U. RITTER,[1] N.G. TSIERKEZOS,[1] YU.I. PRYLUTSKYY,[2] V.V. CHEREPANOV,[3]
A.I. SENENKO,[3] A.A. MARCHENKO,[3] A.G. NAUMOVETS[3]

[1] Ilmenau University of Technology, Institute of Chemistry and Biotechnology
  (25, Weimarer Str., Ilmenau 98693, Germany; e-mail: vvch2000@ukr.net)
[2] Taras Shevchenko National University of Kyiv, Department of Biophysics
  (64, Volodymyrska Str., Kyiv 01601, Ukraine)
[3] Institute of Physics, Nat. Acad. of Sci. of Ukraine
  (46, Nauky Ave., Kyiv 03680, Ukraine)

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STRUCTURE AND ELECTROCHEMICAL PROPERTIES OF AQUEOUS SUSPENSIONS OF FUNCTIONALIZED SINGLE- AND MULTIWALLED CARBON NANOTUBES

The structure of single- and multiwalled carbon nanotubes (CNTs) functionalized with carboxyl groups in water is investigated by means of atomic force microscopy. The electrochemical properties of the water systems containing both types of CNTs are investigated, by using the cyclic voltammetry and electrochemical impedance spectroscopy techniques. The results may be useful for the clarification of the mechanisms of specific biological activities of CNTs and their applications in various fields of nanobiotechnology.

K e y w o r d s: carbon nanotubes, aqueous suspensions, atomic force microscopy, cyclic voltammetry, electrochemical impedance spectroscopy.

1. Introduction

Single- and multiwalled carbon nanotubes (further referred as SWCNTs and MWCNTs, respectively) consist of sp^2-hybridized carbon atoms and are known to exhibit unique chemical and physical properties [1–3]. Namely, they are chemically and thermally stable, possess a high mechanical strength, thermal and electric conductivities, and a large specific surface area, and, in particular, can be successfully applied to biomedicine as materials for the fabrication of biosensors [4–6] as well to biomedical imaging [6–8]. Functionalized CNTs can also be used as molecular carriers for the in vitro and in vivo drug delivery [9, 10] as unique transfection vectors for the gene delivery [11–15] and for the cancer treatment [5, 16–19].

The functionalization of CNTs improves their solubility in water and biocompatibility. Thus, the functionalized CNTs are promising novel materials for a variety of biomedical applications. Aqueous suspensions of CNTs can be used as target therapeutic agents, including biomolecules, on the disease sites. For understanding the mechanisms of specific biological activities of CNTs and for their successful applications to nanobiotechnologies, a comprehensive study of CNTs structure in water is necessary. Particularly important is to ascertain, in which form CNTs are bioactive, as single tubes or in the form of CNT-aggregates [20].

The aim of the present work is to investigate the structure of aqueous suspensions of SWCNTs and MWCNTs functionalized with carboxyl groups using the atomic force microscopy (AFM) technique. In addition, the electrochemical properties of aqueous suspensions of both types of CNTs are studied by means

of the cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) techniques.

2. Materials and Methods

The SWCNTs were synthesized by means of the arc-discharge technique between two graphite electrodes in the helium atmosphere (700 mbar). A hollow in the anode was drilled and filled with a catalytic powder (graphite, 1% Y_2O_3, 4.2% NiO). The arc-discharge was performed at a current of 150 A. The contaminants, such as amorphous carbon and metallic catalyst particles were removed after the treatment with boiling HCl (6 M) in a reflux condenser. MWCNTs were synthesized by chemical vapour deposition (CVD) in a furnace at 900 °C with the decomposition of benzene using ferrocene as a catalyst. The ferrocene solution (1% w/w) was introduced to a furnace through a syringe with a flow rate of 0.2 ml/min. The synthesis process was performed using argon as a carrier gas.

The structure characterization of the products was carried out by means of scanning electron microscopy (SEM; FEI XL30 LaB_6) and high-resolution transmission electron microscopy (HRTEM; Philips TECNAI 20 S-TWIN).

The functionalization (carboxylation) of SWCNTs and MWCNTs was done by the HNO_3 (3 M) treatment. Functionalized SWCNTs and MWCNTs were suspended in distilled water by the ultrasonication (UZDN-1 U42 (Russia); 21 kHz; 0.68 A, processing time 90 s at constant heat removal). The content of surface carboxyl groups (<3 wt.%) was estimated by means of X-ray photoelectron spectroscopy [21]. The maximum concentration of functionalized SWCNTs and MWCNTs in water was 30 μg/ml.

