrexone of 1718cm^{-1} to around region 1620cm^{-1} and ester ketone group to around region 1700cm^{-1}.The bands at about 1000cm^{-1},1200cm^{-1} can be attributed to symmetric and asymmetric stretching modes COC ether in antagonist drug with CNTs,bands at about 1150-1190cm^{-1},1400cm^{-1} to stretching mode C-O ester,bending vibration C-H loaded drug with CNTs,respectively.

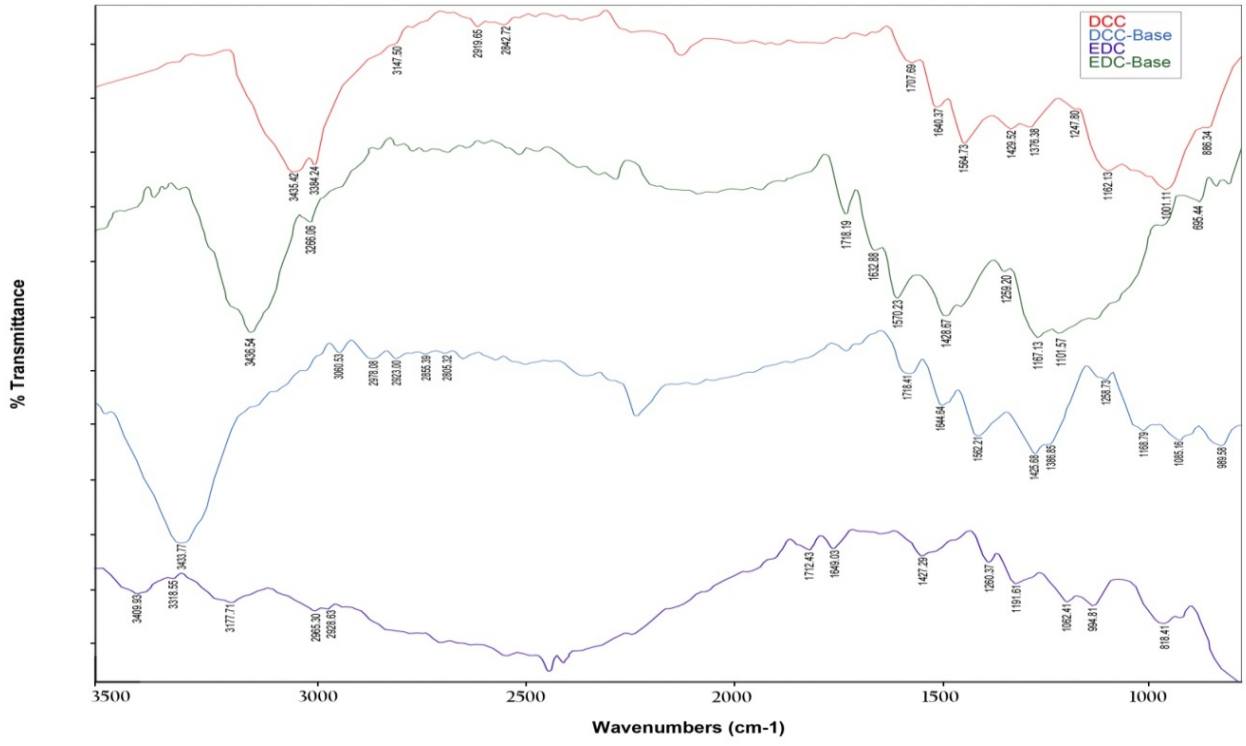


Figure1: FT-IR of drug of Naltrexone to MWCNT-COOH by carbodiimide reagents,DCC, DCC-Base, EDC,EDC-Base

