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# Investigation the Improvement of Teniposide Solubility by Incorporation into Acid Treated Carbon Nanotube and Dispersed by Hydrophilic Polymer

**Raid M. Al Abood[*,1], Mowafaq M. Ghareeb[*] and Alaa A. Abdulrasool[*]**

[*]Department of Pharmaceutics, College of Pharmacy, University of Baghdad, Baghdad,Iraq.

## Abstract

Single walled Carbon nanotubes (SWCNT), as nano-needle structures, are good candidates as nanocarrier delivery systems that can carry drug to the site of action especially for chemotherapy. Teniposide is an anticancer drug, but it has a problem of low solubility. In this study, carbon nanotubes properties were improved by pre-functionalized of carbon nanotubes via carboxylation with strong acids and then functionalized through attaching to polymer and copolymer. Concurrently, a proper polymer-copolymer combination has been selected by the homogeneity and UV-Visible spectroscopy.

The best formula (F19) is further studied utilizing Fourier transoform infra red, scanning electron microscopy, Transmittance electron microscopy, and the solubility of teniposide.

The results showed that the best dispersibility obtained in the formula that used polyvinyl alcohol (PVA) as polymer and polyethylene oxide (PEO) as copolymer at a ratio of 1:1. The solubility of incorporated teniposide in the selected formula is increased by 11.5 fold. Accordingly, it can be concluded that the acid treated carbon nanotube and linked by polymer improve the pharmaceutical properties of the carrier and actively increase the potency of drug.

**Key words: Acid treated carbon nanotubes, Teniposide, Hydrophilic polymers.**

# دراسة تحسين الذوبانية لعقار التنوبوسايد بأدراجه بأنابيب نانوية كربونية المعالجة حمضيا وتشتيته ببوليمر مائي

**رائد مظلوم العبود *[،1] ، موفق محمد غريب* و علاء عبد الحسين عبد الرسول ***

*فرع الصيدلانيات ،كلية الصيدلة، جامعة بغداد، بغداد،العراق.

## الخلاصة

إن الأنابيب النانوية الكربونية أحادية الجدار ، والتي تتكون من هياكل إبرية نانوية ، مرشحه جيدة لأنظمة التوصيل النانوية التي يمكنها نقل العقار إلى موقع الفعالية الخاص بها. عقار التنوبوسايد هو دواء مضاد للسرطان ، لكنه يعاني من مشكلة قلة الذوبان.

في هذه الدراسة ، تم تحسين خصائص الأنابيب النانوية الكربونية عن طريق إضافة مجموعة كربوكسيلية بالتفاعل مع الأحماض القوية ثم تم تفعيل الأنابيب الكربونية من خلال الإلحاق بالبوليمر و البوليمر المساعد. تركيبة بوليمر-بوليمر مساعد المناسبة تم اختيارها من خلال التجانس والتحليل الطيفي المرئي للأشعة فوق البنفسجية.

لأفضل الصيغ تم إجراء دراسات إضافية باستخدام فحص امسح الضوئي السطحي والمسح الضوئي الخلالي والأشعة تحت الحمراء و ذوبانية التنوبوسايد.

أظهرت النتائج أن أفضل تشتت تم الحصول عليه في الصيغة التي استخدمت بولي فنيل الكحول كبوليمر وبولي أثيلين أوكسيد كبوليمر مساعد بنسبة ١: ١. تمت زيادة قابلية الذوبان للتينيبوسيد في الصيغة المحددة بمقدار ١١,٥ أضعاف. وبناءً على ذلك ، يمكن الاستنتاج أن الأنابيب النانوية الكربونية المعالجة بالحمض والمرتبطة بالبوليمر تعمل على تحسين الخصائص الصيدلانية للناقل وتزيد من فاعلية الدواء.

**الكلمات المفتاحية:انابيب نانوية كربونية معالجة حمضيا ، التنوبوسايد ،بوليمر مائي .**

## Introduction

Carbon nanotubes (CNTs) represent promising tools as drug nanocarriers. When drug delivered to the body intended, carbon nanotubes combine the properties of nanoparticles, both the biocompatibility of liposomes or polymeric nanoparticles and the stability of inorganic nanoparticles such as gold and silica ones. They give the potentiation of modification where different particles involving protein, enzymes nucleic acids and drugs can be affixed [(1)].

One of the important anticancer semisynthetic drugs is teniposide, which belongs to a family of topoisomerase inhibitors. Their action is through stabilization of topoisomerase-DNA cleavable complexes by hindering the DNA relegating step of the catalytic reaction. The problem of teniposide is the solubility, since it is water insoluble [(2)].

[1]Corresponding author: E-mail: raedmadloom@yahoo.com
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Carbon nanotubes can be used as a carrier for teniposide, since it could enhance the solubility, improve the penetration to cancer cells, and decrease the side effect by targeting [3].

Pristine CNT has a smooth surfaces and not soluble in aqueous media that need to pre-functionalization by acid treatment and functionalized with polymer[4].

The selection of proper polymers depends on the properties of the polymer. The proper polymers must be nontoxic biocompatible and improve the CNTs properties like stability, dispersibility, facile electrochemical polymerization, ion exchange with the medium, good capacity to enhance adhesive coatings, increase the porosity, and drug loading efficiency [5].

The polymer is wrapping around CNTs body that will improve the aqueous dispersibility of CNT [6].

Different water-soluble polymers like polyvinyl alcohol (PVA) and polyethylene glycol (PEG) of different molecular weight (1500, 1000, and400) were used to increase the dispersibility and enhance cell penetration. This work was performed to improve the properties of nanocarriers and consequently of the drug solubility.