The state of CNTs was examined using the AFM technique ("Solver Pro M" system; NT–MDT, Russia). The samples were deposited onto a freshly cleaved mica substrate (V-1 Grade, SPI Supplies) by the precipitation from an aqueous suspension droplet. The suspension was subjected to the mechanical treatment in an ultrasonic bath for 10 min before being applied to the mica substrate. Afterward, the suspension heterogeneity was not observed. The region of the substrate covered by a suspension drop ("spot") was ~1 mm in diameter. The AFM measurements were performed after the evaporation of the solvent. Several sites within the "spot" were se-

lected. The visualization of samples was carried out in the semicontact (tapping) mode of AFM. NSG10 (NT–MDT) probes were used.

The electrochemical properties of CNTs water suspensions were investigated by means of the CV and EIS techniques. The CVs and EIS spectra were recorded using a Zahner computer-controlled system (model IM6/6EX). The experiments were carried out, by using a three-electrode cell configuration. The working electrode was a glassy carbon (active surface area 0.24 cm^2), the counter electrode was a platinum plate, and the reference electrode was Ag/AgCl (saturated KCl). A three-compartment electrochemical cell specially designed to minimize the distances between the electrodes with a total solution volume of about 20 ml was used in all experiments. Potassium chloride (KCl) (1.0 mol/l) was used as a supporting electrolyte. Before each measurement, the solution was purged with high purity argon in order to eliminate the interference from oxygen. The CVs were recorded in the potential range from −1.0 to +1.0 V (*vs.* Ag/AgCl) with scan rates ranging from 0.02 to 0.12 V/s. The EIS spectra were taken in the frequency range from 0.10 Hz to 100 kHz. All measurements were carried out at room temperature (21.0 °C).

3. Results and Discussion

3.1. AFM study

The comprehensive knowledge of a degree of aggregation of CNTs in aqueous suspensions is very important for their biomedical application, since it is assumed that the toxicity of CNTs is correlated with their aggregation ability [5, 21, 22]. Thus, it is important to determine the optimal concentration of CNTs in water that does not lead to their aggregation. In order to characterize the composition of CNTs aqueous suspensions, AFM studies were performed.

According to the obtained electron microscopic images (Figs. 1, *a* and 1, *b*), the diameter and the length of SWCNTs are 0.5–2 nm and 1–5 μm, respectively. The SEM and TEM images of MWCNTs (Figs. 1, *c* and 1, *d*) demonstrate that their inner and outer diameters are 8–12 nm and 20–80 nm, respectively, while their length is about 1–40 μm.

AFM measurements showed that the water suspensions of SWCNTs were in the non-coagulated state at a concentration of 5 μg/ml (Fig. 2, *a*). From

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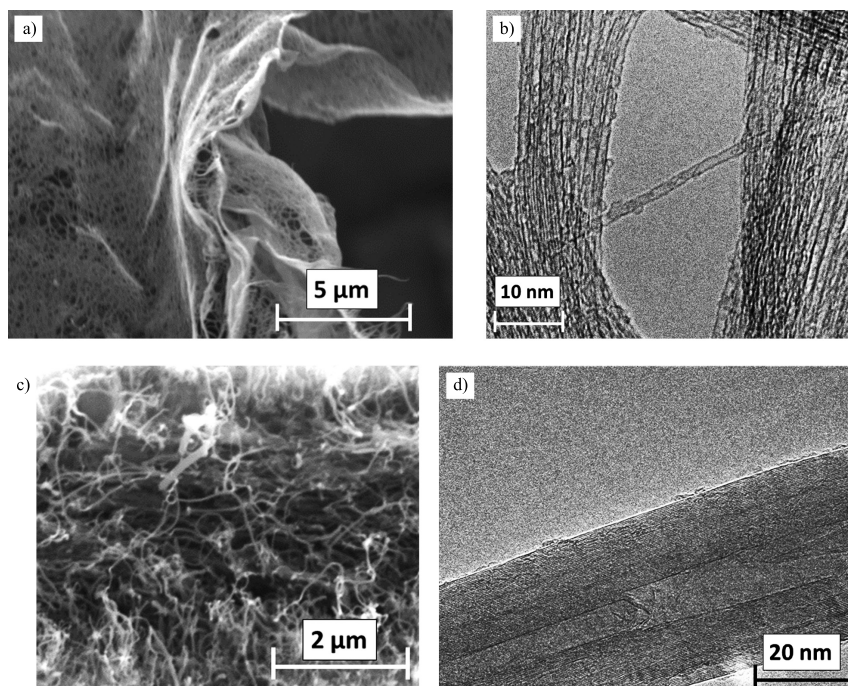
Fig. 1. SEM and TEM images of SWCNTs (*a*, *b*) and MWCNTs (*c*, *d*)

