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Original Research Article

Preparation and Characterization of Desmopressin Peptide Attached Multi-Walled Carbon Nanotube

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HIGHLIGHTS

- The conversion of carboxylic groups to oxalyl chloride on the surface of MWCNTs.
- The conversion of carboxylic groups to O-acylisourea on the surface of MWCNTs.
- DCC and oxalyl chloride activate groups on MWCNTs for nucleophilic substitution.
- Desmopressin is a cyclic nonapeptide with one disulfide bridge.
- The Covalent attachment of desmopressin to functionalized MWCNTs.

ABSTRACT

Desmopressin, a synthetic analogue of vasopressin, has many applications in medicine including diabetes insipidus, night bedwetting, and hemophillia A. In this work, the attachment of desmopressin to multi-walled carbon nanotubes (MWCNTs), functionalized by HNO_3 and H_2SO_4 treatment was first used to remove the unwanted catalyst from MWCNTs and meanwhile introduced carboxylic acid groups onto the surface of MWCNTs. These carboxylic groups were then used as reaction sites for the attachment of desmopressin peptide to MWCNTs. The reagents used for the attachment were oxalyl chloride and dicyclohexylcarbodiimide (DCC). The Covalent attachment of desmopressin to functionalized MWCNTs was confirmed by Fourier Transform infrared spectroscopy (FT-IR), Raman scattering, and Field Emission Scanning Electron Microscopy (FESEM).

Keywords:
Covalent attachment
Desmopressin peptide
Multi-walled carbon nanotubes
Functionalization

Introduction

Over the last twenty years, carbon nanotubes (CNTs) have received considerable attention from many researchers due to their interesting properties and wide applications. In addition to outstanding mechanical characteristics, CNTs exhibit excellent electrical and thermal properties. A characteristic property of pristine carbon nanotubes is their very low solubility in any solvent. In this work, this limitation can be overcome by a proper covalent functionalization (Dyke et al., 2006). Water solubility is an extremely useful property for CNTs since it provides applications in various fields of electronics, tissue engineering, or biomaterials studies (Endo et al., 2008). Reported strategies for increasing water solubility of CNTs include the introduction of hydrophilic functional groups to CNTs, such as carboxyl, hydroxyl, or sulfonate groups (Kharisov et al., 2009). A common technique to improve dispersion and to realize the great capability of CNTs is through chemical functionalization, which is often used relatively to generate functional groups on the surface of CNTs. The covalent side-wall modifications of nanotubes have been well described in several review papers (Banerjee et al.,

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2005; Tasis et al., 2006). In general, major approaches include: (1) amidation or esterification of carboxylated CNTs. (2) side-wall covalent attachment of functional groups directly to pristine CNTs. Modification of the surface morphology plays an important role in enhancing the sorption capacity of CNTs. Activation of CNTs under oxidizing conditions with chemical reagents such as HNO_3, $KMNO_4$, H_2O_2, NaOCl, H_2SO_4, KOH, and NaOH have been widely reported. During activation, metallic impurities and catalyst support materials are dissolved and the surface characteristics are altered due to the introduction of new functional group (e.g., COOH, OH, Carbonyl, OSO_3H, lactones) (Smith et al., 2001; Lu et al., 2006; Rao et al., 2007; Gao et al., 2008; Katsumata et al., 2008; Lu et al., 2008; Liu et al., 2008; Xu et al., 2008; Yang et al., 2008; Gao et al., 2009; Loudon et al., 2015). The acids are relatively unreactive with nucleophiles. The chlorination is one possible route to make the carboxylic acid group to be a more reactive derivative such as an acyl chloride moiety. Yet another route would be to employ reagents, *i.e.* carbodiimide derivatives that selectively activate and prepare carboxyl group for nucleophilic substitution towards amide or ester formation (Lu et al., 2004; Marshall et al., 2006). The purpose of this work is to generate carboxylic acid moieties at the ends and side wall defect sites of carbon nanotubes by two different ways and examine which way is preferred for the next process of binding to ligand. This subject has been reported and analyzed by FTIR, Raman, and FESEM techniques, in this work. Desmopressin is a cyclic nonapeptide with one Disulfide Bridge containing the sequence Mpr-Tyr-Phe-Gln-Asn-Cys-Pro-D-Arg-Gly-NH2. Desmopressin is an analogue of the human antidiuretic hormone, vasopressin. It mimics the action of vasopressin in the kidneys to increase the reabsorption of water thereby reducing the volume of urine produced (walse et al., 1998). Desmopressin peptide has been attached to the functionalized MWCNTs through covalent bond. The MWCNTs have been prepared by chemical vaporization deposition method and the MWCNTs were functionalized with carboxylic groups using acid oxidation treatments. These carboxylic groups were further treated with oxalyl chloride and dicyclohexylcarbodiimide to introduce acyl chloride groups via acyl formation and intermediates as O-acylisourea groups, respectively, and then, acyl chloride and intermediates as O-acylisourea groups on MWCNTs were treated with desmopressin peptide.

