

Investigation of functionalizing effect by PVP, PEG4000, or PEG6000 on dispersability of carbon nanotube as promising drug delivery system

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Abstract

Carbon nanotube as hollow nanoneedle structure has great interest by researchers due to their unique chemical, physical, and biological properties as one of the most promising nanocarriers for drug delivery to their site of action. Purification and carboxylation of single walled carbon nanotube (SWCNT) using different ratios of $H_2SO_4:HNO_3$ which confirmed by Fourier transform infrared spectroscopy analysis to obtain carboxylated SWCNT (CSWCNT). Polymer linked CSWCNT was prepared using polyethyleneglycol (PEG) of different molecular weight (4000 or 6000) or polyvinylpyrrolidone30K (PVP) as polymer while polyethyloxide (PEO) or dextran 70 as copolymer (copo) at different ratios of 1:1 and 1:5 of CSWCNT: polymer-copolymer. Characterization of prepared polymer-CSWCNT by visual observation of homogeneity as well as separation tendency after centrifugation for three hours was studied. The dispersability percent of the formulation was calculated by using UV-Visible Spectrophotometer at 880nm. Also, particle size of CSWCNT was measured.

The results indicate that the best ratio of $H_2SO_4:HNO_3$ is 3:1 in CSWCNT2 for optimum carboxylation and best polymer for functionalization is PEG4000 comparing to PEG6000 which is better than PVP. The best ratio of CSWCNT to polymer-copolymer was 1:5 while best copolymer was PEO comparing to dextran which is better than polymer alone without copolymer. Finally, the best formula (F8) of polymer linked CSWCNT of improved physical properties can be considered a good candidate as targeted drug delivery system for important drugs.

Key words: Carbon-nanotube, functionalization, PVP30K, PEG4000, or PEG6000

INTRODUCTION:

Nanoparticles have particles size range of 1-1000 nm that either solid or dispersion in a liquid. The preference in properties of nanoparticles over the large particles are due to fact that as the size of particle decreases, there is an ascendancy of the behavior of atoms on the outer surface of the particle over those in the interior of the particle (as the size reduces, the number of atoms on the surface increases relative to those in the interior). This effect alters the properties of the particles at this size grade in the minor of the interaction of particles with particles of other materials(1).

In contemporary time the nanoparticle witness unprecedented growth in research and applications at the area of drug delivery, that due to the bright role of nanoparticles in solvate the problems of drugs involving, solubility, permeability, targeted delivery to site of action, therapeutic effectiveness, and plasma fluctuations of drugs(2).

From these nanoparticles, carbon nanotubes (CNTs) which are sheets of graphite rolled into tubes with bonds at the end of the sheet forming the bonds that close the tube. The CNT has attractiveness over other nanoparticles by its unique physical, chemical, mechanical and thermal properties initiating from the small size, cylindrical structure, and high aspect ratio of length to diameter(3).

Nanotubes resemble promising possible drug nanoparticles carrier presently under study. When drug delivery to the body is intended they combine the properties of nanoparticles, the biocompatibility of liposomes or polymeric nanoparticles with the stability of inorganic nanoparticles such as gold and silica ones.

Carbon nanotubes are good carriers for drugs in the organism as they are hollow and the needle- like shape of CNT enable the penetration to the cells with drugs(4).

The problem of pristine CNT that are of smooth surfaces which miss overhanging bonds make them inert chemically, and it is insoluble aqueous or any solvent types due to the strong $\pi-\pi$

interactions between the individual tubes. This problem can be solvate by process of functionalization which might require pre-functionalization treatment by strong acid in order to the opening of the nanotube caps as well as the formation of holes in the sidewalls that enable the process of functionalization. The treatment of pure CNTs with a mixture of HNO_3/H_2SO_4 was suggested to be an efficient method for carboxylation(5).