one to several dozen of SWCNTs were found in the 10×10 μm^2 area of the mica substrate. With respect to their sizes, SWCNTs could can be split into two groups: the first one included the SWCNTs with a diameter of 0.6–0.7 nm and a length of 2–3 μm, while the second group comprised the SWCNTs with diameters of 1.0–1.5 nm and lengths up to 5 μm (note that these SWCNTs were non-uniform in thickness, which could reach a height of 2 nm).

The AFM studies demonstrated that the number of MWCNTs within the above scanned area was significantly less as compared with that of SWCNTs. To increase the statistical sampling, this area was broadened from 10 to 20 μm. According to the AFM image (Fig. 2, *b*), the diameters of individual MWCNTs in water suspensions lay in the range from 16 to 80 nm, while their characteristic length was about 1–3 μm at a concentration of 5 $\mu g/ml$.

3.2. Electrochemical measurements

The exact knowledge of the purity of CNTs is also important in view of their application to nanobiotechnology. It is supposed that the toxicity of CNTs, as well as their ability to generate the reactive oxygen species under the light irradiation, may be related to the presence of residual metal catalyst impurities within the CNTs structure [5, 22, 23]. Our electrochemical investigations by means of the CV and EIS techniques were undertaken in order to estimate this effect.

The CVs recorded for aqueous SWCNT and MWCNT suspensions on a GC electrode using KCl as an electrolyte (1.0 mol/l) at a scan rate of 0.10 V/s are exemplified in Fig. 3. As expected, the redox responses were observed for both types of CNTs in aqueous suspensions and were probably due either to the presence of oxygen-containing groups bound to the CNTs or to remaining metal catalyst impurities stemming from the CNTs fabrication process [24]. It is also possible that the impurities produced during the CNT purification (acid treatment) could enhance as well the electrochemical activity of CNTs. This effect was more pronounced in a SWCNT water suspension. Namely, in the investigated potential range from −1.0 to +1.0 V (*vs.