Materials and Methods

Chemicals and instruments

Multi-walled carbon nanotubes purchased from neutrino Co. (Tehran, Iran) were synthesized by catalytic chemical vapor deposition, and the purity of the received MWCNT materials was ≥ 95%. The length of these MWCNTs was 30µm. These specification details were given by the manufacturer. Concentrated nitric acid (HNO_3, 65%), concentrated sulfuric acid (H_2SO_4, 98%), and H_2O_2 (30% w/v) were obtained from Merck Co and used as received. The desmopressin peptide was obtained from Jaber Ebne Hayyan pharmaceutical Co (Tehran, Iran). Dicyclohexylcarbodiimide (DCC), dimethylformamide (DMF), and tetrahydrofuran (THF) were provided from Merck Co. Anhydrous THF and DMF were prepared using Na with benzophenone and molecular sieve (0.4nm diameter, 2mm length), respectively. FTIR spectra were recorded using a Thermo Nicolet Nexus 870. Raman spectra were obtained using Nicolet Dispersive Raman spectrometer with a Nd:YLF laser (532nm). Morphology of MWCNTs was characterized by Scanning Electron Microscopy (SEM) instrument (Fillips xl30).

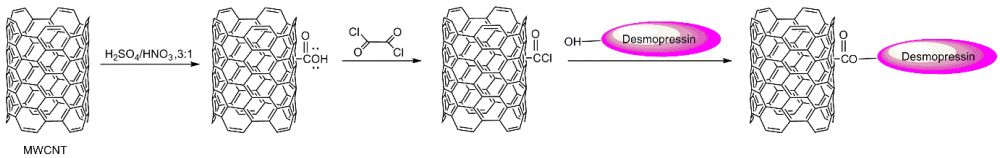
Pretreatment of MWCNTs by reflux method

Raw-MWCNTs (0.1g) were placed into a round bottom flask and treated with a mixture concentrated H_2SO_4 (98%) and HNO_3 (65%) (v/v 3:1). The suspension was refluxed for 12h with stirring. After cooling to room temperature, MWCNTs were added drop wise to 300mL of cold deionized water (DI). Subsequently, the reaction medium was centrifuged at 3500 rpm for 20 minutes. The supernatant was decanted off, and the vials were refilled with DI, and centrifuged again under identical conditions. Finally, the MWCNTs were filtered through a cellulose ester membrane (0.45µm porous), and thoroughly rinsed with DI until the pH value of the filtrate was above 5. The oxidized MWCNTs were then dried at 50°C in a vacuum oven for 24h.

Attachment of desmopressin peptide to MWCNT-COCl

As seen in (Scheme 1), 100 ml of anhydrous DMF and 1ml oxalyl chloride to MWCNTs-COOH (0.2g) in 250 ml round bottom flask were added with stirring, to obtain MWCNT acyl chloride. To MWCNTs acyl chloride (0.1g) in a flask, 2 ml anhydrous DMF, 50 µL anhydrous triethyl amine, and 0.07 g desmopressin peptide, under argon gas, were added. The mixture was sonicated at 30 °C for 30 min and then stirred for 24h at room temperature. Next, the reaction mixture was filtered through a 0.25 µm pore-sized PTFE membrane filter.

The black solid was collected on the filter and washed with 120 ml anhydrous DMF and anhydrous



Scheme 1. The attachment of desmopressin peptide to MWCNT-COOH by oxalyl chloride reagent.

THF. The resulting material was dried at 35 °C in a vacuum oven for 5 h.