The use of proper ratio of mixture of HNO_3/H_2SO_4 lead to carboxylated the surface on CNT at the defect site of nanotubes body as well as the strong acid treatment lead to create a new defects site of CNT that can help in stable attachment with polymer and enhance the properties of CNT and prepared it for functionalization by polymer.

The main approaches of use polymer to disperse and exfoliate carbon nanotubes and graphene. The dispersion of CNTs is a crucial to retaining the electronic attributes of the tubes. The re-dispersion of CNT are an important factor because CNT dispersions usually aggregate easily and therefore make additional processing very difficult. So the functionalization by soluble polymer is required for dispersion and separation the bundles of carbon nanotubes into homogeneous dispersion(6).

The selection of polymer depend on the properties of polymer and the causative of it utilize, which in general must be nontoxic biocompatible and improve the CNTs properties as stability, dispersibility, facile electrochemical polymerization, ion exchange with the medium, good capacity to enhance adhesive coatings, increase the porosity and drug loading efficienc(7).

The dispersion effect is due to the structure, electrical charge and chemical properties of CNTs by polymers addition. The polymer is wrapping around CNTs body that will improve the aqueous dispersibility of CNT(8).

Different water soluble polymer like poly vinylpyrrolidone (PVP) or polyethylene glycol (PEG) to increase the dispersibility and enhance cell penetration.

MATERIAL AND METHODS:**Materials**

Single walled carbon nanotube (SWCNTs) was purchased from Hongwu International Group Ltd, China. Polyethylene glycol 4000 was purchased from Sinopharm Chemical reagent, China. Polyethylene glycol 6000 was purchased from Reagent World TM, USA. Polyvinylpyrrolidone30k was purchased from Hi media, India. Dextran 70 was purchased from Guanjie Chemical reagent, China. All other ingredients used in the study were analytical grade.

Purification and carboxylation of SWCNT

Query the amount of 1 g SWCNTs in 80 ml of different ratio of H₂SO₄(18.4M) and HNO₃(15.7M) as shown in table (1) in tightly close glasses container; ultrasonication in bath sonicator for about 6 hours at 70°C and conserve overnight, then centrifugation steps at 4000 rpm were performed to remove big agglomerates and bundles, after sonication and centrifugation, dilution with deionized water in order to support the filter paper from the strong acids, filtrated through 0.2 μm polycarbonate filters(Whatman Ltd.) then continually wash with deionized water by vacuum-assisted filtration until the pH of elute reached to 7, the filter paper that contains the precipitant was dried by using hot air ovens for a night then the SWCNT collected for the next step as shown in figure1(9–16).

Table 1: Ratios of acids used for carboxylation of SWCNT

Symbol	H ₂ SO ₄ : HNO ₃ ratio	ml of H ₂ SO ₄	ml of HNO ₃
CSWCNT1	4:1	64.0	16.0
CSWCNT2	3:1	60.0	20.0
CSWCNT3	2:1	53.3	26.7
CSWCNT4	1:1	40.0	40.0
CSWCNT5	1:2	26.7	53.3
CSWCNT6	1:3	20.0	60.0
CSWCNT7	1:4	16.0	64.0

**Figure 1:** Steps of carbon nanotubes purification and carboxylation**Characterization of CSWCNT****Fourier transform infrared spectroscopy**

The FTIR was done by using FTIR-7600 of lambda company spectrometer on a wavelength range from 400 to 4000 cm⁻¹ using KBr disc to identify the exact structure of samples and compared with the references(17–20).

Calibration curve of selected CSWCNT in aqueous solution

The calibration curve of optimized CSWCNT in aqueous media was performed in the presence of sodium dodecyl sulfate (SDS) as a dispersing agent in all samples as well as in blank.

