CISPLATIN FUNCTIONALIZATION OF MULTIWALL CARBON NANOTUBES

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Multiwall carbon nanotubes (MWCNTs) functionalization can improve anti-tumor properties of cisplatin (CDDP). Carboxyl and amino groups were chosen to improve the properties of cisplain. This was evidenced by infrared spectroscopy (FTIR) and transmission electron microscopy (TEM) by adding cisplatin to the carboxyl (-COOH) and amino (-NH_2) functionalized nanotubes. Platinum ions released in simulated body fluid (SBF) were measured by inductively coupled

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1. Introduction

Embedding the cisplatin (CDDP) is an outstanding strategy in the discovery of new synthetic drugs. Carbon nanotubes (CNT) are customizable of carbon atoms and are forming a tubing diameter within the range nanometers and a length of up more than a thousand times the diameter. Both types of carbon nanotubes with a single wall and with multiple walls have the unique properties such as electron conductivity, thermal stability, chemical composition and the capacity to cross membrane and enter living cells.[1-3]

These observations as to their nano size and shape suggest the possible application of MWCNTs injected with the drug and intravascular nanovectors.[4-6]. This system supply should be addressed directly to malignant cells, avoiding normal ones because of the body's antibody creation. Biocompatibility of carbon nanotubes which is subject of many controversies,[6] can be increased by functionalizing them with various functional groups such as carboxyl –COOH and amino –NH_2.

Notwithstanding its efficacy, cisplatin reduced stability is one of the leading term to use and is bound to plasma proteins responsible for complex establishment that causes nephrotoxicity and kidney filtration difficulty[7]. It has been already reported by Ajima et al. [8] that cisplatin functionalized the inner

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space of single wall carbon nanohorns: horn-like shape tubes of about 2 nm in diameter (formed by a single graphene sheet) that associate in spherical aggregates with a diameter of 80 nm. Feazell et al.[9] exploited the delivering potential of single-wall carbon nanotubes (SWCNTs) by loading an inert platinum (IV) form of this anti-neoplastic[10] compound on the external tube shell upon a covalent linkage; the drug was then released as active platinum (II) form upon reduction reaction in the endosomes and lysosomes acidic environment. Instead, we exploited the hollow structure of CNTs to transport the guest molecules which are embedded upon capillary wetting process[11-15].

Nowadays, the topical knowledge in the field related to cisplatin clinical experiments concludes as a need investigations in order to reduce cisplatin toxicity.In the spirit of such need the objective of this research is to compare two ways of cisplatin functionalization of multiwall carbon nanotubes with different potential of platinium ion release in time.

2. Materials and methods

2.1. Materials

Multiwall Carbon Nanotubes (MWCNTs) were purchased from Sigma Aldrich having more than 90% carbon basis and D x L 10-15 nm x 0.1-10 μm, produced by Catalytic Chemical Vapor Deposition (CCVD). Oxidation was made using a mixture of 98% sulfuric acid (Merck). The ethylenediamine (EDA) modifier agent was supplied by Fluka. We have used saline solution and cisplatin, and all reagents were not further purified.

2.2. MWCNTs functionalization

The use of MWCNTs as controlled release systems can be achieved by binding to surface proteins or anticancer agent carbon nanotubes (have the ability

to penetrate the cell membrane). MWCNTs (0.6 g) were dispersed in 98% sulfuric acid and ulatrasonicated at 50°C for 6 h to obtain MWCNT-COOH [16]. Functionalization of MWCNTs reduced toxicity. 0.1 mg MWCNT-COOH were ultrasonically prepared with thionyl chloride $SOCl_2$ (50 ml) for 30 minutes at room temperature. The suspension obtained was refluxed under magnetic stirring at room temperature for 48 hours and then filtered. The filtrate was washed with tetrahydrofuran (THF) and dried at room temperature for 20 minutes. MWCNTs-$SOCl_2$ were immersed in ethylenediamine at room temperature for 10 hours. The mixture was washed with tetrahydrofuran and filtered. The filtrate was dried at 80° C for 10 hours. Cisplatin (20 mg) was added to a solution of

MWCNT-COOH and MWCNT-NH_2 (0.5 mg) dispersed in 5 ml saline. They were ultrasonicated for 48 hours at 50° C and filtered (Fig. 1).