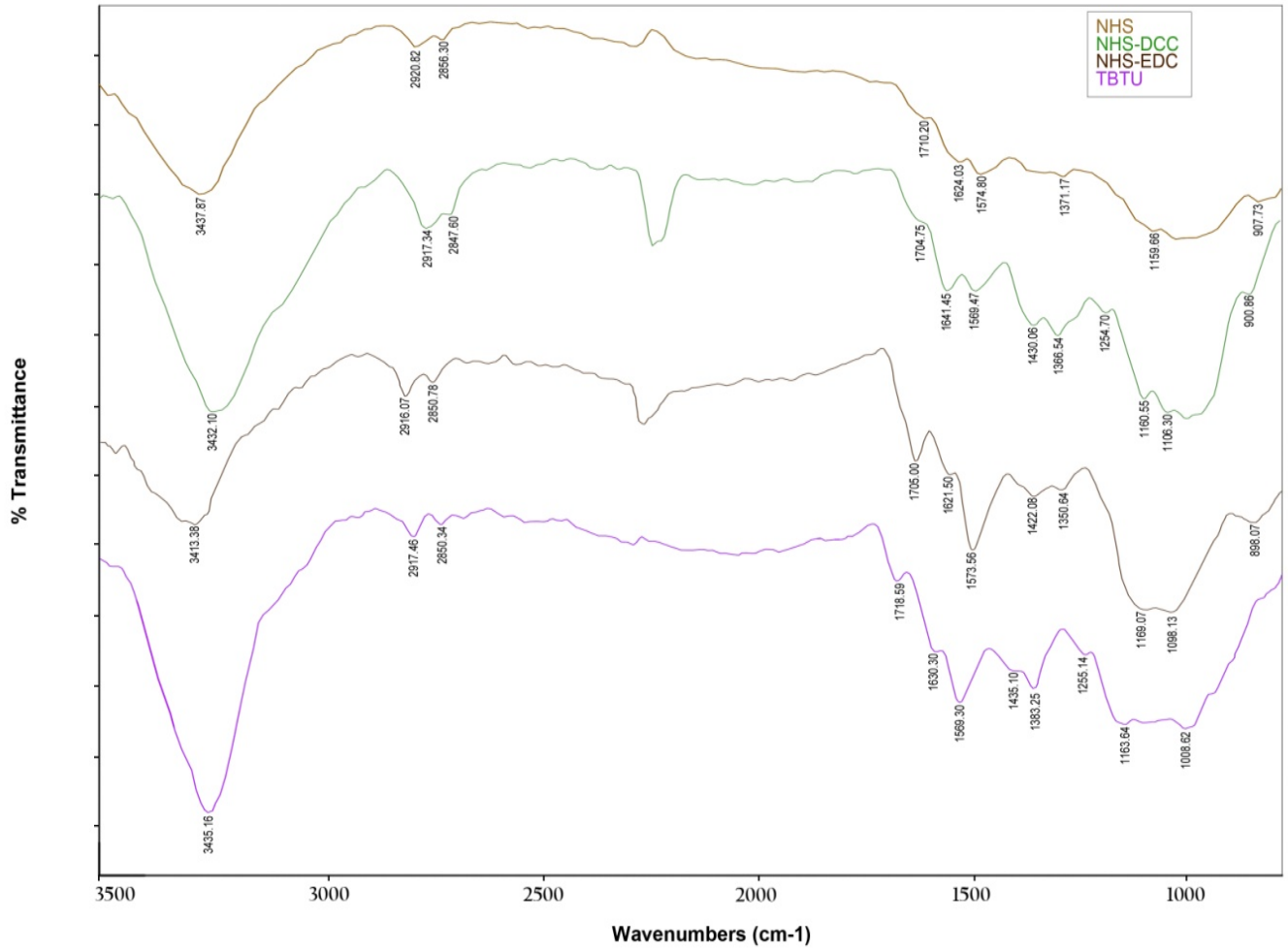


Figure 2: FT-IR of drug of Naltrexone to MWCNT-COOH by carbodiimide reagents,NHS,NHS-DCC,NHS-EDC,TBTU

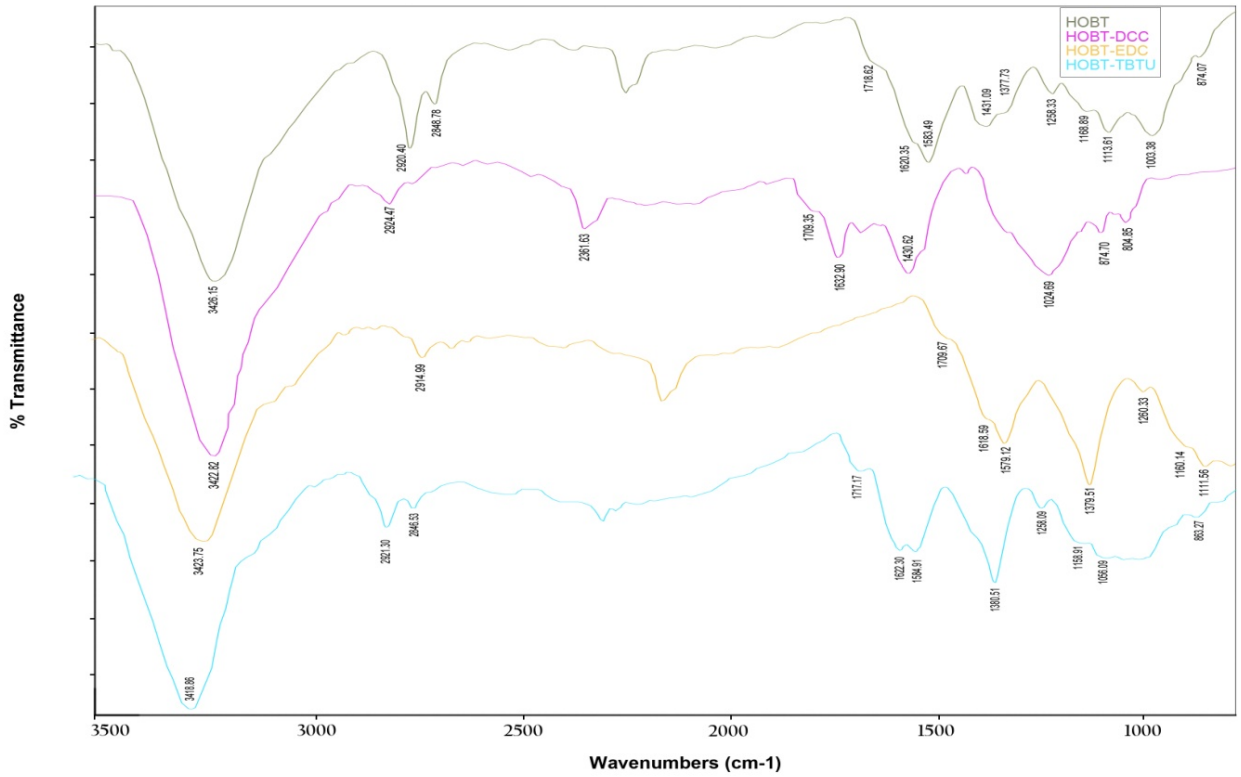


Figure3: FT-IR of drug of Naltrexone to MWCNT-COOH by carbodiimide reagents,HOBT, HOBT-DCC,HOBT-EDC,HOBT-TBTU