The solutions were prepared by mixing 10 ml of 1.5%w/v aqueous SDS solution in a glass vial with different weight of CSWCNT to give a concentration of

(0.005,0.01,0.015,0.02,0.02.5 and 0.03)μg/ml. The solutions were sonicated for 60 minutes in path sonicator at 25°C; Then the dispersed sample solution was centrifuged at 4000rpm for 30 min then the supernatants were measured at 880 nm(21,22). The absorbances measured were recorded and plotted versus the respective concentrations.

Preparation of polymer linked CSWCNT

For the polymeric coating of SWCNTs a different polymer (po) and co-polymer (copo) where used in order to enhance the dispersing capacity of SWCNTs and increase drugs loading. From these polymer polyethyleneglycol (PEG) of different molecular weight (4000 or 6000) or polyvinylpyrrolidone30K (PVP) while polyethyloxide(PEO) or dextran 70 as copolymer(copo) were used at different ratios as shown in the table (2).

Table 2: Composition of polymer-copolymer used for functionalization of CSWCNT

Symbol	Polymer	Co Polymer	Ratio(polymer: copolymer)	Ratio of CSWCNT: polymer-copolymer
F1	PEG6000	PEO	10:1	1:1
F2	PEG6000	PEO	10:1	1:5
F3	PEG6000	Dextran	10:1	1:1
F4	PEG6000	Dextran	10:1	1:5
F5	PEG6000	NIL	10:0	1:1
F6	PEG6000	NIL	10:0	1:5
F7	PEG4000	PEO	10:1	1:1
F8	PEG4000	PEO	10:1	1:5
F9	PEG4000	Dextran	10:1	1:1
F10	PEG4000	Dextran	10:1	1:5
F11	PEG4000	NIL	10:0	1:1
F12	PEG4000	NIL	10:0	1:5
F13	PVP30K	PEO	10:1	1:1
F14	PVP30K	PEO	10:1	1:5
F15	PVP30K	Dextran	10:1	1:1
F16	PVP30K	Dextran	10:1	1:5
F17	PVP30K	NIL	10:0	1:1
F18	PVP30K	NIL	10:0	1:5

Preparation of CSWCNT-PVP

One gram of polyvinylpyrrolidone (PVP) was dissolved in 50 ml of warm deionized water with simple shaking to form a solution; the copolymer prepared by same method and was added in different ratio.

In a 50ml vial, 100mg of CSWCNT were dispersed in 20 ml deionized water by sonication for 1hour, the polymer-copolymer solution added to CSWCNT dispersion drop by drop with sonication in path sonicator for 3 hour.

The CSWCNT-(PVP-COPO) suspension were centrifuged for about 15minute at 4000 rpm; the temperature kept at around 25 °C (room temperature), the supernatant solution was collected to liquidation the dispersion from the large particle. Then filtration with vacuum and washed with deionized water six times in order to get rid of the access non attached PO-COPO with CSWCNT. The filtrate then dried by the oven for 12 hours and kept in a dry close container for the next step(23,24).

Preparation of CSWCNT-PEG

A hundred milligrams of polyethylene glycol of different molecular weight (4000, 6000) was added into the 20ml deionized water in a glass vial and sonicated for 30 minutes in bath sonicator, and the copolymer was added in different ratio.

A hundred milligrams of CSWCNT were 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 was changed every 30 min to avoid overheating and kept at 25°C.

The polymer-CSWCNT suspension was centrifuged for about 15 minute at 4000 rpm; the temperature kept at around 25°C (room temperature), the supernatant solution was collected and separated 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 CSWCNT with the polymer.

Finally, filtrate was dried by hot oven overnight, and the powder was collected in a tightly close container for the next step(25,26).

Characterization of polymer-CSWCNT

Qualitative dispersability 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 well take to quantitative measurement of dispersability(27,28).The centrifugation was performed by Hermle Z200A centrifuge.