## Material and Methods

### *Materials*

Single-walled carbon nanotube (SWCNT) purchased from Hongwu International Group Ltd, China, teniposide from Xi'an Health Biochemical Technology Co., Ltd. China, Polyethylene glycol 1500 from Thomas Baker, India, polyethylene glycol 1000 from Sinopharm Chemical reagent, China, Polyethylene glycol 400 from Hi-Media Laboratories, India, Poly vinyl alcohol146000 from Sinopharm Chemical reagent, China, Polyethylene oxide 10000 from SM chemical SDMPHD, Malaysia. Dextran 70 from Guanjie Chemical reagent, China. All other ingredients used in the study were analytical grade.

### *Methods*

#### *Purification and carboxylation of SWCNT*

Exactly one gram of SWCNT was mixed with 80 ml mixture of $H_2SO_4$ (18.4M) and $HNO_3$ (15.7M) 3:1 in tightly close glass container. The mixture was sonicated in bath sonicator for 6 hours at 70℃and conserved overnight. The dispersion was centrifuged at 4000 rpm for 15 minutes to remove the large agglomerates and bundles. After sonication and centrifugation steps, the dispersion was filtrated through 0.2 µm polycarbonate filters (Whatman Ltd.) that diluted with deionized water in order to protect the filter paper from the strong acids, the filtrate continually washed with deionized water by vacuum-assisted filtration until the pH of eluting reached to 7. The filter paper that contains the precipitant was dried using hot air ovens for a night then the SWCNT collected for the next step [7–10].

#### *Preparation of polymer linked CSWCNT*

For polymeric coating of CSWCNT, a different polymer (po) and copolymer (copo) were used in order to enhance the dispersing capacity of SWCNTs and increase drugs loading. The used polymers include polyethylene glycol (PEG) of different molecular weight (1500, 1000, and 400) and polyvinyl alcohol 146000 (PVA), while polyethylene oxide (PEO) or dextran 70 as a copolymer (copo) was used at different ratios as shown in the Table (1).

#### *Preparation of CSWCNT-PEG*

A hundred milligram of polyethylene glycol of different molecular weight (1500, 1000, and 400 ) was added to 20ml deionized water in a glass vial and sonicated for 30 minutes in a bath sonicator, and the copolymers were added by the same method at different ratios. A hundred milligram of CSWCNT has dispersed in deionized water with sonication for 1 hour, and then the CSWCNT dispersion was added to the polymer-copolymer solution and further sonication for 4 hours in a bath sonicator at room temperature. The water in the bath sonicator changed every 30 min to avoid overheating, and the temperature kept at 25℃. The polymer-CSWCNT suspension was centrifuged for about 15minute at 4000 rpm; the temperature kept at around 25℃ (room temperature), the supernatant solution was collected and cleared the dispersion from the large particle. The product was filtrated through 0.1 µl filter membrane and washed with deionized water six times to exclude the unconjugated polymer. Finally, the filtrate was dried by hot oven overnight, and the powder was collected in a tightly closed container for the next step [11,12].

#### *Preparation of CSWCNT-PVA*

Three grams of PVA were weighed in a closed container and solubilized by 30 ml of deionized water at 70 ℃ with continuous shaking in a water bath with a shaker for 10 hours; the solution obtained left to cool into the room temperature. The copolymer linking was done by the same method at different ratios with ultrasonication. In a conical flask, 20 mg of CSWCNT dispersed in 5 ml of deionized water by sonication for 1hour to give dispersion. The CSWCNT dispersion was added to the PVA/copolymer solution drop by drop with continuous stirring at different ratio and sonicated for 3hour in order to prepare CSWCNT/polymer nanocomposites. The functionalized SWCNT (CSWCNT-polymer) suspension was centrifuged for about 15minute at 4000 rpm; the temperature kept at around 25 ℃(room temperature), the supernatant solution was collected to liquidation the dispersion from the large particles. Then, the mixture filtrated to eliminate the unconjugated polymer. Finally, CSWCNT-polymer composites were cast in Petri dishes and left to dry by hot oven for 12 hours[13,14].

***Incorporation of teniposide into CSWCNT-polymer***

A fifty milligram of teniposide was added into 20ml acetone in closed container with continuous shaking in shaker water path for around 4 hours at 30℃ until clear solution was obtained. In 100 ml tightly close container, 50 mg of CSWCNT-polymer was dispersed in 50 ml deionized water by sonicator for 1 hour. The solution of teniposide was added to the dispersion of CSWCNT-polymer. The mixture was further sonicated for 6 hours at 30℃, conserved overnight in Petri dishes until the acetone was evaporated from the mixture in hot oven at 60 ℃ for 3hour. The teniposide-CSWCNT-polymer dispersion was filtrated and dried [15–17].

**Table (1) Composition of polymer-copolymer used for functionalization of CSWCNT**

| Symbol | Polymer | Co Polymer | Ratio polymer: copolymer | Ratio of CSWCNT: polymer-copolymer |
|---|---|---|---|---|
| **F1** | PEG1500 | PEO | 10:1 | 1:1 |
| **F2** | PEG1500 | PEO | 10:1 | 1:5 |
| **F3** | PEG1500 | Dextran | 10:1 | 1:1 |
| **F4** | PEG1500 | Dextran | 10:1 | 1:5 |
| **F5** | PEG1500 | NIL | 10 | 1:1 |
| **F6** | PEG1500 | NIL | 10 | 1:5 |
| **F7** | PEG1000 | PEO | 10:1 | 1:1 |
| **F8** | PEG1000 | PEO | 10:1 | 1:5 |
| **F9** | PEG1000 | Dextran | 10:1 | 1:1 |
| **F10** | PEG1000 | Dextran | 10:1 | 1:5 |
| **F11** | PEG1000 | NIL | 10 | 1:1 |
| **F12** | PEG1000 | NIL | 10 | 1:5 |
| **F13** | PEG400 | PEO | 10:1 | 1:1 |
| **F14** | PEG400 | PEO | 10:1 | 1:5 |
| **F15** | PEG400 | Dextran | 10:1 | 1:1 |
| **F16** | PEG400 | Dextran | 10:1 | 1:5 |
| **F17** | PEG400 | NIL | 10 | 1:1 |
| **F18** | PEG400 | NIL | 10 | 1:5 |
| **F19** | PVA | PEO | 10:1 | 1:1 |
| **F20** | PVA | PEO | 10:1 | 1:5 |
| **F21** | PVA | Dextran | 10:1 | 1:1 |
| **F22** | PVA | Dextran | 10:1 | 1:5 |
| **F23** | PVA | NIL | 10 | 1:1 |
| **F24** | PVA | NIL | 10 | 1:5 |