3-2-Raman Spectroscopy

Raman spectrum is divided to three area.first area is in range of situated at 0-500cm^{-1} that can be considered radial breathing mode (RBM).The second area is in range of situated at ~1000-1600cm^{-1} that can be considered first order Ramanspectrum. The third area is in range of situated at~1610-3500cm^{-1} that can be considered second order Raman spectrum.first order Raman spectrum is consisted of disorder band(D-band) and graphitic band(G-band).The D-band at approximated 1000-1400cm^{-1} is an A_{1g} breathing mode. This mode is generally attributed to the defects in the curved graphite sheet,SP^3 carbon.The D-band is considered to indicate disorder in the graphitic lattice or defects in nanotubes [27,28,29].The G-band at 1500-1600cm^{-1} is the E_{2g} model corresponding to the movement in opposite direction of two neighboring carbon atoms in a graphitic sheet.This model indicates the presence of crystalline graphitic carbon in MWCNTs [30]. The second Raman spectrum is consisted of D′-band and G′-band. D′-band and G′-band are overtone G-band and D-band,respectively. D′-band and G′-band are in range of situated at~1610-2000cm^{-1} and situated at~2400-2900cm^{-1},respectively. The ratio of intensity of the D and G band,$R=I_{D/IG}$ ratio,can be used to evaluate the relative extent of structural defects or relative degree of functionalization.commonly,the relative intensity ratios of D to G bands ($I_{D/IG}$)are utilized as an approach to monitoring the purity and functionalization of MWCNTs.An increase in the D-band intensity (I_D)has been shown to be an indication of side wall SP^2-SP^3 hybridization from covalent binding of functional various moieties [30].The intense D mode is observed when symmetry of the hexagonal SP^2 bonded lattice is disrupted,so it may determine the covalent functionalization,but may also be due to the presence of anamorphous carbon in the tested sample. In this work, the following table has been designed which shows peaks D-band and G-band and relative degree of functionalization of synthetic samples in Raman spectroscopy.For a MWCNT-COOH sample,a strong peak at around 1563cm^{-1}(G-band) represents a high frequency E_{2g} Raman scattering mode of SP^2 hybridized carbon material,bonded carbon atoms in a two-dimensional hexagonal lattice similar to the vibration of carbon atoms in a graphitic layer.a disordered structure induced peak at around 1327cm^{-1}(D-band)may be associated with the presence of defects in the hexagonal graphitic layer [30,31].are reported which with esterification functionalized carbon nanotube by carbodiimide reagent in this work,peaks D-band and G-band of MWCNTs-COOH are transposed to high frequency region.This high frequency region is reported for synthetic samples in the following table1.This subject can be confirmed by covalent attachment Naltrexone to functionalized carbon nanotubes by

Raman spectroscopy.Figs 4,5,6 present Raman drug of Naltrexone to MWCNTs-COOH by carbodiimide reagents.

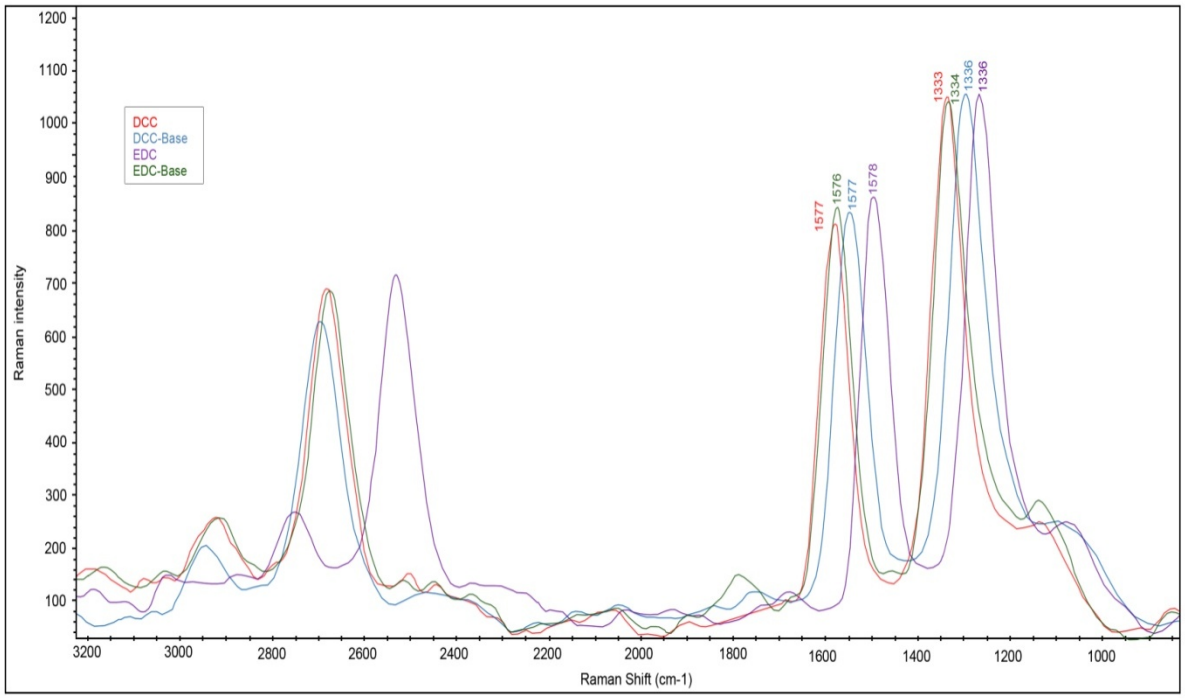


Figure 4: Raman drug of Naltrexone to MWCNT-COOH by carbodiimide reagents,DCC,DCC-Base, EDC,EDC-Base

