Published in final edited form as:

Biomaterials. 2012 February ; 33(5): 1455–1461. doi:10.1016/j.biomaterials.2011.10.060.

Cisplatin@US-tube Carbon Nanocapsules For Enhanced Chemotherapeutic Delivery

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Abstract

The use of chemotherapeutic drugs in cancer therapy is often limited by problems with administration such as insolubility, inefficient biodistribution, lack of selectivity, and inability of the drug to cross cellular barriers. To overcome these limitations, various types of drug delivery systems have been explored, and recently, carbon nanotube (CNT) materials have also garnered attention in the area of drug delivery. In this study, we describe the preparation, characterization, and *in vitro* testing of a new ultra-short single-walled carbon nanotube (US-tube)-based drug delivery system for the treatment of cancer. In particular, the encapsulation of cisplatin (CDDP), a widely-used anticancer drug, within US-tubes has been achieved, and the resulting CDDP@US-tube material characterized by high-resolution transmission electron microscopy (HR-TEM), energy-dispersive spectroscopy (EDS), X-ray photoelectron spectroscopy (XPS), and inductively-coupled optical emission spectrometry (ICP-OES). Dialysis studies performed in phosphate-buffered saline (PBS) at 37 °C have demonstrated that CDDP release from CDDP@US-tubes can be controlled (retarded) by wrapping the CDDP@US-tubes with Pluronic-F108 surfactant. Finally, the anticancer activity of pluronic-wrapped CDDP@US-tubes has been evaluated against two different breast cancer cell lines, MCF-7 and MDA-MB-231, and found to exhibit enhanced cytotoxicity over free CDDP after 24 hours. These studies have laid the foundation for developing US-tube-based delivery of chemotherapeutics, with drug release mainly limited to within cancer cells only.

Keywords

Cisplatin; cancer chemotherapy; nanotechnology; single-walled carbon nanotubes; drug delivery

1. Introduction

Chemotherapy is one of the main treatments for both localized and metastasized cancer, and it is typically used in conjunction with surgery and/or radiotherapy. For chemotherapy, it is

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desirable to deliver a sufficient quantity of drug to malignant cells, while minimizing undesirable side effects. However, the efficacy of many drugs is hindered due to complications with administration such as limited solubility, rapid elimination, inefficient distribution, inability to cross cellular barriers, and the inability to differentiate between normal cells and cancer cells. Cis-dichlorodiammineplatinum(II) (cisplatin, CDDP) is one of the most potent and widely-used anticancer drugs for the treatment of a variety of solid tumors, including testicular, ovarian, bladder, cervical, head and neck, oesophageal, and small-cell lung cancers [1,2]. The biological activity of CDDP is derived from its interactions with DNA since CDDP interferes with the normal transcription and replication processes of the cell which results in cell death [2,3]. Despite its success, the clinical use of CDDP and its derivatives are limited due to severe side effects including nephrotoxicity, neurotoxicity, ototoxicity, nausea, and vomiting [4]. Thus, the development of efficient drug delivery systems that selectively increase the concentration of chemotherapeutics, such as CDDP, in diseased cells while limiting their concentration in normal cells continues to be of great interest.

Carbon nanotubes (CNTs) are a well-known material with unique chemical and physical properties that make them potentially desirable for many applications [5-8]. In particular, recent studies have indicated that biomedical applications of CNTs are a potentially promising area of study for the treatment of cancer [9-17]. While the toxicological effects of CNTs themselves have been widely debated in the literature [18-21], one recent study [22] has shown that highly-purified single-walled carbon nanotubes (SWCNTs), and ultra-short single-walled carbon nanotubes (US-tubes) are well-tolerated by Swiss mice, even at very high doses ($\approx 0.5 \text{ g kg}^{-1} \text{ b.w.}$) [22]. In addition, CNTs are considered to be relatively bioinert, even though one study has reported that they can be degraded *in vivo* by neutrophils [23]. Finally, CNT materials, whether derivatized or underivatized, have been observed to be eliminated from animals via the kidneys [22,24].