Quantitative dispersability measurement for polymer-CSWCNT

The dispersability percent of the formulation was calculated by measuring the absorbances by UV-Visible Spectrophotometer (Cary100 UV, Varian, Australia) at 880nm of 0.015 µg/ml concentration before and after centrifugation at 6000 rpm for 15 min according to the following equation(29):

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

Particle size determination of CSWCNT

The aqueous dispersion of CSWCNT before and after polymer linking was prepared in a suitable amount of ultrapure water (1:100) to get a suitable scattering intensity. The mean of particle sizes was estimated by Brookhaven Instruments Corp90 PLUS (ZetaPlus Particle Sizing, NY, Software, Version 5.34). The measurements were at a fixed scattering angle of 90° at room temperature (25°C)(30).

RESULTS AND DISCUSSION:

Purification and carboxylation of SWCNT

Till now none of the techniques that used in the synthesis of SWCNTs produce a pure carbon, the production of SWCNTs create a lot of impurities besides the SWCNTs, the most common impurities, metallic catalysts and amorphous carbonaceous species. These impurities might disturb the physicochemical properties of SWCNTs, so it is necessary to purifying 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. Makes them undesired in pharmaceutical peruses since intact SWCNTs are not readily reachable to the biological system(31).

To overcome these limitations, chemical oxidation in order to purification and pre-functionalization 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(32). Oxidation of SWCNTs surfaces is generally a starting point in different functionalization proficiencies that enhances dispersability in various solvents and increases the intensity of the interface amongst SWCNT and the polymers.

Oxygen-incorporating 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(33).

The ratio of H₂SO₄ to HNO₃ effect the oxidation of SWCNT, in high HNO₃ ratio the dispersion of SWCNT increase as well as the efficiency of elimination of metallic impurities. These effects might be due to that the oxidation of SWCNTs occur at defects found on body of SWCNTs and these defects either original which takes place in synthesis process or newly created due to harsh effects of strong acid specially nitric acids which represent an important factor in oxidation process.

For formulation that contain high percentages of HNO₃ as in formulations CSWCNT3 to CSWCNT7 show coalesce formation and increase damaged SWCNTs that lead to elevate the percentage of weight loses after the centrifugation steps, which might be due to the high percentage of HNO₃ lead to intercalation of carbonyl and carboxyl group in SWCNTs that lead to bunch SWCNTs together and came with bundle scurf and engraving of the surfaces which lead to organization of extra amorphous carbon nanoparticles covering the remaining smaller bundles of SWCNT which lead to increase wastage that need more afford to remove as well as cost effectiveness since high quality SWCNTs are costly materials as shown in figure (2)



Figure 2: Damaged and coalesced SWCNT

At low concentration of HNO₃ as in CSWCNT1 the dispersion of SWCNT in water was less due to the defects site generated from sulfuric acid is lesser than from nitric acid, so the oxidation of SWCNT will be lesser. The optimum ratio of H₂SO₄/HNO₃ is 3:1 in CSWCNT2. The filtration elutes of H₂SO₄/HNO₃ at high and low concentrations of nitric acid are shown in figure (3).

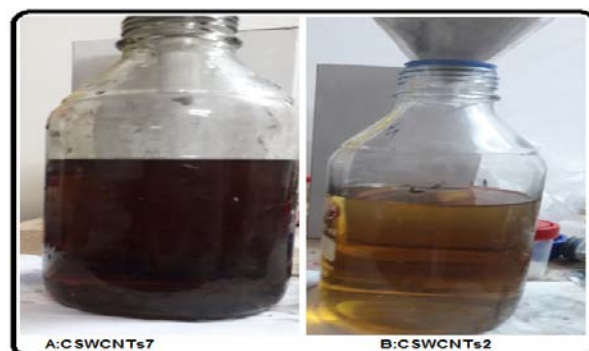


Figure 3: Eluent of an oxidation product of SWCNT; a) from CSWCNT7, b) from CSWCNT2

Fourier transform infrared spectroscopy

The Fourier transform infrared spectroscopy (FTIR) can be employed in characterization of functional group engaged with carbon nanotubes(confirm the presence of -COOH group on the purified SWCNTs), ensure the chemical reactions occur, it is an appropriate technique that identifies and confirm the chemical fundamental interaction within the SWCNTs(17-19).