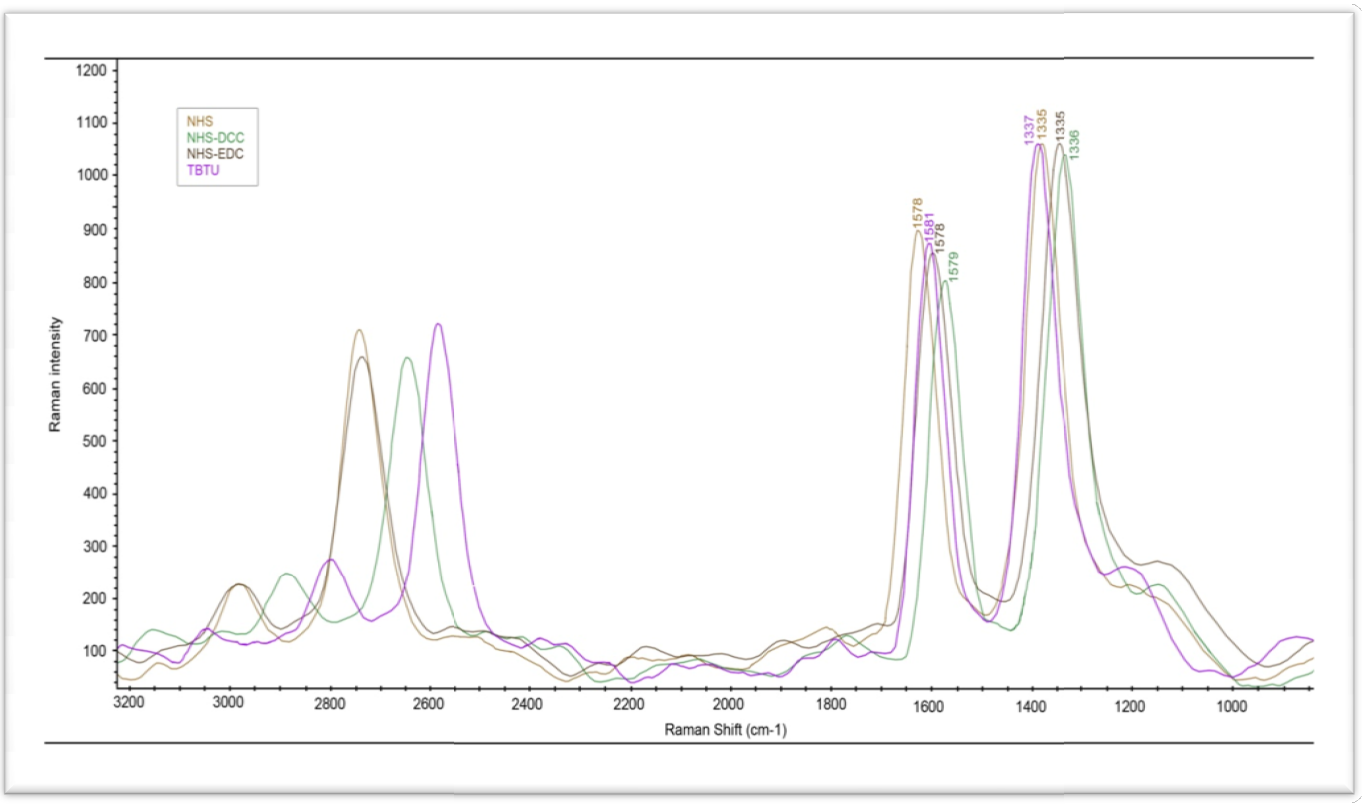


Figure 5: Raman drug of Naltrexone to MWCNT-COOH by carbodiimide reagents,NHS, NHS-DCC,NHS-EDC,TBTU

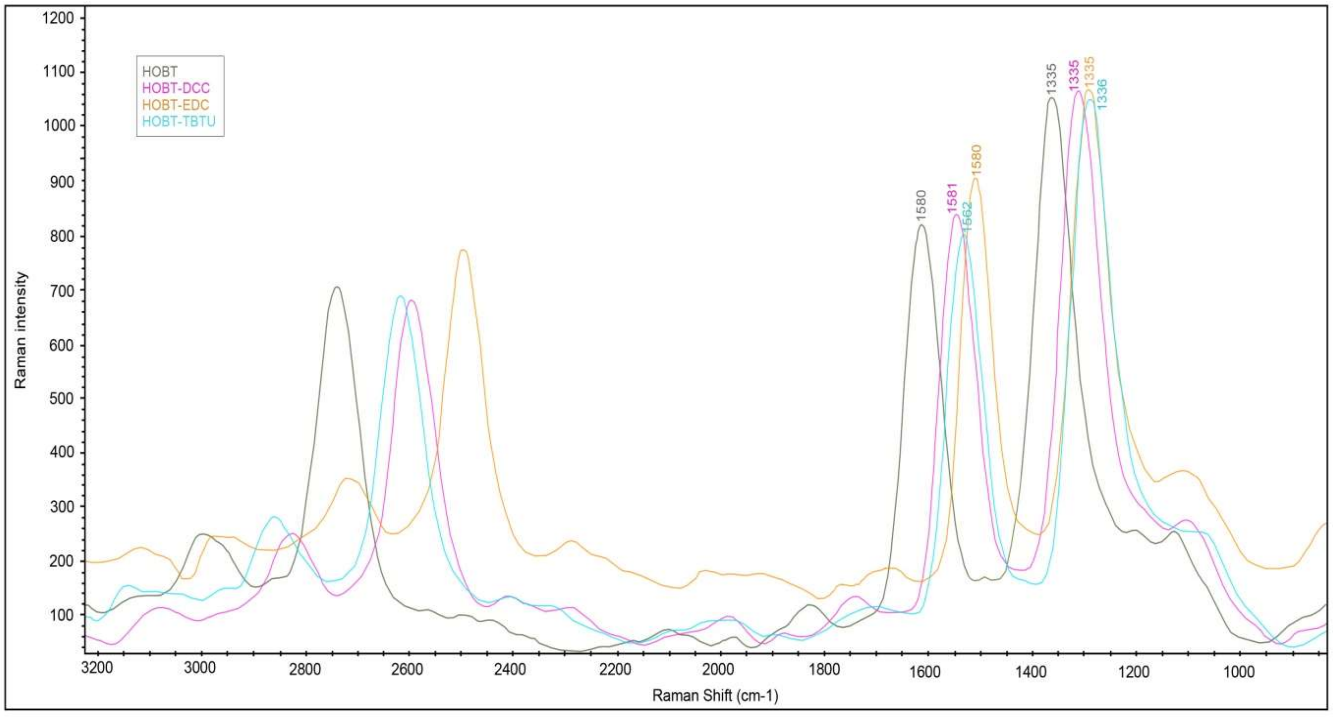


Figure 6: Raman drug of Naltrexone to MWCNT-COOH by carbodiimide reagents,HOBT, HOBT-DCC,HOBT-EDC,HOBT-TBTU