Thus, although a variety of drug delivery systems, including microspheres [25,26], silica nanoparticles [27,28], dendrimers [29], polymeric micelles [30-32], liposomes [33-35], and carbon nanohorns [11,36], can be used as drug delivery agents, CNTs offer a number of special properties which suggest that they too might be engineered into desirable drug delivery platforms to complement other more typically-used materials. To date, a number of CNT based drug delivery systems have been explored. In these systems, drug molecules mainly have been attached onto the surface or sidewalls of the nanotubes either by specific adsorption or by covalent bonding [10,37]. In addition to surface attachment, it has been shown that small drug molecules can also be embedded [38] or encapsulated [39] within SWNT materials. In this study, we report the use of US-tube capsules, 20-80 nm in length and ca. 1.4 nm in diameter, as a drug delivery platform for CDDP (Figure 1). US-tubes, which are produced from full-length SWCNTs via a fluorination and pyrolysis procedure [40], seem ideal candidates for drug encapsulation and delivery *in vivo* due to their short and relatively uniform-lengths (ca. 95% $\leq 50 \text{ nm}$) which might help them avoid the reticuloendothelial system (RES), while enhancing their cellular uptake properties and eventual elimination profiles. In addition, US-tubes have already been shown to encapsulate other materials of medical interest such as Gd^{3+} -ion clusters to produce a high-performance MRI contrast agent [41], I_2 molecules to make an X-ray contrast agent [42], and $^{211}\text{AtCl}$ molecules to prepare a new agent for α -radiotherapy [43].

In this report, we describe the preparation, characterization, and *in vitro* testing of a new US-tube-based drug delivery platform for the treatment of cancer, which allows for the possible design of a cancer-specific enzyme-activatable US-tube-based drug delivery platform that releases its drug cargo within cancer cells only.

2. Materials and methods

2.1. Preparation of US-tubes

US-tubes were prepared from full-length SWCNTs (purchased from Carbon Solutions Inc., CA, USA), which were produced by the electric-arc discharge method, as previously reported [40]. To remove amorphous carbon and metal catalyst impurities, US-tubes were first bath-sonicated in concentrated HCl for 1 h, then collected by filtration on a glass filter and finally washed several times with distilled water. These purified US-tubes were then chemically reduced by a metallic Na⁰/THF reduction procedure to create individual US-tubes [44].

2.2. Encapsulation of CDDP within US-tubes

Chemically debundled US-tubes (20 mg) were dispersed in DI water (20 mL) via bath-sonication for 1 h. CDDP (20 mg) was then added to the US-tube suspension and vigorously stirred for 24 h. After stirring, the solution was left undisturbed overnight whereupon CDDP-loaded US-tubes (CDDP@US-tubes) flocculated from solution. After removing the supernatant solution, CDDP@US-tubes were collected on a glass filter. To remove the excess CDDP from the outer surface of the US-tubes, the CDDP@US-tubes were washed forty times with DI water (total of 60 mL) until all exterior CDDP was removed, as judged from Pt analysis on the filtrate aliquots by ICP-OES (see below). The collected CDDP@US-tubes were then dried in an oven at 80 °C.

2.3. Characterization of CDDP@US-tubes

To confirm the encapsulation of CDDP within the US-tubes, HR-TEM studies were performed using a JEOL 2000 FX microscope equipped with an energy-dispersive spectrometer (EDS) and operated at 200 kV. Conventional and HR-TEM imaging methods were used. Precautions were taken to minimize structural changes in the sample caused by electron-beam heating effects. One such precaution was the use of a cold sample stage which kept the sample temperature in the range of –70 °C to –50 °C. The HR-TEM samples were prepared by placing a small amount of CDDP@US-tube sample on a copper grid coated with amorphous carbon holey film. No solvents were used during the procedure.

To confirm that CDDP was removed from the exterior surface of US-tubes by washing, washings were collected and analyzed by ICP-OES. Elemental analyses of CDDP@US-tube samples were carried out by multiple analytical techniques: XPS, EDS, and ICP-OES. For XPS analysis, solid samples were placed on indium foil (Sigma-Aldrich) and then examined using a PHI Quantera Spectrometer with photo and Auger emissions produced via a monochromatic Al K α X-ray source with a pass energy of 140 eV for survey and 30 eV for high-resolution scans. EDS measurements were carried out using an OXFORD EDS system installed in a JEOL-2100F TEM operating at 200 kV. Samples for EDS measurements were prepared via bath sonication in methanol for a few min, and a drop of dispersed solution was then placed on a TEM copper grid. ICP-OES analyses were made using a Perkin Elmer Optima 4300 DV instrument with a CDD detector. The quantity of CDDP internalized within the US-tubes was quantified by measuring the Pt concentration of each sample. Samples for ICP-OES measurements were prepared by digesting the CDDP@US-tubes with 26% HClO₃, as previously described [45]. The platinum signal was observed at 265.95 nm. Five scans were performed for each sample, and the concentration was determined from the average of scans. Yttrium was used as the internal standard.