The FTIR of pristine SWCNT was performed, and the peaks appear at 1634cm^{-1} which due to C=C double bond as shown in figure (4). For acid treatment, quite a number of new peaks appear that not found in pristine SWCNT. The bands due to the C=O stretch are very conspicuously seen in the range 1730cm^{-1} for the carboxylated SWNT, which can be attributed to the acid carbonyl-stretching mode.

Other bands also were seen in this sample are very low one at 2950cm^{-1} and another at 3433cm^{-1} , that are characteristic of C-H and O-H stretches respectively. The C-C vibrations occur due to the internal defects, and the O-H vibration is associated with the amorphous carbon because amorphous carbon easily forms a bond with atmospheric air (20,21) .

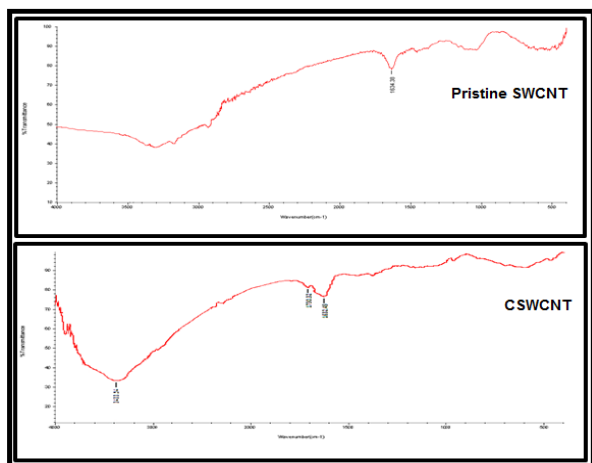


Figure 4: FTIR spectra of pristine SWCNT (top) and carboxylated SWCNT (down)

Preparation of polymer-CSWCNT

In order to improve the SWCNT properties like aqueous dispersability, dimensional firmness, chemical stability, permeability, targeting in body tissues, flexibility, polymer or mixture of polymer and copolymer has been used for this purpose. The hypothesis to form complexes between SWCNT and different types of polymers drastically enhanced the features of solubility of SWCNT, that the solubility in different solvents can be modulated according to the type of bond, polymers types and the moieties of polymer that interact with the tubes(34).

The polymers when wrapping up around the SWCNTs usually by either hydrophobic or aromatic interactions toward the SWCNT surfaces, and displayed the hydrophilic groups on the outer surface toward the aqueous or polar solvents. However, in non-covalent bond types the bounded moiety might be dissociated from the surfaces due to the structures of SWCNT that have strong Vander Waals between the tubes moiety, consequently, release of polymers exposing their hydrophobic surface which have a tendency to aggregation. While, by presence of active group on the surfaces of SWCNT the bond gets stronger and free from this drawback, which depends on presence of structural defects on the SWCNT surfaces.

The phenomenon of polymer driving force to disperse the SWCNT that due to the steric repulsion force of between polymer moiety, once the polymer that attached to the outer surface of the SWCNT, it provide an enough repulsive potential that might stabilize the dispersion.

In the situation of nonionic polymers, based on poly(oxyethylene) copolymers, dispersion efficiency is because of their hydrophilic similarity(35).

Characterization of CSWCNT-polymer

Qualitative dispersability measurement of CSWCNT-polymer

The uniform dispersion system of CSWCNT-polymer in aqueous phase resemble the main challenge, since the improvement of dispersion properties might leads to ameliorated mechanical, electrical, and optical properties of dispersion, also the interfacial adhesion between the CSWCNT and (po-copo) which is one of essential factor in formulation can be evaluated according to dispersion degree.