***Characterization of polymer-CSWCNT***

***Qualitative dispersibility measurement of polymer-CSWCNT***

The dispersion of polymer-CSWCNT was qualitatively measured by two approaches; the visual observation of homogeneity, as well as centrifugation for three hours at 6000 rpm and the formula that can pass the centrifugation step was recorded [18,19].

***Quantitative dispersibility measurement for polymer-CSWCNT***

The dispersibility percent of the formulation was calculated by measuring the absorbance by UV-Visible Spectrophotometer at 880nm of 0.015 µg/ml concentration before and after centrifugation at 6000 rpm for 15 min according to the following equation[20] :

$$Dispersability\ \% = \frac{conc.of\ polymer - CSWCNT\ after\ centrifugation}{conc.of\ polymer - CSWCNT\ before\ centrifugation} \times 100\%$$

***Characterization of selected teniposide- CSWCNT-polymer formula***

***Fourier transform infrared spectroscopy***

The Fourier transform infrared spectroscopy (FTIR) was performed on a wavelength range from 400 nm to 4000 $cm^{-1}$ to identify the structural changes that may happen to the samples in comparison with the references [21–24].

***Scanning electron microscopy***

The sample for Scanning Electron Microscopy (SEM) was measured using VEGA3 SEM

***Transmission electron microscopy***

Thin section of the sample was prepared by a process known as ultramicrotomy, sections of 50-70 nm thickness were collected on metal mesh 'grids'; and TEM studies were performed[25,26].

***Solubility study of teniposide in optimized formula***

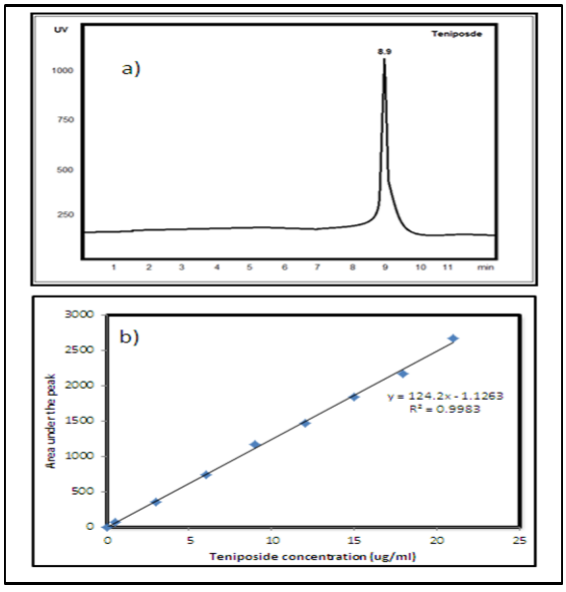
Saturated solutions were prepared by adding excess of teniposide to deionized water, and shaking by the shaker water bath for 48 hours at 25°C under constant vibrations.

Similarly, saturated solutions were prepared by adding excess of teniposide CSWCNT-polymer to deionized water for the purpose of comparison. The saturated solutions were shaked by the shaker water bath for 48 hours at 25°C under constant vibrations. The dispersions were filtered through a 0.45 µm filter, diluted suitably and analyzed by HPLC. Three determinations ± S.D were carried out for each sample to calculate the solubility of the drug

## Results and Discussions

***Calibration curve of teniposide by HPLC***

The calibration curve of teniposide was performed with mobile phase acetonitrile: water (36:64) by series of dilution of teniposide in acetonitrile and the retention time was found to be 8.9 minutes. When the area under the peak was plotted against the concentration, straight line was obtained as shown in figure 1. The square correlation coefficient (R2) was found to be (0.9983), which give a signal of good linearity.




**Figure(1) HPLC a) The chromatogram of teniposide b) Teniposide calibration curve in HPLC**

***Preparation of teniposide formulation***

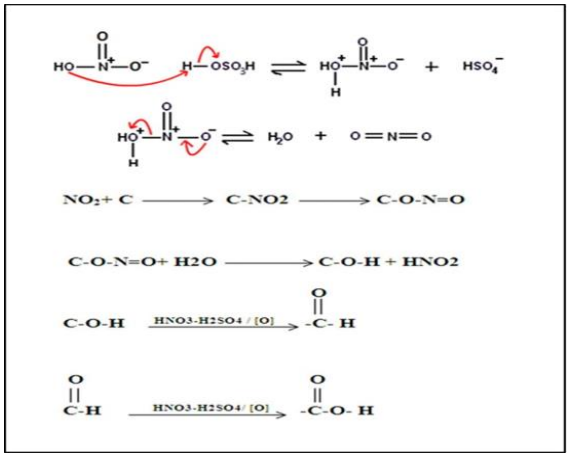
***Purification and carboxylation of SWCNT***

The Synthesized SWCNT in all techniques available until now produces SWCNT with impurities. These impurities were produced during the production of pristine SWCNTs. The most common impurities involved metallic catalysts and amorphous carbonaceous species.