2.4. CDDP release studies from CDDP@US-tubes

CDDP release studies from US-tubes were performed by enclosing the CDDP@US-tubes inside a dialysis-membrane (20,000 MW) cylinder, which was immersed in PBS at 37 °C for

one week. During immersion, the solid CDDP@US-tube sample in the dialysis-membrane cylinder was sampled periodically by filtration and collection on a glass frit filter, followed by washing with distilled water to remove PBS. The Pt concentrations of solid CDDP@US-tube samples were then determined by ICP-OES. CDDP release with time from CDDP@US-tube samples was also determined after dispersing the CDDP@US-tube samples in a 0.17 % (w/v) Pluronic F-108 solution via probe sonication for two min to produce Pluronic-F108-wrapped CDDP@US-tubes (W-CDDP@US-tubes). Pluronic-F108 is a neutrally-charged, non-cytotoxic surfactant that is commonly used to administer carbon nanotube materials for in vitro and in vivo testing [45]. Release of CDDP from W-CDDP@US-tubes and CDDP@US-tubes was then compared.

2.5. Cell viability studies

The cytotoxic activity of CDDP released from W-CDDP@US-tubes was evaluated against two different breast cancer cell lines (MCF-7 and MDA-MB-231) by using MTT cell proliferation assay (Invitrogen). This colorimetric assay quantifies cell proliferation and viability by measuring the reduction of the tetrazolium component (MTT) into an insoluble formazan product by the mitochondria of viable cells. The cells were cultured and grown in Minimum Essential Medium (MEM) supplemented with 10 % FBS, 1 % penicillin/streptomycin, and 0.15 % insulin (10 mg mL^{-1}) at 37°C under a humidifying atmosphere containing 5 % CO_2 . The cells were then seeded onto 96-well plates at a density of 5×10^3 cells well^{-1} in 150 μL MEM. After 24 h of incubation to allow cell adhesion, the medium in each well was replaced with 150 μL of new medium containing the serial dilution of either CDDP, W-CDDP@US-tubes, or W-US-tubes at various concentrations (5-50 μM), and cells were then incubated at 37°C in 5 % CO_2 for 24 h, and 48 h. An equivalent US-tube concentration of CDDP@US-tubes and US-tubes was used for each of the concentration point by calculating the US-tubes concentration of CDDP@US-tubes from Pt wt % values obtained by ICP-OES analysis. After incubation for each time period, the medium in each well containing CDDP, W-CDDP@US-tubes, or W-US-tubes was aspirated and the cells washed with phosphate buffered saline (PBS). For cell viability measurements, 100 μL of fresh medium (free of phenol red) and 10 μL of MTT reagent (5 mg mL^{-1} in PBS) was added to each well and incubated for 4 h to allow the formation of formazan crystals. After removing all but 25 μL of medium from the wells, 50 μL of dimethyl sulfoxide (DMSO) was added to each well (to solubilize the formazan) and mixed thoroughly and incubated at 37°C for an additional 10 min. The absorbance values were measured using a microplate reader (Bio-Rad Model 680) at 570 nm to determine relative viabilities.

2.7. Cell internalization rate studies

To determine the comparative cell internalization rate of free CDDP and W-CDDP@US-tubes into MCF-7 cells, the cells were plated on a cell culture dishes at 5×10^5 cell/dish and allowed to reach up to 70-80% confluence. After removing the medium in each dishes, the attached cells were first washed with PBS and then incubated with free CDDP and W-CDDP@US-tube samples in MEM at 10 μM CDDP concentration for various times (3, 6, 12, 24, and 48 h). After each incubation time, the cells were washed with PBS, collected from the dishes by lifting up with trypsin-EDTA, counted with a cell counter (Cellometer Auto T4), and prepared for ICP-OES analysis to quantify Pt in each sample.

2.8. Statistical evaluation

Unless otherwise noted, all data are expressed as mean $\pm$ standard deviation (SD) of at least three independent experiments. Wherever appropriate, the statistical significance of data were analyzed using the Microsoft Excel statistical package. A one-tail homoscedastic unpaired *t*-test was used to determine statistical significance; a *p*-value of less than 0.05 ($p < 0.05$) was considered statistically significant.