Sample	D-Band(Hertz)	G-Band	Relative Functionalization degree
MWCNT-COOH	1326	1569	1.35
DCC	1333	1577	1.25
DCC-Base	1336	1577	1.27
EDC	1336	1578	1.15
EDC-Base	1334	1576	1.20
NHS	1335	1578	1.15
NHS-DCC	1336	1579	1.30
NHS-EDC	1335	1578	1.20
HOBT	1335	1580	1.22
HOBT-DCC	1335	1581	1.20
HOBT-EDC	1335	1580	1.15
HOBT-TBTU	1336	1562	1.30
TBTU	1337	1581	1.17

Table 1: D-band,G-band, Relative functionalization degree

3-3-Thermo gravimetric analysis (TGA)

Gravimetric analysis is a type of testing performed on samples that determines changes in weight in relation to a temperature program in a controlled atmosphere.Such analysis relies on a high degree of precision in three measurements:Weight,temperature,and temperature change.Thermalgravimetric analysis is the act of heating a mixture to a high enough temperature so that one of the components decomposes into a gas,which dissociates into the air. It is a process that utilized heat and stoichiometry ratios to determine the percent by mass ratio of a solute.If the compounds in the mixture that remain are known,Then the percentage by mass can be determined by taking the weight of what is left in the mixture and dividing it by the initial mass. Functionalized MWCNT were tested by thermogravimetric analysis.TGA was performed under Nitrogen. Samples were heated from room temperature to 800°C at a scan rate of 20 °C/min. In TGA graphs of MWCNT-COOH and samples,two distinct decompositions are observable.The first one (bellow 450°)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 MWCNTs by Naltrexone and carbodiimide reagents at 450°C is about 10-12%.The first decomposition temperature and second decomposition temperature functionalized MWCNT by Naltrexone and carbodiimide reagents are 250°C and 450°C in spectrum,respectively [32].

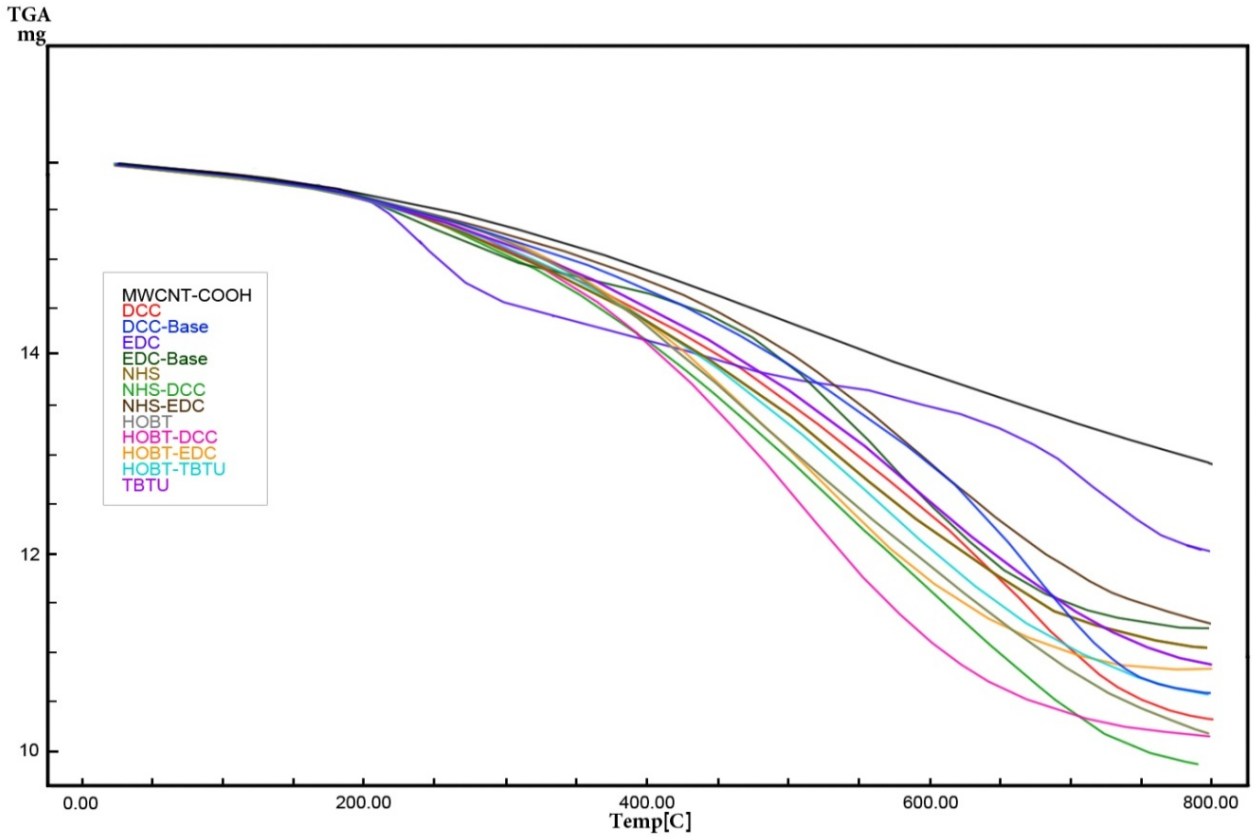


Figure7: Thermo gravimetric analysis drug of Naltrexone to MWCNT-COOH by carbodiimide reagent

3-4-Field Emission Scanning Electron Microscopy (FESEM)The figure 8,9,10 present Field Emission Scanning Electron Microscopy drug of Naltrexone to MWCNT-COOH by carbodiimide reagents,DCC,DCC-Base,EDC-Base, NHS, NHS-DCC,HOBT-DCC,respectively.According to our FESEM results,black strands in the figure show strands of MWCNTs,and white strands show functionalization on surface of CNTs.It can be reported which diameters of CNTs increase with functionalization [33].