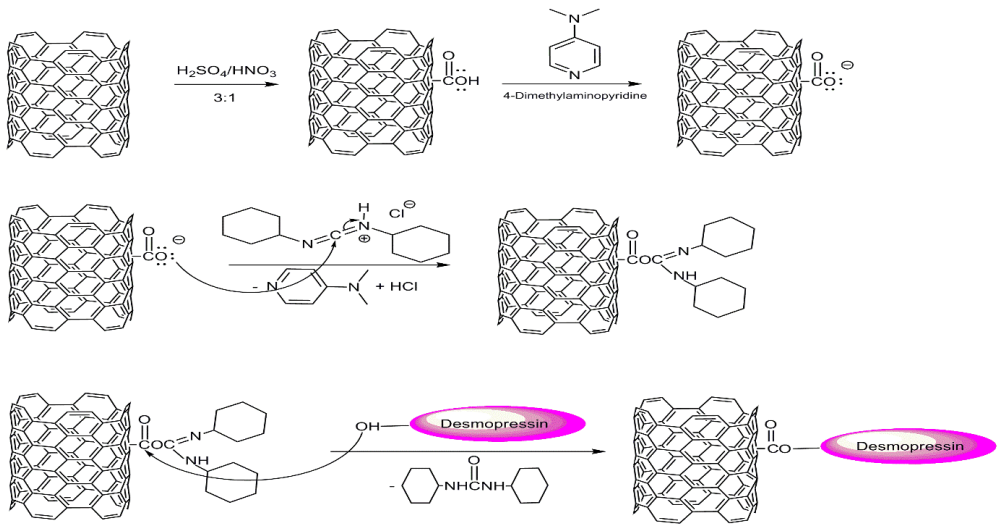
Attachment of desmopressin peptide to MWCNTs-COOH

As seen in (Scheme 2), to MWCNTs-COOH (0.2 g) in 250ml round bottom flask 100 ml of anhydrous DMF and 0.1g 4-Dimethylaminopyridine (DMAP) were added; the mixture was stirred for 40 min under argon gas. By adding DCC (0.3 g) to the mixture, the flask was sonicated for 30 min to give a homogeneous suspension under argon gas. Then, by desmopressin peptide addition, the flask was stirred for 24hr under a stirring condition. Next, the reaction mixture was filtered on a PTFE membrane filter and the resulting solid on filter was washed with excess anhydrous THF and DMF, and dried at 50°C in a vacuum oven for 8hr.

Results and Discussion

Attachment of desmopressin on MWCNT and its confirmation by FTIR

The FTIR spectra of MWCNTs samples in range 500-3500 cm^{-1} are shown in Fig.1. The bands around 1690-1700 cm^{-1},1090-1200 cm^{-1},3300-3400 cm^{-1} in spectra can be attributed to the stretching vibration of C=O, stretching vibration C-O, and contribution OH stretching vibration of the COOH groups, respectively (Kovtyukhova et al., 2003). Also bands at about 1640-1650 cm^{-1} can be attributed to the MWCNT C=C graphitic stretching mode that is infrared-activated by extensive side wall functionalization. The bands at about 1500-1550 cm^{-1} can be assigned to (C=C) stretching of benzenoid rings. The bands at about 2800-2980 cm^{-1} and at about 3100 cm^{-1} can be related to symmetric and asymmetric stretching vibration of C-H, and stretching vibration =C-H in rings of CNTs,



Scheme 2. The attachment of desmopressin peptide to MWCNT-COOH by carbodiimide reagent (DCC).

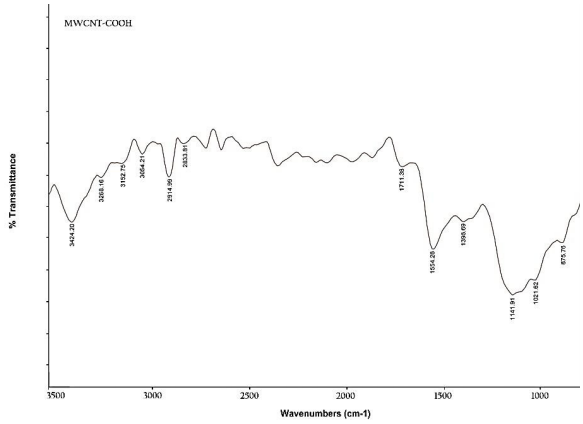


Figure 1. FTIR spectrum of MWCNT-COOH.

respectively (Wang et al., 2007). As shown in Fig. 1, the band at 1711.38 cm^{-1} can be attributed to stretching vibration C=O of COOH and in Fig. 2 and Fig. 3, the bands at 1718.77 and 1718.68 cm^{-1} can be attributed to stretching vibration C=O of esters, and this bands confirm the attachment of desmopressin peptide to the functionalized MWCNTs.