These impurities disturb the physicochemical properties of SWCNTs, so it is necessary to purify SWCNTs before using it. Moreover, owing to the firm Vander Waals interactions, SWCNTs usually trapped with each other, that make them insoluble in most solvents. This makes them undesired in pharmaceutical peruses since intact SWCNTs are not readily reachable to the biological system [27].

To overcome these limitations, chemical oxidation, in order to be purified and pre-functionalized by oxidation to give carboxyl groups on surfaces, is the most predominant strategy applied to SWCNTs. Moreover, oxidation can be viewed as a bridge between physical and chemical properties of SWCNTs[28]. Oxidation of SWCNTs surfaces is generally a starting point in different functionalization proficiencies that enhances dispersibility in various solvents and increases the intensity of the interface amongst SWCNT and the polymers. Oxygen incorporated with carbonyl, carboxylic acid, and hydroxyl components introduced easily by acid treatment. Moreover, it can purify the SWCNTs by dissolving the impurities in strong acids and filtrate to get rid of impurities with the strong acid [29].

The exact mechanism of carboxylation might not be too obvious, since CNTs have aromatic properties as well as aliphatic properties, on the principle ground these are not ideal $sp^2$ hybridizations but that they have something in between $sp^3$ and $sp^2$ hybridization related to curve surface and so display varied chemical reactions but are difficult to execute because of stability. The expected mechanisms for carboxylate group formation were that the concentrated nitric and sulfuric acid both could act as oxidizing agents. Some carbon compounds ( aliphatic type ) got nitration on carbon center by nitric acid and these nitro group isomerized to isonitro group, i.e. from C-$NO_2$ to C-ONO and the later on hydrolysis readily form C-OH that further oxidized to form an aldehyde and finally carboxyl group as shown in scheme 1 [30,31].



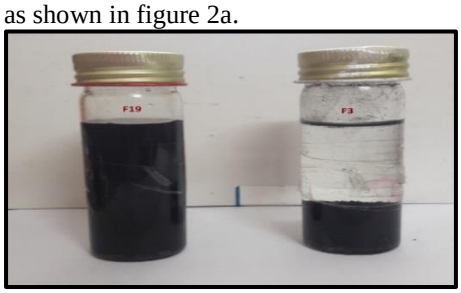

**Scheme (1) Scheme for expected carboxylation steps of carbon nanotubes**

For these reasons and in order to get a lower degree of entanglement, a mixture of concentrated $HNO_3$ and $H_2SO_4$ was used at ratio of 3:1 for oxidation of SWCNT[32].

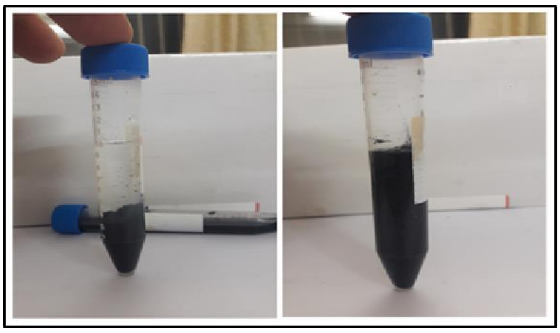
***Qualitative dispersibility measurement of CSWCNT-polymer***

The uniform dispersion system of CSWCNT-polymer in aqueous phase resembles the main challenge, since the improvement of dispersion could lead to ameliorated mechanical, electrical, and optical properties of dispersion. In addition, the interfacial adhesion between the CNT and (po-copo) is one of essential factors in formulation that could be evaluated according to dispersion degree. Several factors can influence the dispersibility like the degree of carboxylation, type of po-copo, ratio of po-copo to CSWCNT, ratio of po to copo, particle size, electrical properties of interfaces, bundle formation and aggregation, interfaces and wettability all the factor detect whether the dispersion is homogenous or not [33]. Different po-copos with different molecular weight and ratios were scanned in different parameters in order to optimize the proper CSWCNT-polymer to further study as shown in Table 2.

From Table 2, the consistency of formulation was varied from homogeneous to non-homogenous and this depends on the dispersibility of the system. The high dispersibility will support the dispersing particle, and support the system from breakoff [34,35], as shown in figure 2a.




(a)



(b)

**Figure (2) a) The dispersibility of homogenous (F19) and non-homogenous (F3) prepared CSWCNT-polymer b) Results of prepared CSWCNT-polymer centrifugation test; a) not pass b) pass.**

**Table(2) Consistency and separation tendency after centrifugation of CSWCNT-polymer**

| Symbol | Consistency after sonication and overnight preserved | Separation tendency after centrifugation for 3 hour |
|---|---|---|
| **F1** | Homogenous | Pass |
| **F2** | Homogenous | Pass |
| **F3** | Non-homogenous | Not pass |
| **F4** | Homogenous | Pass |
| **F5** | Non homogenous | Not pass |
| **F6** | Homogenous | Pass |
| **F7** | Homogenous | Pass |
| **F8** | Homogenous | Pass |
| **F9** | Homogenous | Pass |
| **F10** | Homogenous | Pass |
| **F11** | Homogenous | Pass |
| **F12** | Homogenous | Pass |
| **F13** | Non-homogenous | Not pass |
| **F14** | Homogenous | Pass |
| **F15** | Non-homogenous | Not pass |
| **F16** | Non-homogenous | Not pass |
| **F17** | Non-homogenous | Not pass |
| **F18** | Non-homogenous | Not pass |
| **F19** | Homogenous | Pass |
| **F20** | Homogenous | Pass |
| **F21** | Homogenous | Pass |
| **F22** | Homogenous | Pass |
| **F23** | Homogenous | Pass |
| **F24** | Homogenous | Pass |

For formulations that contained PEG1500 as polymer, most formulations show homogeneity. And by decreasing the molecular weight of PEG to 400, the homogeneity disturbed this might be due to the lower steric hindrance effect of polymer on the formulation with small molecular weight PEG and the bundle of SWCNT start to format [36,37]. The presence of PEO as copolymer will enhance the dispersibility due to the increment in the functional groups and this will lead to better dispersibility.