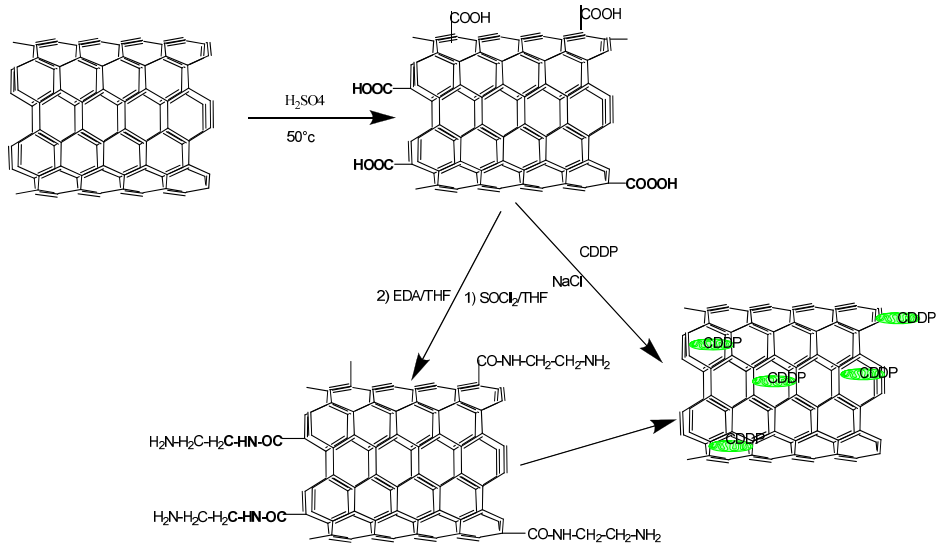

Fig. 1. Functionalization of MWCNTs with cisplatin

2.3. Methods of characterization

FTIR spectra of functionalized MWCNTs were registered on a Perkin Elmer, Spectrum 100 equipment in 400÷4500 cm^{-1} range with 4 cm^{-1} resolution and 32 scans.

Nano-sized particles were investigated using TEM analysis with a microscope Philips EM-410, 60kV.

An ICP-MS, ELAN DRC-e Perkin Elmer SCIEX U.S.A. was used for platinum determinations. The detection limit was 0.001μg.g^{-1}. The signals were recorded in simulated body fluid (SBF) with the composition given in Table 1 according to Kokubo formula [17].

Table 1.

SBF chemical composition

NaCl	KCl	$CaCl_2$	$NaHCO_3$	$Na_2HPO_2 \cdot 2H_2O$	$MgCl_2.6H_2O$	KH_2PO_4	$MgSO_47H_2O$	Glucose
8g/L	0.4g/L	0.18g/L	0.35g/L	0.48g/L	0.10g/L	0.06g/L	0.10g/L	1g/L

3.Results and discussion

3.1. FTIR measurements

FTIR spectroscopy is a very useful tool to show the presence of functional groups on the surface of MWCNTs, the bands allocated to groups: -CH, amino and CDDP in the two samples are almost identical.

-COOH functionalized sample bands for -CH functional groups range from: 3307.55 to 2136.89 cm^{-1} for CDDP range: 723.81 to 86.86 cm^{-1} as shown in Fig. 2.

-NH_2 functionalized sample bands for -CH functional groups are between: 3304.73 to 2134.36 cm^{-1}, for grouping amines between: 163.31-1186.34 cm^{-1} and for CDDP between: 694,94-93.28 cm^{-1} as shown in Fig. 3.

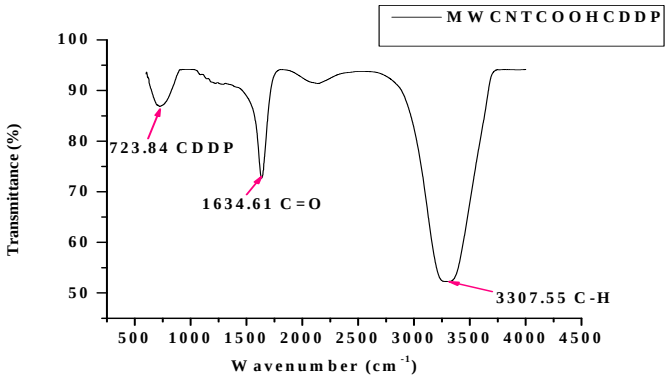

Fig. 2. FTIR spectra for MWNT-COOH-CDDP

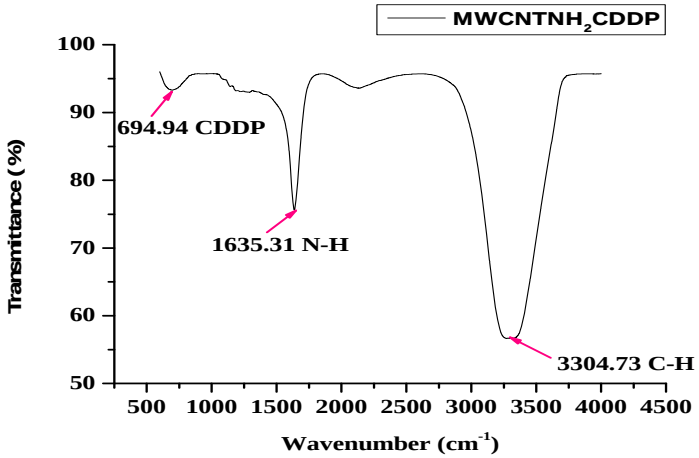

Fig. 3. FTIR spectra for MWNT-NH_2-CDDP

As we see from Fig. 4 and in Table 2 the two bands attributed CDDP characterizing functional groups are almost identical.