Characterization of desmopressin-MWCNT by Raman scattering

Raman spectrum is divided to three areas. The first area in range of 0-500 cm^{-1} can be considered as a radial breathing mode (RBM). The second area in range of 100-1600 cm^{-1} can be considered first order Raman spectrum. The third area in range of 1610-3500 cm^{-1} can be considered second order Raman spectrum. The first order Raman spectrum consists of disorder band (D-band) and graphitic band (G-band). The D-band at approximately 1000-1400 cm^{-1} is an A_{1g} breathing mode. This mode is generally attributed to the defects in the curved graphite sheet, sp^3 carbon, or other impurities. The D-band is considered to indicate disorder in the graphitic lattice or defects in nanotubes (Zhang et al., 2003; Osswald et al., 2006; Chen et al., 2009). The G-band at 1500-1600 cm^{-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 (Chen et al., 2005). The second

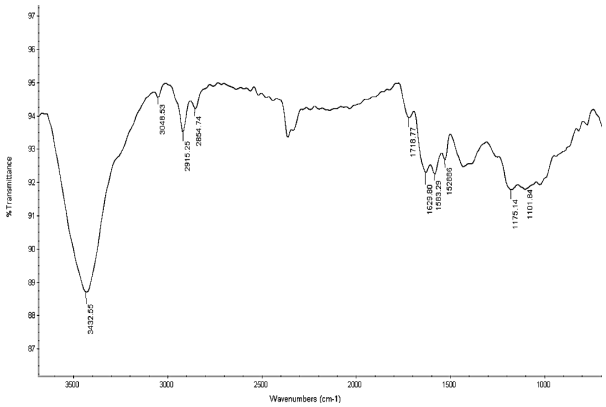


Figure 2. FTIR spectrum of desmopressin-MWCNT-COOH coupled by oxalyl chloride reagent.

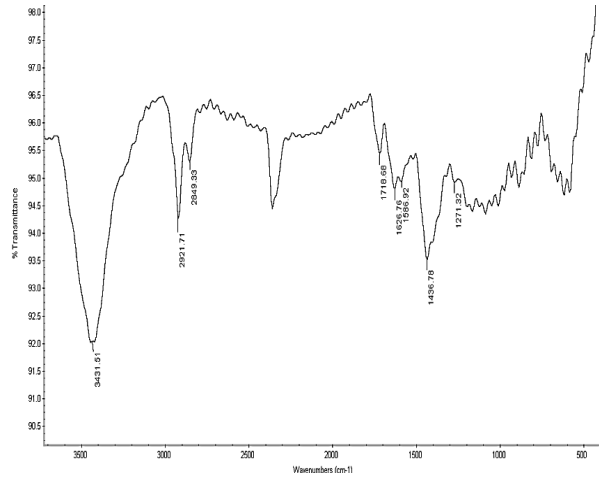


Figure 3. FTIR spectrum of desmopressin- MWCNT-COOH coupled by carbodiimide reagent (DCC).

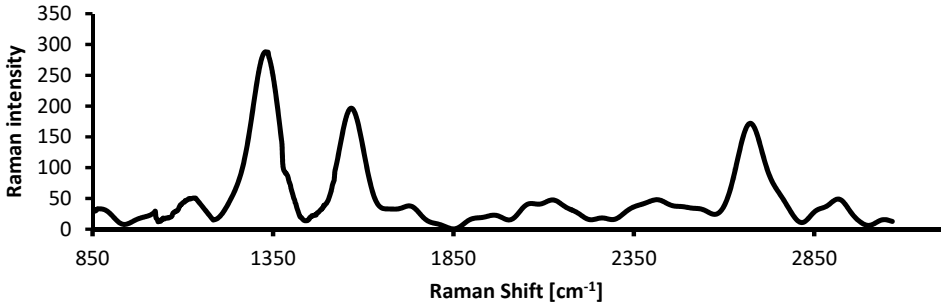


Figure 4. Raman spectra of MWCNT-COOH.