3. Results & Discussion

3.1. Encapsulation of CDDP within US-tubes

The encapsulation of CDDP within US-tubes has been achieved as detailed in the Materials and Methods Section and as outlined in Figure 1. After an extensive washing procedure ($>20\times$) with DI H_2O , 10 mL aliquots of each washing were collected and analyzed for Pt content by inductively-coupled plasma optical emission spectrometry (ICP-OES) to determine CDDP release over time. As shown in Figure 2, the majority of CDDP was removed by the first few washings with water. Since no additional removal of CDDP was observed after 20 washings, it was concluded that all exterior CDDP was removed by 20 washings.

After the extensive washing procedure, the CDDP@US-tubes were collected by filtration and dried in an oven at 80°C . To determine the quantity of CDDP within the US-tubes, the Pt concentration was determined via ICP-OES. Because of the limited solubility of CDDP in water, CDDP@US-tube preparation was also attempted in a few different solvent mixtures in which CDDP is more soluble, and the results are summarized in Table 1. Although CDDP has greater solubility in ethanol (EtOH) and dimethylformamide (DMF), greater loading was achieved when water alone was used. For this reason, further preparations were performed in water to enhance CDDP@US-tube yields.

A CDDP@US-tube sample has been characterized by high-resolution transmission electron microscopy (HR-TEM). As seen in Figure 3a, CDDP clusters are observed as dark spots encapsulated along the interior axis of the US-tubes, with both Pt and Cl content confirmed by electron-dispersion spectroscopy (EDS). For comparison, empty US-tube images do not reveal such dark spots (Figure 3b). Since the dimension of an individual CDDP molecule is about $0.4\text{ nm}\times 0.6\text{ nm}\times 0.1\text{ nm}$ [46], CDDP molecules have the ability to easily fit within US-tubes (ca. 1.4 nm in diameter) and cluster at the sidewall defect sites. Based on the CDDP aggregate sizes (black spots) which ranged in length from 0.75-1.0 nm, up to four or five CDDP molecules exist in each aggregate. Earlier theoretical calculations have also concluded that CDDP can easily fit within the confines of SWCNTs [47].

To determine whether the CDDP Pt center remains in its original oxidation state (Pt^{2+}) after encapsulation within US-tubes, the X-ray photoelectron spectroscopy (XPS) spectra of the Pt 4f peaks of CDDP@US-tubes, metallic platinum (Pt^0), and sodium hexachloroplatinate hexahydrate, ($\text{Na}_2\text{PtCl}_6\cdot 6\text{H}_2\text{O}$; Pt^{4+}), were obtained. Figure 4a shows the survey XPS spectrum of CDDP@US-tubes, confirming the presence of platinum, chloride, and nitrogen from CDDP and carbon and oxygen from US-tubes. Figure 4b shows that the Pt 4f peaks of the CDDP@US-tube sample were positioned between the Pt 4f peaks of metallic platinum and $\text{Na}_2\text{PtCl}_6\cdot 6\text{H}_2\text{O}$; confirming that the CDDP@US-tube sample contains Pt^{2+} and not reduce Pt^0 or oxidize Pt^{4+} . For comparison, we also obtained the Pt 4f spectrum of free CDDP (Strem Chemicals) which displayed peaks at 72.30 eV and 75.65 eV. On the other hand, the CDDP@US-tube sample exhibited Pt 4f peaks at 72.90 eV and 76.25 eV, corresponding to a +0.60 eV shift from that of free CDDP sample. This difference apparently arises from the environment of the encapsulating US-tubes. XPS also confirmed a 2:1 N:Cl ratio and a 2:1 N:Pt ratio, suggesting that the extensive washing process with water to prepare the CDDP@US-tube sample nearly halved the amount of chloride ion present in the original CDDP sample, likely replacing them with water molecules [48]. Since the loss of chloride ions from CDDP is desirable before CDDP binds to DNA and other cellular targets [49], the loss of chloride ions in the CDDP@US-tube sample should not affect the efficacy of the Pt^{2+} species released from the US-tubes. EDS analysis was also performed on a solid CDDP@US-tube sample (Figure S1). EDS detected carbon, nickel

(metal catalyst) from US-tubes, copper (from the TEM grid), and platinum and chloride (from CDDP). EDS also determined the same Pt:Cl (1:1) mole ratio as that of the XPS data.