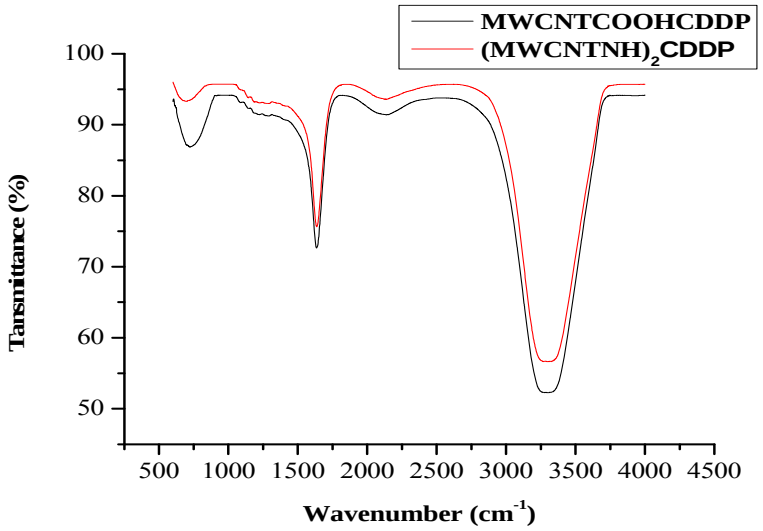

Fig. 4. FTIR spectra for MWNT-COOH-CDDP and MWNT-NH_2-CDDP

Table 2

Allocating the bands

	MWCNT-COOH-CDDP	**MWCNT-NH_2-CDDP**
Correspondence	**Wave number, cm^{-1}**	**Wave number, cm^{-1}**
-OH	3307.55	3304.73
-CH2	2136.89	2134.36
C=C	1414.36	1289.66
C=O	163.61	1635.31
C–N stretching vibration	-	1186.34
N–H distortion	-	1222.50
NH_2 stretching	-	3493.66
CDDP	723.84	694.94

3.2 TEM measurements

Nanosized particles morphologies are investigated using Transmission Electron Microscopy analysis (TEM).

From this analysis, we can observe in Fig. 5 the surface of multiwall carbon nanotubes carboxyl functionalised MWCNTs–COOH further prepared with cisplatin. MWCNTs of curled shape have about 10-20 nm in diameter and 0.1-10 μm length. TEM images shows that grains of -CDDP of 10-20 nm in width and 10-20 nm in length were grown radially originating from a common centre at the intersection of two MWCNTs and perpendicularly to the longitudinal direction of MWCNTs. The encapsulation of cisplatin drug it is very well put in evidence.

In Fig. 6 it is shown the morphologies for multiwall carbon nanotubes amino functionalised further prepared with cisplatin.

The grains of cisplatin are in a range of 4-6 nm in width and 10 nm in length. The surface is not quite homogenous and the encapsulation of cisplatin drug is not very well put in evidence.