Raman spectrum consists 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 the range of situated at~1610-2000 cm^{-1} and situated at~2400-2900 cm^{-1}, respectively. The ratio of intensity of the D-band and G-band $R=I_D/I_G$ ratio, can be used to evaluate the relative extent of structural defects or relative degree of functionalization. Commonly, the relative intensity ratios of D-band to G-band (I_D/I_G) are utilized as an approach to monitor the purity and functionalization of MWCNTs.

The 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 (Chen et al., 2005). D-band and G-band of MWCNTs-COOH are at about 1330 cm^{-1} and 1580 cm^{-1}, which were attributed to the defects and disorder induced modes and tangential mode (Fig 4). The increase in the intensity D-band of MWCNT-g-desmopressin can be related to some damage of the graphite sheet caused by the ester functional group on the surface of CNTs or it might be also due to a difference in energy transfer between the MWCNT-COOH and desmopressin molecules or the influence of the grafted desmopressin on the electronic properties of the MWCNT-COOH (Gao et al., 2005; Brunetti et al., 2008). In this work, we reported that with esterification of the functionalized carbon nanotube using oxalyl chloride reagent and DCC reagent, D-band and G-band peak of MWCNT-COOH were transposed to the high frequency region, as shown for synthetic samples in Fig. 5 and Fig. 6. This subject can be confirmed the covalent attachment of desmopressin peptide to functionalized carbon nanotubes by Raman spectroscopy (Amit et al., 2009).

Preparation of MWCNT and its characterization by Field Emission Scanning Electron microscope

As shown in Figs 7, 8, and 9, Field Emission Scanning Electron Microscope (FESEM) of MWCNTs-COOH and MWCNTs-peptide are presented. According to our FESEM results, black strands in the pictures show strands of MWCNTs, and white strands show the functionalization on side wall and end of CNTs and the covalent attachment of desmopressin peptide

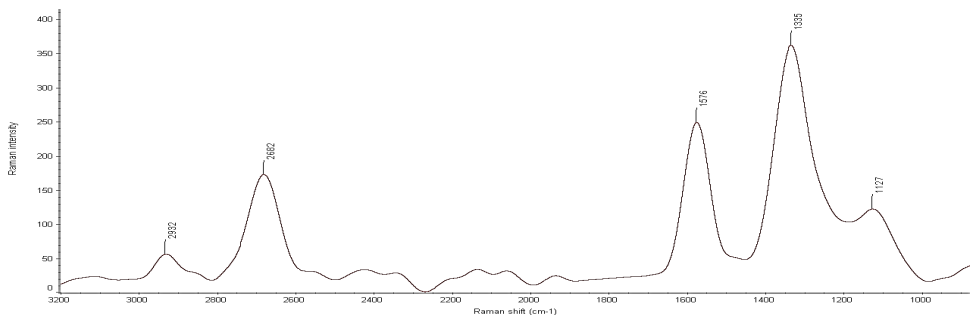


Figure 5. Raman spectrum of desmopressin-MWCNT-COOH coupled by oxalyl chloride reagent.

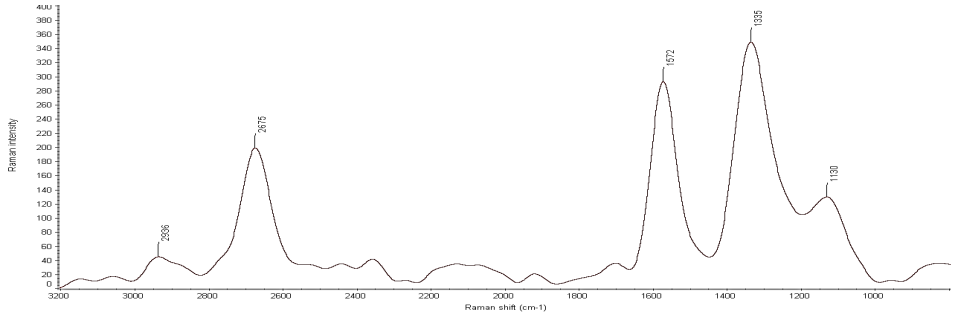


Figure 6. Raman spectrum of desmopressin-MWCNT-COOH coupled by carbodiimide reagent (DCC).