* Ag/AgCl), the reduction peak at about −0.322 V (*vs.* Ag/AgCl) and the oxidation peak at about +0.742 V (*vs.* Ag/AgCl) were observed for SWCNTs. But, for MWCNTs, only the shoulder-shaped cathodic peak could be seen at about −0.334 V (*vs.* Ag/AgCl). The obtained
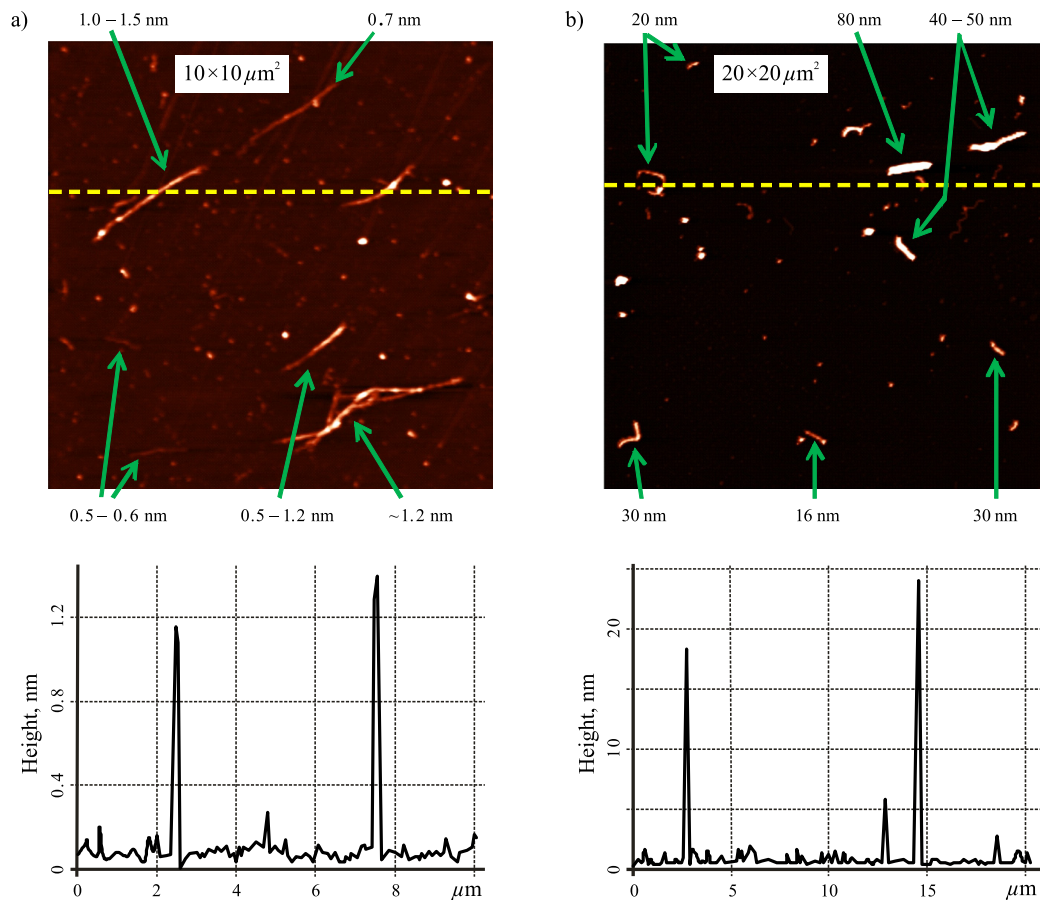
Fig. 2. AFM images of SWCNTs (*a*) and MWCNTs (*b*) deposited from an aqueous suspension (concentration: 5 μg/ml) on the mica substrate (tapping mode). Topographic images for a given profile (dashed line) are shown on the top. The diameters of some CNTs are indicated by arrows
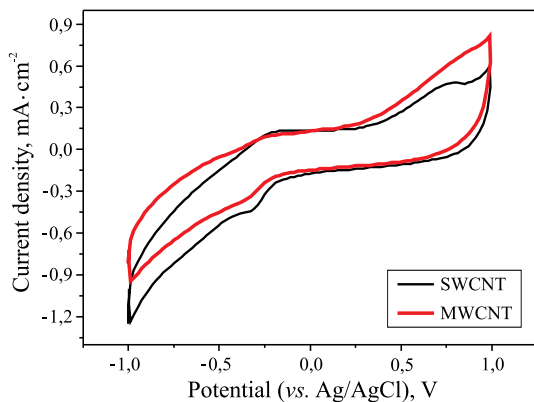
Fig. 3. CVs recorded for aqueous SWCNT and MWCNT suspensions (1.0 KCl) on a GC electrode (0.24 cm^2) at $v =$ $= 0.10$ V$\cdot$s^{-1}

impedance data provided a further support for the CV-results. Actually, the recorded EIS spectra for aqueous SWCNT and MWCNT water suspensions (Fig. 4) exhibited that the barrier for the electron transfer is significantly lower in the case of SWCNTs as compared with MWCNTs. The estimated charge transfer resistance for MWCNTs ($\sim$26 k$\Omega\cdot$cm^{-2}) was found to be by a factor of $\sim$130 higher than that determined for SWCNTs ($\sim$0.20 k$\Omega\cdot$cm^{-2}), by demonstrating a much faster electron transfer kinetics in the SWCNT water suspension. The better electrochemical performance of the aqueous SWCNT suspension may be caused by a larger amount of residual metal catalysts impurities, which they contain. These findings demonstrate that the existence of oxygen-
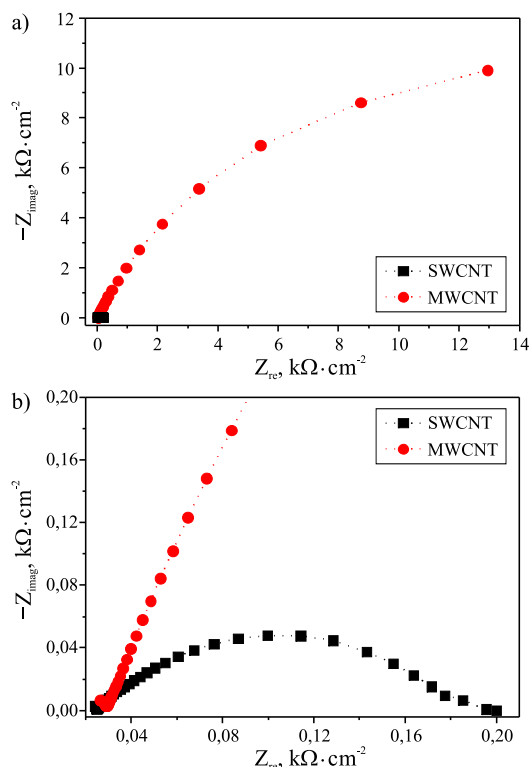
Fig. 4. EIS spectra recorded for aqueous SWCNT and MWCNT suspensions (1.0 KCl) on a GC electrode (0.24 cm^2) in the frequency range 0.1 Hz−100 kHz (*a*); zoom of EIS spectra in the high frequency region (*b*)