For formulations that contained PVA as a polymer, the results of most samples are homogenous. This might be due to the structure of PVA that tends to stretch around the CSWCNT, and effective interfacial interaction might occur between PVA and CSWCNT [38,39]. The use of dextran as a copolymer had not improved the consistency which may be due to decreasing in the interfacial area between CSWCNT and polymer that lead to decrease in lyophilized area around the SWCNT and enhance tube-tube bundling [40]. For PEO copolymer, the consistency might improve this effect due to the formation of stable blends between PVA and PEO owing to van der Waals type bond that gives steric hindrance preventing bundle formation [41].

The results of homogeneity estimation obtained visually showed in figure 2a. In order to confirm the homogeneity of CSWCNT-polymer in aqueous media, the efficiency of sonication step, and the distinction whether the CSWCNT-polymer aggregated or dispersed. The centrifugation step is necessary at 6000 rpm for one hour and the formulation that can pass this step without separation into two layer will subjected to further studies as explained in table 2 and figure 2b [42,43].

***Quantitative dispersibility measurement of CSWCNT-polymer***

The quantitative measurements of dispersibility were performed and the results are shown in Table 3.

From Table 3, it was observed that the formulations F2, F8, F12, F19, and F20 exhibited a high dispersion percent. In this type of dispersion, the concentrated area between CSWTNTs and polymers behaved in a lyophilic manner due to the helical wrapping of hydrophilic polymers around the SWCNT body that were thermodynamically favored through the removal of the hydrophobic interfaces between SWCNT sidewall and the aqueous medium. It suggests a stable wrapping of polymer around the SWCNT where the bundle aggregation are very little compared to formula F3 and F23 which established less dispersion percent due to the conception that the polymer in these formulation showed less ability to coat the SWCNT and prevent the bundle formation [37,44–46].

**Table (3) The dispersibility percentage of prepared polymer linked CSWCNT**

| Symbol | dispersibility efficiency percent ± S.D. |
|---|---|
| **F1** | 84.00±1.63 |
| **F2** | 97.00±0.82 |
| **F3** | 33.00±1.63 |
| **F4** | 84.33±1.25 |
| **F5** | 25.33±1.25 |
| **F6** | 86.33± 1.25 |
| **F7** | 84.00± 1.41 |
| **F8** | 93.00±1.40 |
| **F9** | 84.33±2.05 |
| **F10** | 85.00±2.16 |
| **F11** | 86.67±2.05 |
| **F12** | 92.67±1.25 |
| **F13** | 27.00±2.94 |
| **F14** | 84.00± 1.41 |
| **F15** | 14.00± 1.63 |
| **F16** | 25.67± 4.50 |
| **F17** | 13.67±1.25 |
| **F18** | 17.00±0.82 |
| **F19** | 95.33±0.94 |
| **F20** | 97.33±1.25 |
| **F21** | 89.67±2.36 |
| **F22** | 96.33±1.25 |
| **F23** | 77.33±1.25 |
| **F24** | 89.00±1.41 |

***Characterization of selected teniposide-CSWCNT-polymer***

***Fourier transform infrared spectroscopy***

The FTIR performed to pure teniposide as well as to teniposide in final selected formulation and bands are shown in table 4. It was found that the characteristic bands are similar in both spectra.

**Table(4)FTIR bands for reference teniposide and measured.**

| Type of band | Reference(47) value | Measured value |
|---|---|---|
| **Carbonyl stretch vibration of the stained trans lactone ring** | 1785 $cm^{-1}$ | 1780 $cm^{-1}$ |
| **OH stretch vibration of the phenolic** | 3540 $cm^{-1}$ | 3533 $cm^{-1}$ |
| **OH stretch vibration of the sugar OH** | 3400 $cm^{-1}$ | 3398 $cm^{-1}$ |
| **Aromatic bands** | 1605 and 1485 $cm^{-1}$ | 1609 and 1484 $cm^{-1}$ |
| **C-O stretch vibrations** | 1230 and 1100 $cm^{-1}$ | 1233 and 1087 $cm^{-1}$ |

From the FTIR of teniposide-CSWCNT-(PVA-PEO), the (1233and1093) $cm^{-1}$ C-O stretching vibration belongs to teniposide as well as

the PVA. For (1480 and 1382) $cm^{-1}$, the band belongs to C-C, which is found in all component of formulation. The 1638 $cm^{-1}$ belongs to aromatic C=C stretching bands, for SWCNT as well as for teniposide. The 1729 $cm^{-1}$ is the stretching C=O carbonyl group, which indicated the presence of carboxylic acid results from carboxylation of SWCNT and the broad area between 3240 $cm^{-1}$ and 3640 $cm^{-1}$ belongs to OH groups of phenolic and alcoholic OH that are widely found in teniposide in formulation as well as carboxyl group and PVA [47]. The results of FTIR study indicate that the presence of teniposide characteristic peaks as well as the carboxyl group and alcoholic OH, which might confirm the stability of the formulation.