3.2. Release studies of CDDP from CDDP@US-tubes

Release of CDDP from a drug delivery system is an important parameter that should occur in a controlled fashion. To test the release of CDDP from CDDP@US-tubes, we prepared a sample in which the CDDP@US-tube sample was dispersed via probe sonication for two minutes in an aqueous 1.7 mg mL^{-1} Pluronic-F108 surfactant solution. The pluronic-wrapped CDDP@US-tube sample (W-CDDP@US-tubes) was expected to slow CDDP release since the sidewall defects and ends of US-tubes should be, at least, somewhat blocked by surfactant. Dialysis was performed on both CDDP@US-tubes and W-CDDP@US-tubes in phosphate-buffered saline (PBS) at 37°C . The CDDP release rate for these two samples was investigated by determining the Pt content of samples over time by ICP-OES analysis. Figure 5 displays the comparative release profiles of CDDP from CDDP@US-tubes and W-CDDP@US-tubes. From the obtained data, we observed that the W-CDDP@US-tube system leaked only 6.1 % CDDP in 24 h and only 16.9 % over one week. However, unwrapped CDDP@US-tubes showed a much greater rate of release, with almost 50 % of CDDP released in the first 3 h and about 80 % after one week. From this data, we infer that the release of CDDP from W-CDDP@US-tubes is retarded because the pluronic wrapping around the US-tubes helps prevent drug release.

3.3. In vitro cytotoxicity studies

A series of *in vitro* studies have been conducted to determine the cytotoxicity profiles of W-CDDP@US-tubes using the well-established MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) cell proliferation assay. The performance of W-CDDP@US-tubes was evaluated against two different breast cancer cell lines (MCF-7 and MDA-MB-231), which are known to be resistant to CDDP treatment [50], at 24 h and 48 h incubation times, and their efficacies compared to that of free CDDP. Figure 6 displays the comparative cytotoxicities of CDDP, W-US-tubes, and W-CDDP@US-tubes in a dose- and time-dependent manner. The cytotoxicity of CDDP and W-CDDP@US-tubes both increased with increasing CDDP concentration. While the US-tubes alone have a relatively small effect on cell viability for concentrations $\leq 20 \mu\text{M}$, the W-CDDP@US-tubes clearly exhibited greater cytotoxicity for both cell lines and even greater cytotoxicity when compared to free CDDP at all CDDP concentrations. Although, the W-CDDP@US-tubes displayed greater efficacy for each incubation time than free CDDP at an equivalent dose, the efficacy of the W-CDDP@US-tube sample was greater at 24 h than 48 h. To investigate this result, we decided to determine the Pt concentration per cell for the MCF-7 cell line treated with CDDP and W-CDDP@US-tubes as a function of time. These results are given in Figure S2 which demonstrate that the cells treated with W-CDDP@US-tubes have significantly more Pt than cells treated with free CDDP over the first 24 h time period, however by 48 h, the Pt concentration in per cell is about the same for both W-CDDP@US-tubes and free CDDP. Thus, the US-tube structure apparently assists the delivery of free CDDP from W-CDDP@US-tubes into the cells over the first 24 h. In addition, the release rate of CDDP from W-CDDP@US-tubes *in vitro* is likely greater than in only PBS (ca. 6% after 24 h) because previously observed aggregation of US-tubes within stem cells has indicated the removal of the Pluronic coating from US-tubes upon passage through the cell membrane [45]. All of the above results taken together demonstrate that W-CDDP@US-tubes are more cytotoxic than free CDDP at the 24 h time point ($p < 0.05$). Since both cell lines used in this study are resistant to CDDP, and it is well known that reduced drug uptake is a major cause of CDDP resistance in cancer cell lines [3,49], the present data suggests that CDDP@US-tube materials help to overcome CDDP resistance by increasing drug accumulation in resistant cells.

From this encouraging result, we believe that it might be possible to control CDDP release from CDDP@US-tubes by wrapping them with covalently-attached activatable cancer-specific peptide sequences instead of surfactant molecules. Recently, a catalytic method to functionalize US-tubes with various amino acids and peptides was reported which improved their solubility, biocompatibility, and biological targeting capabilities [51]. In future work, using this functionalization protocol, a peptide chain, containing an enzyme-specific substrate sequence for a cancer cell antigen, such as the well-known prostate specific antigen (PSA), will be synthesized and covalently attached to CDDP@US-tubes [51]. This peptide chain should cover the sidewall defect sites and ends of the US-tubes as a sheath and help prevent premature drug release until the peptide is cleaved upon PSA activation within prostate cancer cells, as shown schematically in Figure S3. Using this strategy, peptide-wrapped CDDP@US-tubes could become a smart drug delivery platform that would activate CDDP drug release within cancer cells after intravenous administration.