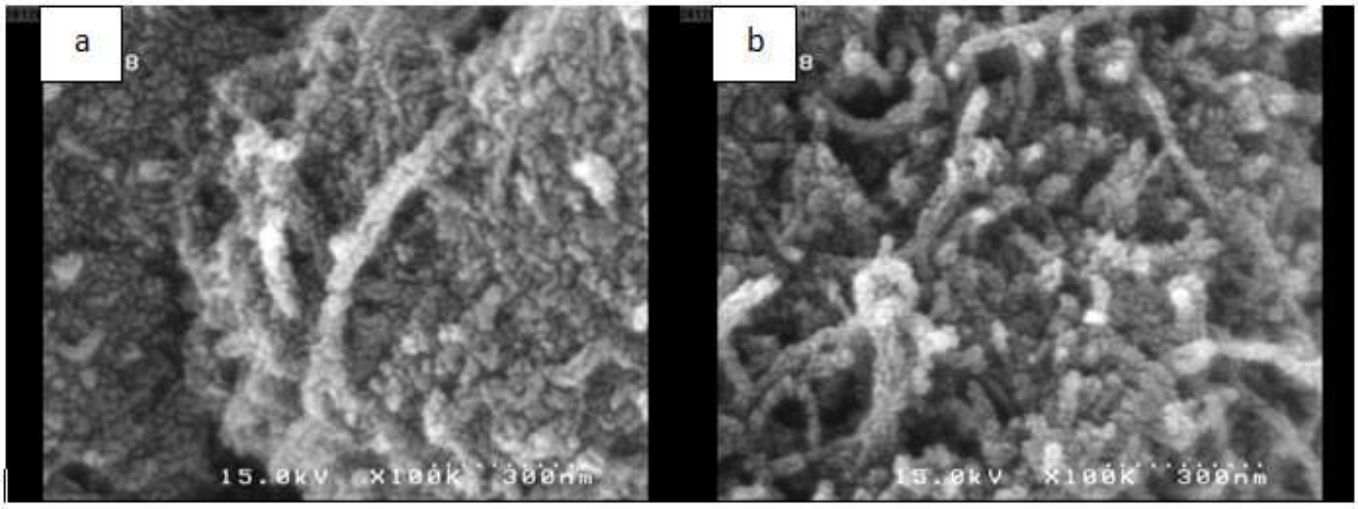


Figure 8: (a) Field Emission Scanning Electron Microscopy DCC,(b)DCC-Base

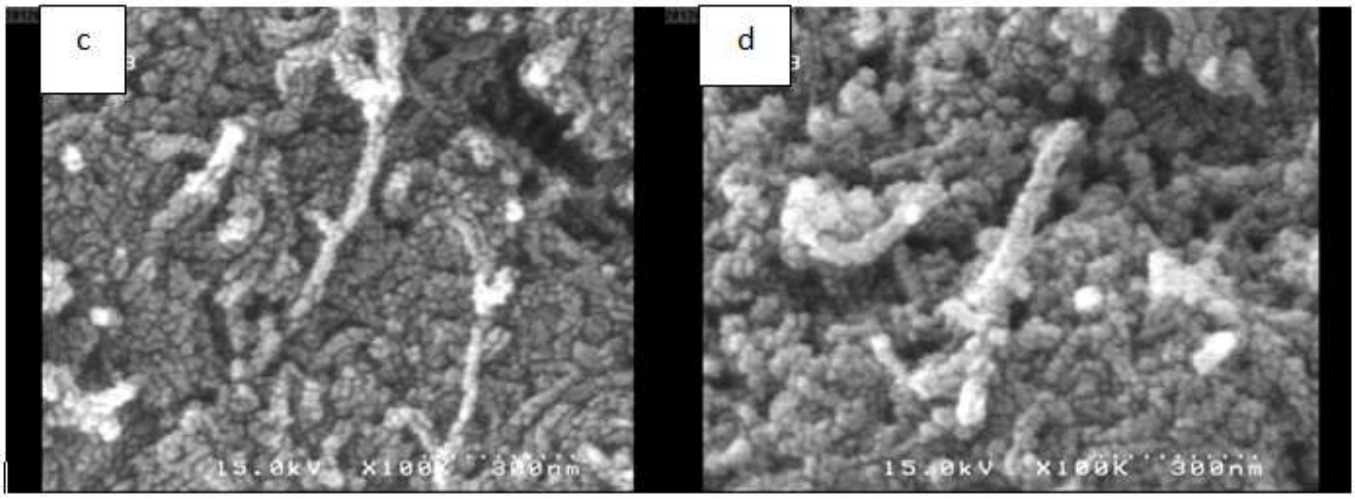
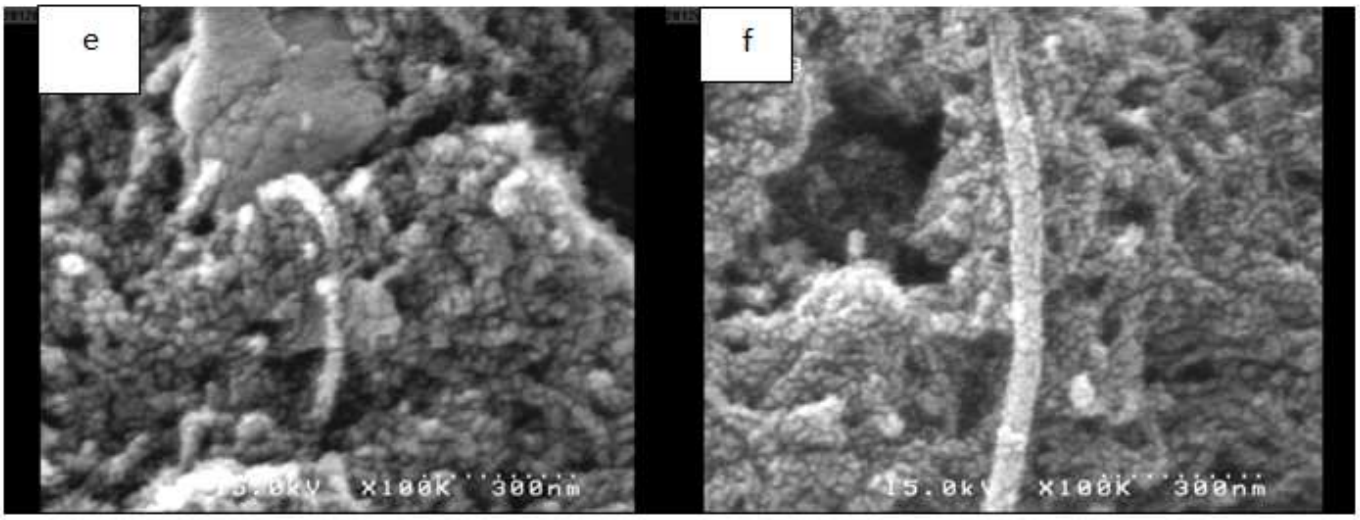