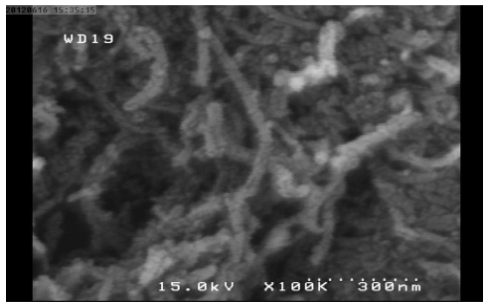


Figure 7. FESEM pattern of MWCNT-COOH, black strands in the pictures show the strands of MWCNTs, and white strands show carboxylic groups on the surface of MWCNTs.

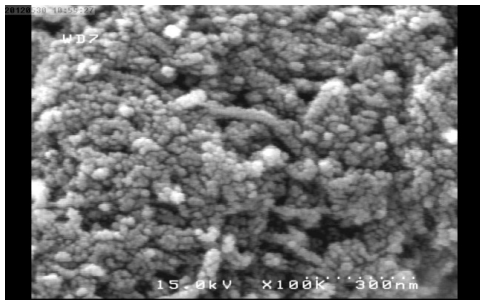


Figure 8. FESEM pattern of desmopressin-MWCNT-COOH coupled by oxalyl chloride reagent, black strands in the pictures show the strands of MWCNTs, and white strands show the covalent attachment of desmopressin peptide to functionalized MWCNTs by oxalyl chloride reagent.

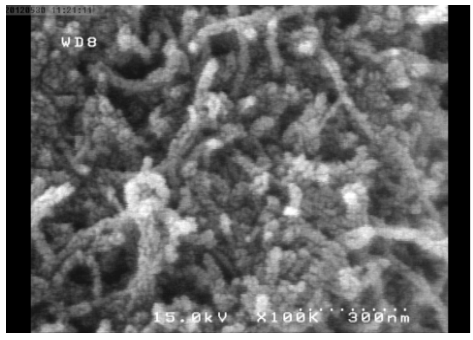


Figure 9. FESEM pattern of desmopressin-MWCNT-COOH coupled by carbodiimide reagent (DCC), black strands in the pictures show the strands of MWCNTs, and white strands show the covalent attachment of desmopressin peptide to functionalized MWCNTs by carbodiimide reagent.

to MWCNTs. It can be reported that the diameters of CNTs increased by the functionalization and covalent attachment of peptide to MWCNTs (Sinani et al., 2005).

Conclusion

Desmopressin peptide has been attached to MWCNTs through covalent attachment to the functionalized MWCNTs. 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 and dicyclohexylcarbodiimide to introduce acyl chloride groups and intermediates as O-acylisourea groups, respectively, and (3) acyl chloride and intermediates as O-acylisourea groups on MWCNTs were treated with desmopressin peptide. FESEM observations clearly confirm the covalent attachment of desmopressin peptide to the functionalized MWCNTs.

The chemical linking of desmopressin peptide to the functionalized MWCNTs has been also confirmed by FTIR spectroscopy. The FT-IR results showed the presence of bands at 1718.77 and 1718.68 cm^{-1}, which can be attributed to the covalent attachment of peptide to the functionalized MWCNTs. In FTIR spectra (as shown in Fig. 2 and Fig. 3), sharpening and spreading of the band around 1700 cm^{-1} indicates a strong link for ester formation between desmopressin peptide and functionalized carbon nanotubes, therefore, dicyclohexylcarbodiimide hydrochloride with 4-dimethylaminopyridine can be reported as a more suitable activation reagent than oxalyl chloride reagent. In Raman spectroscopy, we reported that the peak intensity of the MWCNT-g-Desmopressin peptide was slightly stronger than that of MWCNT-COOH, therefore, the covalent attachment of the peptide to the functionalized MWCNTs has been confirmed by shift to high frequency regions in Raman spectroscopy. All of the evidence presents the covalent attachment of desmopressin drug to MWCNTs.

This drug carrier of desmopressin can be used as an antidiuretic in anti-diuretic hormone (ADH) replacement therapy in the management of central cranial diabetes insipidus, as well as for management of the temporary polyuria and polydipsia following head trauma or surgery. In this work, in the recent future, the attachment of antidiuretic drugs to carbon nanotubes will desire to control increased thirst and too much urination and helps prevent dehydration.

Competing Interests

The authors declare that there is no conflict of interest on this research work.

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