4. Conclusions

In this study, CDDP encapsulation within US-tubes has been confirmed by HR-TEM. Elemental analysis (XPS, and EDS) of CDDP@US-tubes has revealed that about half of the chloride ions of the encapsulated CDDP are replaced with water molecules during the aqueous washing procedure to prepare CDDP@US-tubes. It has also been shown that CDDP@US-tubes release CDDP upon dialysis in PBS at 37 °C much more slowly when CDDP@US-tubes are wrapped with Pluronic-F108 surfactant. Finally, the CDDP@US-tube platform exhibited greater cytotoxicities against MCF-7 and MDA-MB-231 cells at 24 h, when compared to free CDDP. These results suggest that by wrapping CDDP@US-tubes with cancer-specific enzyme-activatable peptide sequences, that cancer-cell-specific chemotherapeutics might be developed using the carbon nanocapsule platform of this study.

Appendix

Additional figures documenting the EDS spectrum of CDDP@US-tubes (Figure S1), the comparative cell internalization rate of CDDP and W-CDDP@US-tubes (Figure S2), and hydrolysis of the peptide sequence on a CDDP@US-tube by PSA (Figure S3), and can be found in the Supplementary Data section.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by The Welch Foundation (Grant C-0627), Ensysce Biosciences, Inc., the Breast Cancer SPORE (Grant P50 CA058183), and National Cancer Institute (Grant R01 CA138197). The authors would like to thank Lesa A. Tran for initial studies, rewarding discussions, and helpful editing, as well as Gabriel J. Villares for cell culture study training and his assistance. A.G. also gratefully acknowledges the Turkish Ministry of Education for financial support.

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Figure 1.
Preparation and purification of CDDP@US-tubes.

Figure 2.

Pt determination by ICP-OES analysis of each collected aliquot of H₂O wash solution for a typical CDDP@US-tube sample. The data are expressed as mean $\pm$ SD of three independent experiment. Error bars may be smaller than symbols.

Figure 3.
HR-TEM images of a) bundled CDDP@US-tubes and b) bundled empty US-tubes.

Figure 4. XPS spectrum of a) CDDP@US-tubes (survey), b) Pt 4f peaks for various samples: i) metallic platinum (Pt^0), ii) CDDP@US-tubes (Pt^{2+}) and iii) $\text{Na}_2\text{PtCl}_6 \cdot 6\text{H}_2\text{O}$ (Pt^{4+}).

Figure 5. Comparative time release profiles of CDDP released from CDDP@US-tubes and W-CDDP@US-tubes (pluronic-F108-wrapped) in PBS at 37 °C. The data are expressed as mean $\pm$ SD of three independent experiments.

Figure 6.

Time and concentration dependency of cell viability studies for MCF-7 and MDA-MB-231 cells incubated with CDDP, W-CDDP@US-tubes, and W-US-tubes. The quantity of US-tubes at each CDDP concentration is equivalent for the CDDP@US-tube and US-tube samples, with the concentration being calculated from their wt% values obtained by ICP-OES for Ni and Y which is contained within US-tube materials as the metal catalysts for carbon nanotube growth. The data are expressed as mean $\pm$ SD for three independent experiments. (*) and (†) indicate a statistically significant difference between W-CDDP@US-tube data compared to free CDDP data (* $p < 0.01$, † $p < 0.05$).

Table 1

Pt content by ICP-OES of CDDP@US-tubes under different loading solvent and washing conditions. EtOH is ethyl alcohol and DMF is dimethylformamide. Table presents the wt% Pt for an average of three trials under each condition and the standard deviation.

Material	Loading Solvent	wt% Pt (after one washing with H ₂ O)	wt% Pt (after 40 washings with H ₂ O)
CDDP@US-tubes	H ₂ O	8.70 ±0.43	6.37 ±0.57
CDDP@US-tubes	H ₂ O:EtOH(1:1)	6.66 ±0.31	4.42 ±0.35
CDDP@US-tubes	H ₂ O:DMF(1:1)	8.51 ±0.34	3.38 ±0.30