Figure 9: (c) Field Emission Scanning Electron Microscopy EDC-Base,(d)NHS



(e)FieldEmissionScanningElectronMicroscopyNHS-DCC,(f)HOBT-DCC

Figure 10: 3-5-Atomic ForceMicroscopy (AFM)

The figure 11 present Atomic Force Microscopy drug of Naltrexone to MWCNT-COOH by carbodiimide reagent DCC with 4-dimethylaminopyridine (DCC-Base). According to our AFM results,Gray strands in the figure show strands of MWCNTs,and white strands show functionalization on surface of CNTs. It can be reported which diameters of CNTs increase with functionalization.

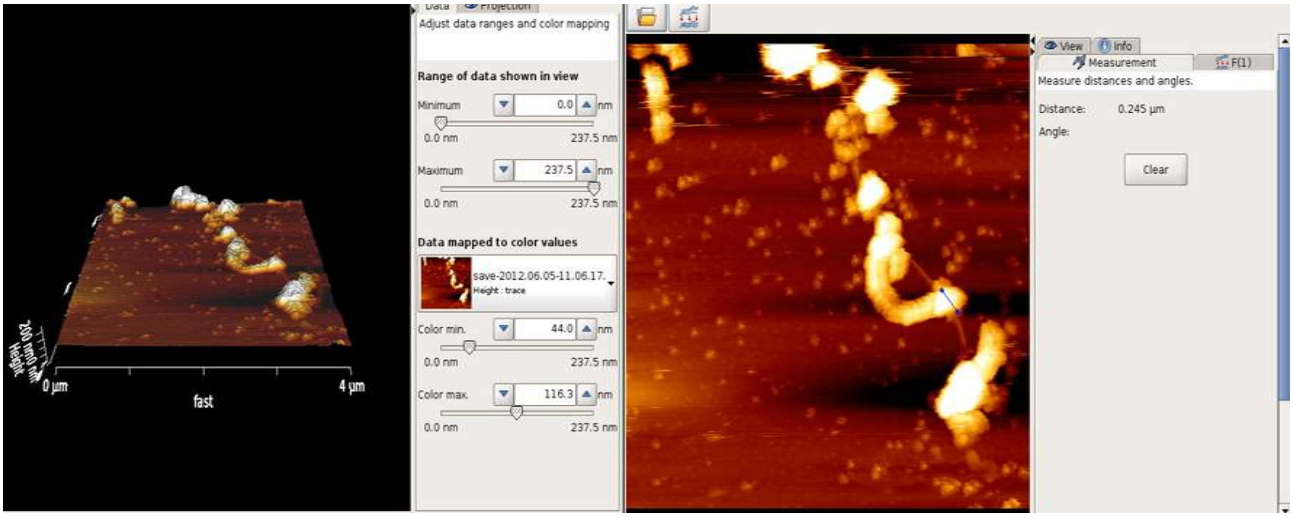


Figure 11: Atomic Force Microscopy drug of Naltrexone to MWCNT-COOH by carbodiimide reagent DCC-Base

3-6-High performance liquid chromatography (HPLC)

200µg/ml neat drug of Naltrexone injected to instrument HPLC,after 50 mg of Nano drug were poured into flask and was added 20ml acidic buffer and then was stirred for 2hr until is hydrolyzed nanodrug.Drug releasing from grafted MWCNT-drug is indeed a hydrolysis reaction,which involves breaking of ester band in acidic buffer.after 2hr,is segregated nanotube and acidic buffer of each other by filter.Then 20 µL drug of hydrolysis injected to instrument HPLC.The attachment quantitative drug to MWCNTs is resulted by considering under surface peak of neat Naltrexone and nanodrug of hydrolysis.We reported the attachment quantitative drug of Naltrexone to MWCNTs by carbodiimide reagents in this work.Also, the covalent attachment drug to functionalized carbon nanotubes can be confirmed by HPLC.The following table has been designed which shows attachment quantitative drug to MWCNTs by carbodiimide reagents, for exampleDi cyclohexyl carbodiimide hydrochloride with 4-dimethylaminopyridine (DCC-Base) are reported as carbodiimide reagent for the most quantitative attachment drug of Naltrexone to MWCNTs in this work. The Table 2 shows quantitative attachment drug of Naltrexone to MWCNTs-COOH by carbodiimide reagents.