containing groups and metal impurities in CNTs suspensions enhances their electrochemical activity.

4. Conclusions

This work reports on the studies of the aqueous suspensions of functionalized SWCNTs and MWCNTs. According to our AFM findings, the SWCNTs at a concentration of 5 μg/ml appear as non-aggregated species with lengths up to 5 μm and diameters in the interval ≈0.5−1.5 nm. Furthermore, the MWCNTs also appear as single non-aggregated species at the same concentration, with lengths up to 3 μm and diameters in the interval 20−80 nm. The electrochemical studies revealed that the SWCNTs within CNT water suspensions studied exhibit a better electrochemical performance (significantly lower barrier and faster kinetics for the electron transfer) as compared with MWCNTs, due, probably, to a greater amount of residual metal catalyst impurities that these particular CNTs contain.

Toxicity is a major concern in using the CNT-based materials for biomedical applications, since they can cause toxic effects in human bodies and animals, which are strongly dependent on the size and the concentration of CNTs, as well as on the amount of metal catalyst impurities [5, 21, 22]. The findings of the present work may contribute to clarifying the mechanisms of specific biological effects of CNTs (membranotropic [25], tumoritropic [18], antihypertensive [26], *etc.*) at the molecular and cellular levels.

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У. Ріттер, Н.Г. Циркезос,
Ю.І. Прилуцький, В.В. Черепанов, А.І. Сененко,
О.А. Марченко, А.Г. Наумовець

СТРУКТУРА І ЕЛЕКТРОХІМІЧНІ
ВЛАСТИВОСТІ ВОДНИХ СУСПЕНЗІЙ
ФУНКЦІОНАЛІЗОВАНИХ ОДНО-
ТА БАГАТОСТІННИХ ВУГЛЕЦЕВИХ НАНОТРУБОК

Р е з ю м е

Методом атомно-силової мікроскопії досліджено структуру одностінних та багатостінних вуглецевих нанотрубок (ВНТ), функціоналізованих карбоксильними групами, осаджених з водних суспензій. Електрохімічні властивості суспензій, що містять обидва типи ВНТ окремо, вивчено методами циклічної вольтамперометрії та спектроскопії електрохімічного імпедансу. Отримані результати можуть бути корисними для з'ясування механізмів специфічної біологічної дії ВНТ та їх застосування у різноманітних сферах нанобіотехнології.

У. Риттер, Н.Г. Циркезос,
Ю.И. Прилуцкий, В.В. Черепанов, А.И. Сененко,
А.А. Марченко, А.Г. Наумовец

СТРУКТУРА
И ЭЛЕКТРОХИМИЧЕСКИЕ
СВОЙСТВА ВОДНЫХ СУСПЕНЗИЙ
ФУНКЦИОНАЛИЗИРОВАННЫХ ОДНО-
И МНОГОСТЕННЫХ УГЛЕРОДНЫХ
НАНОТРУБОК

Р е з ю м е

Методом атомно-силовой микроскопии исследована структура одностенных и многостенных углеродных нанотрубок (УНТ), функционализированных карбоксильными группами и осажденных из водных суспензий. Электрохимические свойства суспензий, содержащих оба типа УНТ отдельно, изучены методами циклической вольтамперометрии и спектроскопии электрохимического импеданса. Полученные результаты могут быть полезными для установления механизмов специфической биологической активности УНТ и их применения в различных областях нанобиотехнологии.