***Scanning electron microscopy for teniposide-CSWCNT-(PVA-PEO)***

The SEM images for teniposide-CSWCNT-(PVA-PEO) are shown in figure 3. The SEM images of teniposide-CSWCNT-(PVA-PEO) had presented the morphology of final formulation in which the nanostructures were seen and the polymer was clearly coated the carbon nanotube. It contained large number of nano chunks due to the affixations of teniposide on ends either of carbon nanotubes or within the body of tubes. The images established that the needle-like body of carbon nanotubes was not affected by the drug attachment [48].

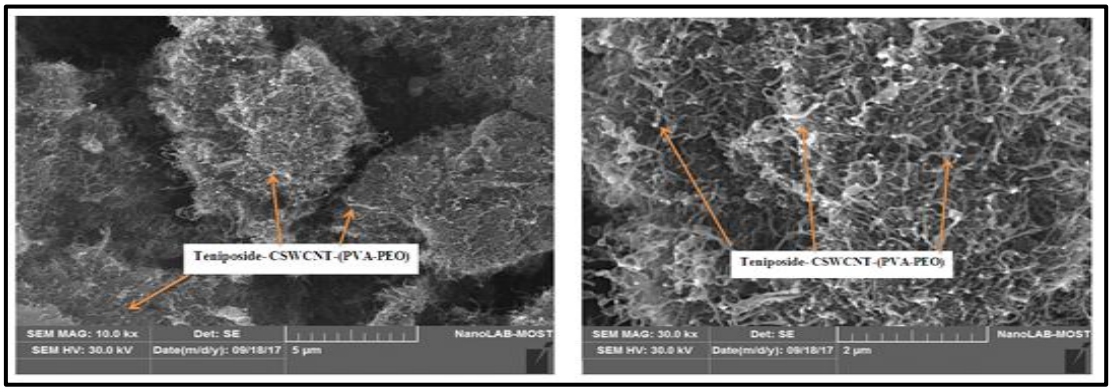



**Figure (3) SEM images of teniposide-CSWCNT-(PVA-PEO)**

***Transmission electron microscopy for CSWCNT-(PVA-PEO)***

The morphology of teniposide-CSWCNT-(PVA-PEO) after dilution was studied using TEM and the results are shown in figure 4, which affirmed that the formula F19 was well dispersed without any bundles or aggregation. In the TEM image, the CNT formula diameter was within 35-40 nm; the teniposide was appeared as dark spots point in the ends of CNTs as well as inside the nanotubes. For the polymer, it appears not too dark as teniposide and spreads around parts of nanotubes.

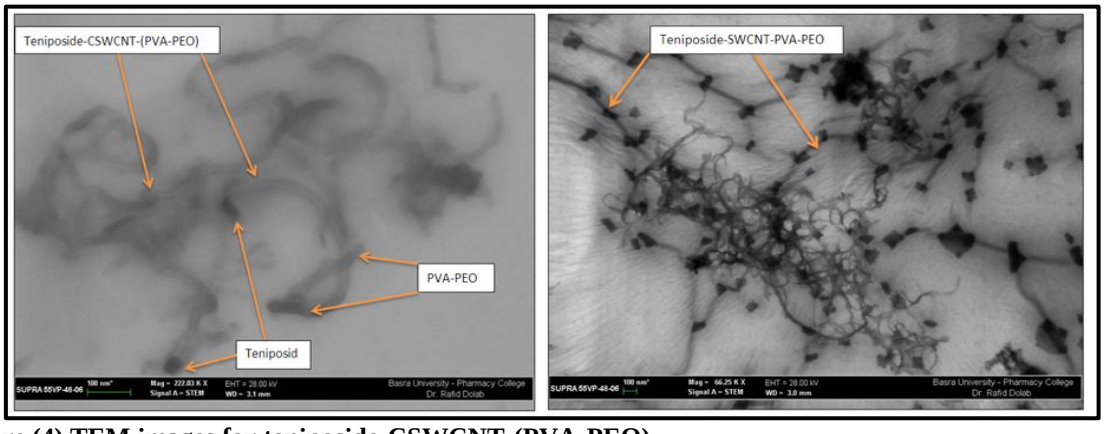



**Figure (4) TEM images for teniposide-CSWCNT-(PVA-PEO)**

***Solubility studies of teniposide in optimized formula***

The solubility is an important parameter in the formulation of poorly soluble drugs. This experiment was done to assess the saturation solubility of pure teniposide and compared it to the incorporated teniposide in carbon nanotubes to find out the improvement of solubility that had achieved from the CSWCNT-polymer.

The result of saturation solubility study of teniposide in water was found to be 0.0527±0.00061 mg/ml while the solubility in teniposide- SWCNT-(PVA-

PEO) was found to be 0.6081±0.01163mg/ml, which around 11.5 folds improvement. This result reveals that pure teniposide has very low water solubility. This fact is expected since teniposide is a BCS class II drug (low solubility, high permeability) with very low water solubility. Similarly, the CSWCNT-(PVA-PEO) was expected to increase the solubility of teniposide since the drug is molecularly dispersed within the CSWCNT-polymer after functionalization, the functionalization of SWCNT is usually required to make them soluble, and also to attach other chemical moieties. These modifications can alter the SWCNT properties, such as the solubility, besides, the size of CWCNT is of nanoparticle size and of a hallow structure which greatly increases the drug surface area exposed to the dissolving media [(49)].

## Conclusions

From the results of this study, it is concluded that the uses of carbon nanotubes can improve the activity of drug through the increments of the solubility as well as the enhancement penetration to the cells, which could be considered as a promising nanocarrier drug delivery system for anticancer drug therapy. The pre-functionalization by carboxylation with $H_2SO_4/HNO_3$ 3:1 ratio and functionalization by polymer/ copolymer mixture linking have improved the physical properties of carbon nanotubes like dispersibility and prevention of bundles of CNT formation. The improvement of dispersibility in this study was the highest in those samples that contain PVA as polymer and PEO as copolymer (F19).