www.sciforce.org Sample	Mean area(n=3)	Drug concentration in the solution nano drug (Microgram/ml)	The amount of drug in the sample(mg/ml)	Percent The amount of drug in the sample
Standard (200microgram/ml)	568150			
1-12 sample				
DCC	664853	234.0413623	.234041362	9.001590858
DCC-Base	800237	281.6991992	.281699199	10.83458458
EDC	204228	71.89228197	.071892281	2.765087768
EDC-Base	682390	240.214732	.240214732	9.239028155
NHS	577772	203.3871337	.203387133	7.822582038
NHS-DCC	372624	131.1709936	.131170993	5.045038214
NHS-EDC	341681	120.2784476	.120278447	4.626094138
HOBT	299956	105.5904251	.105590425	4.061170195
HOBT-DCC	273202	96.17248966	.09617249	3.69894191
HOBT-EDC	603903	212.5857608	.212585761	8.176375415
HOBT-TBTU	335802	118.2089237	.118208923	4.546497065
TBTU	381240	134.2039954	.134203995	5.161692132

Table 2: quantitative attachment drug of Naltrexone to MWCNTs by carbodiimide reagent

The Table 2 shows percent quantitative attachment drug of Naltrexone to MWCNT-COOH by carbodiimide. As can be seen in above table the most quantitative attachment drug of Naltrexone to MWCNTs in this work are reported DCC-Base,DCC,EDC-Base,HOBT-EDC,respectively.The following presentation shows HPLC drug of Naltrexone to MWCNT-COOH by carbodiimide reagents.

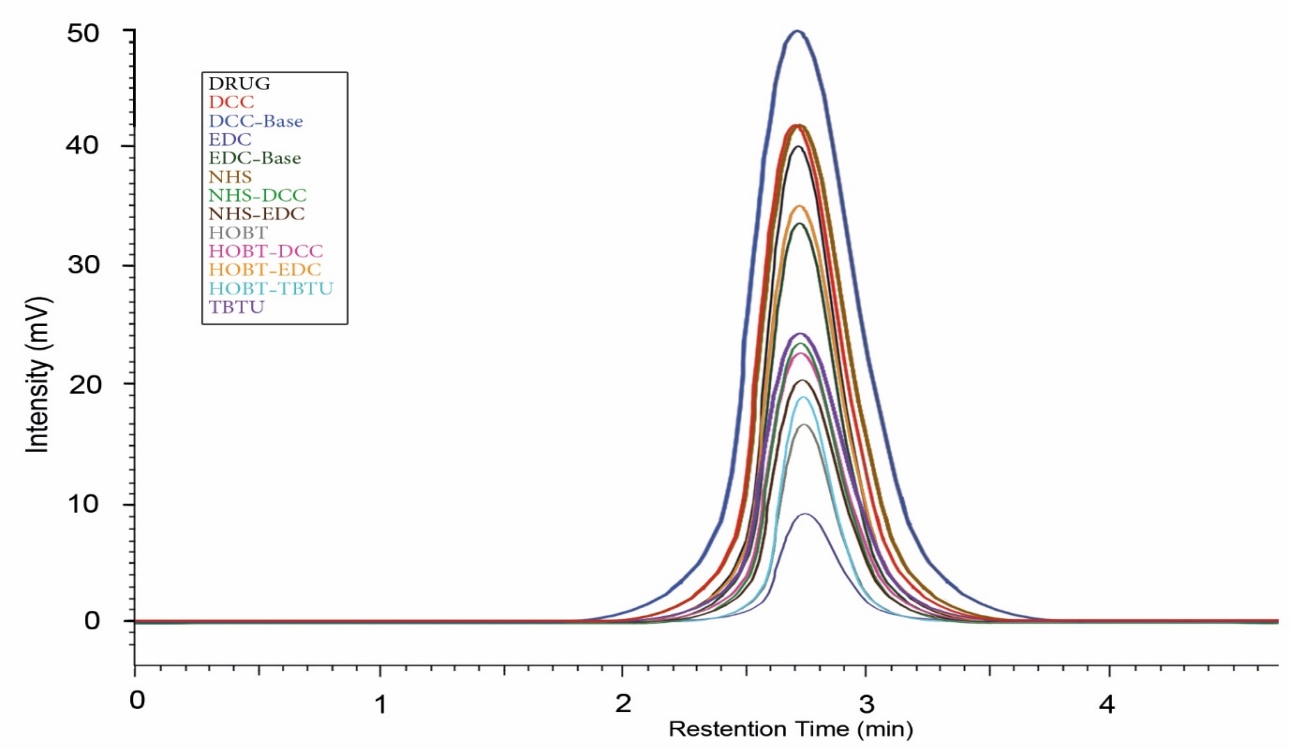


Figure 12 : HPLC drug of Naltrexone to MWCNT-COOH by carbodiimide reagents

Conclusion

In synthetic organic chemistry,compounds containing the carbodiimide functionality are dehydration agents and are often used to active carboxylic acids towards amide or ester formation. Carboxylic acids will react with the carbodiimide to produce the key intermediate as O-acylisourea which can be considered as a carboxylic ester with an activated leaving group. In Raman spectroscopy we have reported which with esterification functionalized carbon nanotube by carbodiimide reagent in this work,peaks D-band and G-band of MWCNT-COOH are transposed to high frequency region.This high frequency region is reported for synthetic samples in the following table1.This subject can be confirmed by covalent attachment Naltrexone to functionalized carbon nanotubes by Raman spectroscopy. In TGA The first one (bellow 450°)can be assigned to ester and Naltrexone,While the second one (above 450°C) is related to nanotube as comparing to MWCNT-COOH thermogram. In FESEM and AFM can be reported which diameters of CNTs increase with functionalization. In HPLC quantitative drug to MWCNTs by carbodiimide reagents, for example Di cyclohexyl carbodiimide hydrochloride with 4-dimethylaminopyridine(DCC-Base) are reported as carbodiimide reagent for the most quantitative attachment drug of Naltrexone to MWCNTs in this work.all of the evidence presents covalent attachment drug of Naltrexone to MWCNTs.

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