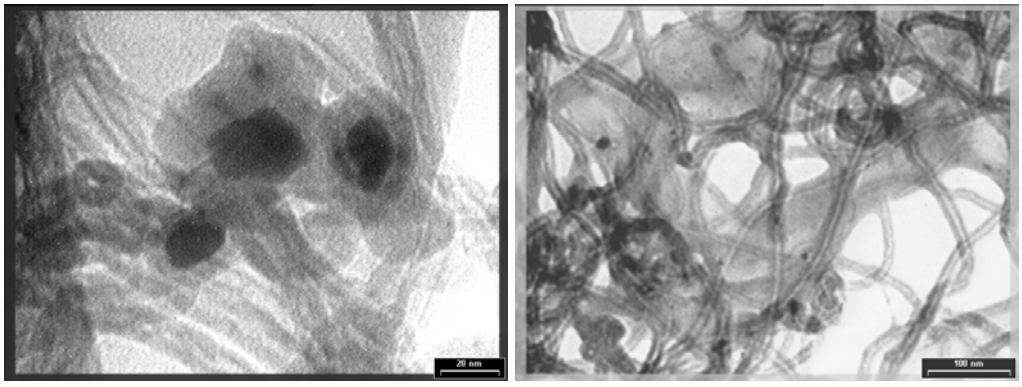

Fig. 5. TEM morphologies for MWCNTs-COOH-CDDP

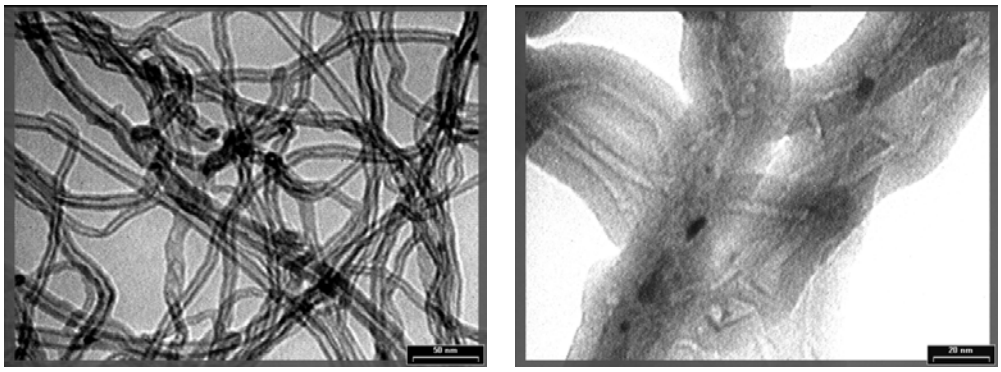

Fig. 6. TEM morphologies for MWCNTs-NH_2-CDDP

3.3. ICP-MS measurements

The samples were introduced in SBF for different periods of time and the solutions were analyzed. Samples were introduced with an *in-situ* nebulizer/vapor generator sample introduction system. The conditions were selected in order to maximize the platinum ion signal while the solution was introduced into the vapor generating system. The generated vapor was then transported to the ICP-MS for platinum determination. After 6, 12 and 24 hours it was measured the quantity of platinum ions released in a solution of simulated body fluid. The results from ions release analysis are shows in Table 3 and in Fig. 7.

Table 3

Pt ions release from cisplatin

Samples	Pt ions release after 6 hours (µg/100µg MWCNTs)	Pt ions release after 12 hours (µg/100µg MWCNTs)	Pt ions release after 24 hours (µg/100µg MWCNTs)
MWCNTs-COOH-CDDP	6.82	12.65	19.12
MWCNTs-NH_2-CDDP	5.24	9.61	12.34

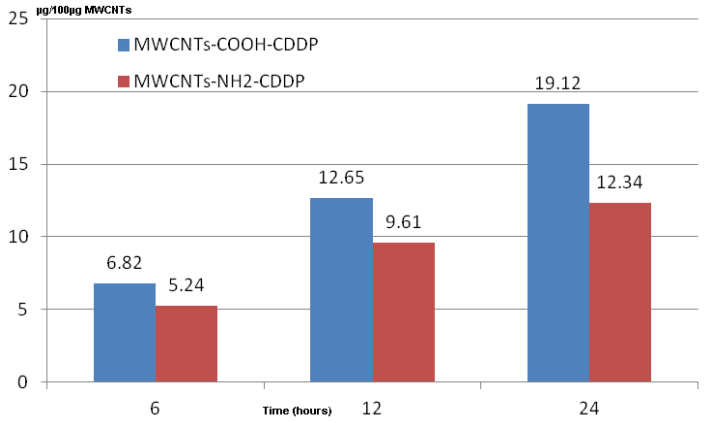

Fig. 7. Platinum ions release determined via ICP-MS measurements for MWCNTs-COOH-CDDP and MWCNTs-NH_2-CDDP samples

The highest value for platinum ions release was observed for MWCNTs-COOH-CDDP samples after 24 hours and the smallest value were observed for MWCNTs-NH_2-CDDP samples after 6 hours of immersion in SBF. The amounts of platinum ions release vary in the following ranges: from 6.82 to 19.12 μg Pt /100 μg MWCNTs for MWCNTs-COOH-CDDP, and from 5.24.3 to 12.34 μg Pt /100 μg MWCNTs for MWCNTs-NH_2-CDDP. This value indicates the possibility for the future positive employment after further analysis.

4. Conclusions

We presented the study on anticancer drug cisplatin which functionalized the -COOH and -NH_2 groups from MWCNTs. After chemical functionalization of MWCNTs with –COOH and –NH_2 groups, put in evidence by FTIR measurements, the procedure resulted in coating of MWCNTs surface with the chemotherapeutic agent. A good coating results for MWCNT-COOH samples. This fact was observed by studying the morphologies of carbon nanotubes by TEM analysis.

The release of CDDP from as-functionalized sample in physiological solution was evaluated by ICP-MS, and the amount of platinum ions released in 24 hours was found greater for MWCNTs-COOH-CDDP. Further study on the influence of the process parameters of the starting material on CDDP release is required.

Acknowledgements

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R E F E R E N C E S

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