## References


1. Kushwaha SKS, Ghoshal S, Rai AK, Singh S. Carbon nanotubes as a novel drug delivery system for anticancer therapy: A review. Brazilian J Pharm Sci. 2013;49(4):629–43.
2. Canada BS, Sultan P, Shawl AS, Abdellah AA, Ramteke PW, Library N, et al. DRUG NAME : Teniposide. Iarc Monogr Eval Carcinog Risks To Humans. 2000;76(August 2011):575–99.
3. Adem Guvena, Irene A. Rusakovab, Michael T. Lewisc and LJW. Cisplatin@US-tube Carbon Nanocapsules For Enhanced Chemotherapeutic Delivery. biometerials. 2012;33(5):1455–61.
4. Goyanes S, Rubiolo GR, Salazar A, Jimeno A, Corcuera MA, Mondragon I. Carboxylation treatment of multiwalled carbon nanotubes monitored by infrared and ultraviolet spectroscopies and scanning probe microscopy. Diam Relat Mater. 2007;16(2):412–7.
5. Sahoo NG, Rana S, Cho JW, Li L, Chan SH. Polymer nanocomposites based on functionalized carbon nanotubes. Prog Polym Sci. 2010;35(7):837–67.
6. GP. Bhagath Singh, Chandu Baburao, Vedayas Pispati H, Pathipati, Narashimha Muthy SP and BGR. Carbon Nanotubes – A Novel Drug Delivery System. Int J Res Pharm Chem. 2012;2(2):523–32.
7. Park T-J, Banerjee S, Hemraj-Benny T, Wong SS. Purification strategies and purity visualization techniques for single-walled carbon nanotubes. J Mater Chem. 2006;16(2):141–54.
8. Agrawalla RK, Paul R, Chakraborty AK, Mitra AK. Synthesis and Optical Characterization of Sulfonated Polyaniline/Single-Walled Carbon Nanotube/Zinc Sulphide Nanocomposite. ISRN Nanotechnol. 2013;2013:1–6.
9. Ciobotaru CC, Damian CM, Iovu H. Single-wall carbon nanotubes purification and oxidation. UPB Sci Bull Ser B Chem Mater Sci. 2013;75(2):55–66.
10. Mahalingam P, Parasuram B, Maiyalagan T, Sundaram S. Chemical Methods For Purification Of Carbon Nanotubes-A Review. J Environ Nanotechnol. 2012;1(1):53–61.
11. Liu Z, Tabakman SM, Chen Z, Dai H. Preparation of carbon nanotube bioconjugates for biomedical applications. Nat Protoc. 2009;4(9):1372–82.
12. Chen S, Jiang Y, Wang Z, Zhang X, Dai L, Smet M. Light-controlled single-walled carbon nanotube dispersions in aqueous solution. Langmuir. 2008;24(17):9233–6.
13. Alghunaim NS. Optimization and spectroscopic studies on carbon nanotubes/PVA nanocomposites. Results Phys. 2016;6:456–60.
14. Castell P, Cano M, Maser WK, Benito AM. Combination of two dispersants as a valuable strategy to prepare improved poly(vinyl alcohol)/carbon nanotube composites. Compos Sci Technol. 2013;80:101–7.
15. Lay CL, Liu HQ, Tan HR, Liu Y. 1. Lay CL, Liu HQ, Tan HR, Liu Y. Delivery of paclitaxel by physically loading onto poly(ethylene glycol) (PEG)-graftcarbon nanotubes for potent cancer therapeutics. Nanotechnology. 2010;21(6):1–10. Delivery of paclitaxel by physically loading onto poly(e. Nanotechnology. 2010;21(6):1–10.
16. DRUG NAME : Teniposide. In: BC cancer agency cancer drug manual. 2014. p. 1–7.
17. Tsai HC, Lin JY, Maryani F, Huang CC, Imae T. Drug-loading capacity and nuclear targeting of multiwalled carbon nanotubes grafted with anionic amphiphilic copolymers. Int J Nanomedicine. 2013;8:4427–40.
18. Jum'h IY, Al Sekhaneh W, Al-Taani H, Albiss BA. Preparation and characterization of high qualitysingle-walled carbon nanotubes using arc discharge technique. Dig J Nanomater Biostructures. 2016;11(2):517–23.
19. Zhao P, Einarsson E, Lagoudas G, Shiomi J. Mechanism Behind Separation of Single-Walled Carbon Nanotubes via Density Gradient Ultracentrifugation Using Co-Surfactant Dispersions. Photon.Tu-Tokyo.Ac.Jp. 2011.