Several factors can be influenced on the dispersability 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 (36). Different polymer-copolymer with different molecular weight and ratio were scanned in different parameters in order to optimize the proper (polymer-copolymer)-CSWCNT to study further as shown in table (3).

Table 3: Consistency and separation tendency after centrifugation of CSWCNT-polymer

Symbol	Consistency after sonication and overnight preserve	Separation tendency after centrifugation for 3 hour
F1	Non homogenous	Not pass
F2	Non homogenous	Not pass
F3	Non homogenous	Not pass
F4	Non homogenous	Not pass
F5	Non homogenous	Not pass
F6	Non homogenous	Not pass
F7	Homogenous	Not pass
F8	Homogenous	Pass
F9	Non homogenous	Not pass
F10	Non homogenous	Not pass
F11	Non homogenous	Not pass
F12	Homogenous	pass
F13	Homogenous	Not pass
F14	Not homogenous	Not pass
F15	Not homogenous	Not pass
F16	Not homogenous	Not pass
F17	Not homogenous	Not pass
F18	Not homogenous	Not pass

From the results listed in table (3), the consistency of formulation was varied from homogeneous to non-homogenous which depend on the dispersibility of the system the high dispersability will support the dispersing particle and support the system from brake out.

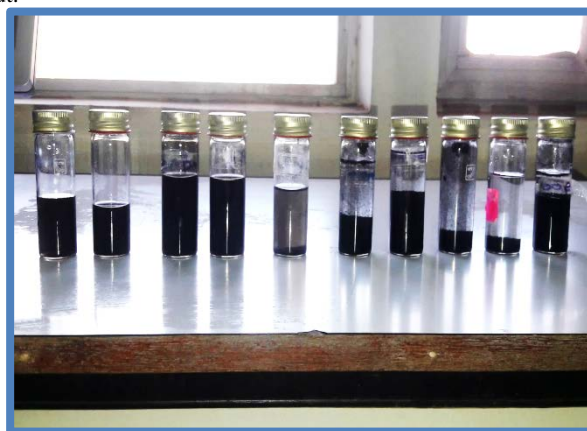


Figure 5: The dispersability of different prepared CSWCNT-polymer

Formulation of CSWCNT-polymer that contain PVP as polymer in all concentration measured with or without different copolymer used show less homogeneity this might due factor like the high polarity of PVP tend to complexation formation with hydrogen donor group like carboxyl group which found in CSWCNT and form cross-link complexes with more than one particles of CSWCNT and bundle forming that lead to non-homogenous tendency also the high molecular weight of PVP might increase the rate of sedimentation of CSWCNT-polymer due to formation of large molecules(37,38).

For the formulation that contains PEG as polymer it was noted that the homogeneity of high molecular weight is low and tends to increase with decreasing molecular weight of PEG to certain limit this might be due to large particles will form. By decreasing the molecular weight of PEG the homogeneity will be better (39,40). The results indicate that the presence of PEO as copolymer will enhance the dispersability due to the increment in the functional group which may be led to better dispersability.

The estimation of homogeneous degree obtained visually as seen in figure (5) and in order to confirm the homogeneity of CSWCNT-polymer in aqueous media, efficiency of sonication step and distinguish whether the CSWCNT-polymer aggregated or dispersed, the analysis by centrifugation step is necessary at 6000 rpm, and the formulation that can pass this step without separated into two layers are taken to the next step as shown in table (3) and figure (6).

The most formulation that preserved its consistency as homogeneous was able to pass centrifugation step except for F7 that is in spite of its homogeneity not pass the centrifugation step which might be due to the size of CSWCNT-polymer (41,42).