**20.** Lee J, Kim M, Hong CK, Shim SE. Measurement of the dispersion stability of pristine and surface-modified multiwalled carbon nanotubes in various nonpolar and polar solvents. Meas Sci Technol. 2007;18(12):3707–12.
**21.** Sawant SD, Baravkar AA, Kale RN. FT-IR spectroscopy: Principle, technique and mathematics. Int J Pharma Bio Sci. 2011;2(1):513–9.
**22.** Misra a, Tyagi P, Singh M, Misra D. FTIR studies of nitrogen doped carbon nanotubes. Diam Relat Mater. 2006;15(2–3):385–8.
**23.** Tan JM, Arulselvan P, Fakurazi S, Ithnin H, Hussein MZ. A review on characterizations and biocompatibility of functionalized carbon nanotubes in drug delivery design. J Nanomater. 2014;2014(1):1–20.
**24.** Chaudhari AA, Ashmore D, Nath S deb, Kate K, Dennis V, Singh SR, et al. A novel covalent approach to bio-conjugate silver coated single walled carbon nanotubes with antimicrobial peptide. J Nanobiotechnology. 2016;14(1):1–15.
**25.** Harris P. Transmission Electron Microscopy of Carbon: A Brief History. J Carbon Res. 2018;4(4):1–17.
**26.** Somani PR, Umeno M. Importance of Transmission Electron Microscopy for Carbon Nanomaterials Research. Mod Res Educ Top Microsc. 2007;2(4):634–42.
**27.** Kaushik BK, Majumder MK. Carbon nanotube based VLSI interconnects: Analysis and design. SpringerBriefs in Applied Sciences and Technology. 2015. 17-37 p.
**28.** Yellampalli S. CARBON NANOTUBES – POLYMER NANOCOMPOSITES. Vol. 63, Synthetic Metals. 2011. 91-110 p.
**29.** Ado Jorio GD, Dresselhaus MS. Carbon nanotubes: Advanced topics in the synthesis, structure, properties and applications. Vol. 11, springer. 2008. 107-110 p.
**30.** Chhabra, Deep A, Kaur R, Kumar R. Functionalization of Graphene using Carboxylation process. Int J Sci Emerg Technol with Latest Trends. 2012;4(1):13–9.
**31.** Wang C, Zhou G, Liu H, Wu J, Qiu Y, Gu B-L, et al. Chemical functionalization of carbon nanotubes by carboxyl groups on stone-wales defects: a density functional theory study. J Phys Chem B. 2006;110(21):10266–71.
**32.** Khanna V, Merugula L, Bakshi BR. Advances in Polymer Nanocomposites. Advances in Polymer Nanocomposites. 2012. 8-14 p.
**33.** Im-emsap W, Siepmann J. Modern Pharmaceutics , Chapter 9 Disperse Systems. 2002. 380-429 p.
**34.** Yilmaz B, Bjorgaard J, Lin Z, Köse ME. Phthalimide containing donor-acceptor polymers for effective dispersion of single-walled carbon nanotubes. Org Commun. 2015;8(3):78–89.
**35.** Matarredona O, Rhoads H, Li Z, Harwell JH, Balzano L, Resasco DE. Dispersion of Single-Walled Carbon Nanotubes in Aqueous Solutions of the Anionic Surfactant NaDDBS. J Phys Chem B. 2003;107(48):13357–67.
**36.** Zeineldin R, Al-Haik M, Hudson LG. Role of polyethylene glycol integrity in specific receptor targeting of carbon nanotubes to cancer cells. Nano Lett. 2009;9(2):751–7.
**37.** Fujigaya T, Nakashima N. Non-covalent polymer wrapping of carbon nanotubes and the role of wrapped polymers as functional dispersants. Sci Technol Adv Mater. 2015;16(2):1–22.
**38.** Naebe M, Lin T, Staiger MP, Dai L, Wang X. Electrospun single-walled carbon nanotube/polyvinyl alcohol composite nanofibers: Structure-property relationships. Nanotechnology. 2008;19(30):1–8.
**39.** Coleman JN, Blau WJ, Dalton AB, Muñoz E, Collins S, Kim BG, et al. Improving the mechanical properties of single-walled carbon nanotube sheets by intercalation of polymeric adhesives. Appl Phys Lett. 2003;82(11):1682–4.
**40.** Kalia S, Sabaa MW. Polysaccharide based graft copolymers. Vol. 9783642365, Polysaccharide Based Graft Copolymers. 2013. 1-353 p.
**41.** Liu GQ. Morphology and thermal behaviour of poly ( methyl methacrylate ) / poly ( ethylene glycol ) / multi-walled carbon nanotubes nanocomposites. Bulg Chem Commun. 2015;47(3):889–94.
**42.** Koh BE. Dispersion of Single-Walled Carbon Nanotubes (SWCNTs) in Aqueous Solution and Reversion of SWCNT Aggregates. 2013.
**43.** Rösner B, Guldi DM, Chen J, Minett AI, Fink RH. Dispersion and characterization of arc discharge single-walled carbon nanotubes – towards conducting transparent films. Nanoscale. 2014;6(7):3695–703.
**44.** Michael J. O'Connell, Sergei M. Bachilo, Chad B. Huffman,Valerie C. Moore, Michael S. Strano, Erik H. Haroz, Kristy L. Rialon, Peter J. Boul, William H. Noon, Carter Kittrell, Jianpeng Ma, Robert H. Hauge, R. Bruce Weisman RES. Band Gap Fluorescence from Individual Single-Walled Carbon Nanotubes. Science (80- ). 2002;297(5581):593–6.
**45.** Woo HS, Czerw R, Webster S, Carroll DL, Ballato J, Strevens AE, et al. Hole blocking in carbon nanotube-polymer composite organic light-emitting diodes based on poly (m-phenylene vinylene-co-2, 5-dioctoxy-p-phenylene vinylene). Appl Phys Lett. 2000;77(9):1393–5.
**46.** Hasan T, Scardaci V, Tan PH, Bonaccorso F, Rozhin AG, Sun Z, et al. Molecular- and Nano-Tubes. 2011. 279-354 p.
**47.** Kattenes J, Bosch van den, J.m. J holtumis, Bult

A. Analytical profiles of drug substances. / Volume 19. 1990. 575-599 p.

**48.** Kwon Y, Shim W, Jeon S-Y, Youk J-H, Yu W-R. Improving dispersion of multi-walled carbon nanotubes and graphene using a common non-covalent modifier. Carbon Lett. 2016;20:53–61.

**49.** Wang T, Zhao P, Zhao Q, Wang B, Wang S. The mechanism for increasing the oral bioavailability of poorly water-soluble drugs using uniform mesoporous carbon spheres as a carrier. Drug Deliv. 2016;23(2):420–8.