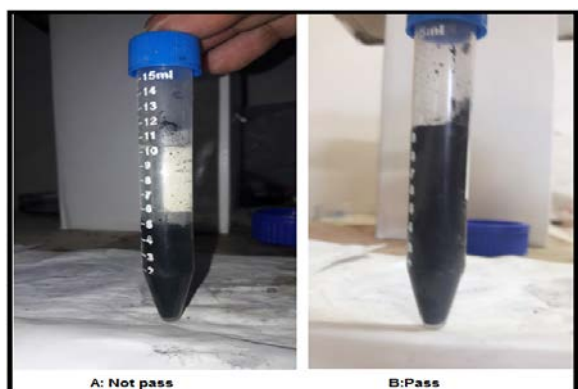


Figure 6: Results of prepared CSWCNT-polymer centrifugation a) not pass b) pass

Calibration curve of CSWCNT in aqueous solution

The calibration curve of CSWCNT2 shows a straight line with a high correlation coefficient (0.9977) as shown in figure (7) which means that the data obeys Beers-Lambert law and useful for quantitative analysis.

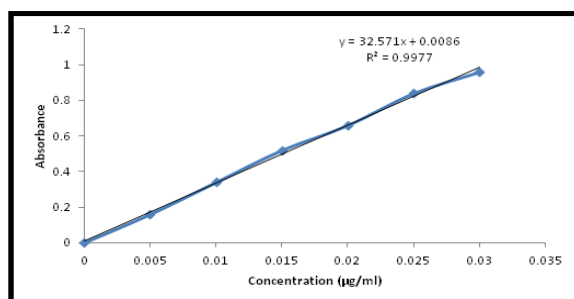


Figure 7: Calibration curve of CSWCNT-polymer in deionized water at 880 nm

Quantitative dispersability measurement of CSWCNT-polymer

In order to measure the percentage of dispersibility of the prepared formulations, it was measured by the spectrophotometer, and the calculated results are shown in the table (4)

Table 4: Dispersability percentage of the prepared polymer linked CSWCNT

Symb ol	Dispersability efficiency % \pm S.D. for prepared CSWCNT-polymer
F1	16.33 \pm 1.25
F2	20.67 \pm 1.89
F3	11.00 \pm 1.63
F4	14.00 \pm 2.16
F5	8.00 \pm 0.82
F6	13.33 \pm 0.94
F7	39.67 \pm 3.40
F8	84.67 \pm 3.09
F9	25.67 \pm 1.70
F10	23.00 \pm 0.82
F11	29.33 \pm 2.62
F12	77.33 \pm 2.05
F13	8.67 \pm 0.47
F14	11.33 \pm 0.47
F15	6.33 \pm 1.25
F16	9.33 \pm 1.25
F17	13.00 \pm 0.82
F18	17.00 \pm 0.82

It was observed that the formulation F8 and F12 exhibit a high dispersion ratio, in this type of dispersion exhibits a lyophilic behavior in concentrated area, the helical wrapping by hydrophilic polymers are thermodynamically favored through the removal of the hydrophobic interface between SWCNT sidewall and the aqueous medium that suggest a stable wrapping of polymer around the SWCNT where the bundle aggregation are very little (40,43–45).

Particle sizes of CSWCNT before and after polymer link

The average particle size of CSWCNT was average of 69.32 \pm 6.73nm while the particle size of polymer linked CSWCNT was in average of 74.67 \pm 8.28nm for the all eighteen formulations which indicate no significant change in particle size after linking of CSWCNT with the polymer with or without copolymer.

CONCLUSIONS

From the results of this study, it can be concluded that linking of CSWCNT by the polymer with or without copolymer improve significantly the physical properties of the promising drug delivery system (CSWCNT) specially the dispersability. It was found that the best ratio of H₂SO₄/HNO₃ is 3:1 in CSWCNT2 for optimum carboxylation. Also, it was found that PEG4000 is the best polymer comparing to PEG6000 which is better than PVP. The best ratio of CSWCNT to polymer-copolymer was 1:5 while best copolymer was PEO comparing to dextran which is better than polymer alone without copolymer. Finally, the best formula (F8) of polymer linked CSWCNT of improved physical properties can be considered good candidate as targeted drug delivery system for important drugs

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