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Evaluation of Histidine Functionalized Multiwalled Carbon Nanotubes for Improvement in the Sensitivity of Cadmium Ions Determination in Flow Analysis

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1. Introduction

The determination of heavy metal ions in environmental, clinical and food samples has been a common concern for several researchers and in special case of aquatic environment, there has been a more stringent regulation standards pertaining to the discharge of heavy metals. Some efforts have been carried out to develop many treatment processes to reduce the disposal of heavy metal ions mainly in water bodies. The different treatment processes include precipitation, membrane filtration, ion exchange and adsorption (Tokaloğlu et al. 2009), however, the levels of heavy metals disposal into water bodies are still rising, mainly in aqueous effluents discharged into aquatic bodies (Mello et al. 2005). As example, lead is considered as the major environmental pollutant and has become the most widely scattered toxic heavy metal in the world as a result of anthropogenic action. It is very well known that lead enters the environment through several industrial activities, such as industrial ammunition, paper board mill, battery manufacturing and coal burning and pigments (Zhan & Zhao, 2003). Lead can cause several effects on central nervous system, blood pressure, kidneys and reproductive system (Needleman, 2004). Similarly to lead, cadmium is also considered as toxic for animals and humans, and it is classified by the International Agency for Research on Cancer as a human carcinogen (IARC, 1993). The National Council for the Environment in Brazil (CONAMA, 2005) established 0.2 mg L⁻¹ as the maximum level of cadmium for effluent discharge in aquatic bodies, while 5.0 and 3.0 µg L⁻¹ are the maximum contaminant levels in potable water allowed by EPA(EPA, 2003) and ANVISA (ANVISA, 2004), respectively. Even at low concentration levels (≤ µg L⁻¹), there is bioaccumulation of heavy metals in aquatic organisms, so there is a growing need for their monitoring in different types of water samples. In addition, the heavy metal ions monitoring can provide both important aspects in water quality and controlling the industrial processes (Martinotti et al. 1995). Thus, the development of analytical methods based on preconcentration systems has commonly been the aim of choice for the determination of trace metals in water and...
biological samples. Moreover, the development of new analytical protocols play an important role in solving instrumental problems commonly associated with low selectivity and sensitivity. Undoubtedly, solid phase preconcentration has been the most technique used for this purpose, owing to its attractive features such as high reproducibility and preconcentration factor, ease of automation and regeneration of solid phase, low cost, simplicity, easy coupling to flow injection analysis (FIA) and high sample throughput (Barbosa et al. 2007).

Basically, preconcentration consists on passing a large quantity of sample through a column containing a solid phase, at which, due to the very low concentration of the analyte in the raw sample, can not be analyzed by simple techniques without this preconcentration step. Moreover, adsorbent extraction can be used for selective retention of some particular chemical forms of a metal, thereby enabling speciation (Campanella et al. 1996, Pyrzyńska et al. 1999). Currently, several solid phase extractors can be found for metal ions preconcentration, such as modified silica (C18), chelating resins, polyurethane foam, activated carbon, fullerenes, modified silica, polymers loaded with chelating reagents and natural adsorbents (Souza & Tarley, 2009). Despite the available solid phase extractors, the development of new solid phase preconcentration systems has still been growing and target of analytical research, where the choice of appropriate extractor is a critical factor to obtain attractive features in preconcentration systems. The new materials developed should present large specific surface area, chemical resistance, high adsorptive capacity, life-time, high clean-up capacity, reversible adsorption and large accessibility of analyte towards the sites of adsorbents. There are numerous attempts on the development of nanomaterials, in which present such characteristics mentioned, but undoubtedly, in the last 20 years, no other nanomaterials have desperted so much attention as carbon nanotubes (CNTs) in the development of solid phase extraction procedures. CNTs can be described as a graphite sheet rolled up into a nanoscale-tube [which are single-wall carbon nanotubes (SWCNTs), or with additional graphene tubes around the core of an SWCNT (which are multi-wall CNTs, MWCNTs)]. CNTs have diameters in the range between fractions of nanometers and tens of nanometers and lengths up to several centimeters with both their ends normally capped by fullerene-like structures, having usually a length-to-diameter ratio \( \leq 10^5 \). The combination of size, structure and topology provides nanotubes with important mechanical and surface properties. However, CNTs are insoluble in organic solvents and inorganic aqueous medium limiting their manipulation in real samples and their walls are not reactive, but their tips can be more reactive. So, several studies have been performed to functionalize the CNTs surface to generate functional groups, such as carbonyl, hydroxyl and carboxylic groups and naturally enhance their dispersion property and adsorption capacity. Studies have demonstrated the excellent performance of these materials as an analytical adsorbent for metal ions, pesticides and phenolic compounds (Liang et al. 2004, Al-Degs et al. 2008, Salam & Burk et al. 2008). Recently, the ability of MWCNTs as a solid adsorbent for the on-line preconcentration and speciation of \( V^{3+} \) and \( V^{5+} \) coupled to electrothermal atomic absorption spectrometry (ETAAS) has been described (Gil et al. 2007). The application of oxidized MWCNTs with nitric acid for cadmium, lead and cobalt preconcentration with posterior determination by atomic absorption spectrometry has been reported (Barbosa et al. 2007, Tarley et al. 2006, Souza & Tarley, 2009). The pH plays a very important role on extraction of metals ions onto oxidized MWCNTs (Liang et al. 2005). It can be explained by functionalization that has occurred on the surface of the carbon structures producing oxygen-containing groups when submitted to chemical oxidation. This oxidation leads to a
reduction of the isoelectric point (IEP) of the MWCNTs to lower pH values. Under these conditions, the sample pH needs to be higher than the IEP of the oxidized MWCNTs (El-Sheikh 2008, Stafiej & Pyrzyńska 2007). The selectivity of raw and oxidized CNTs towards metal ions can be improved by introducing polymers, inorganic oxides and organic compounds onto surface material. The stability, accessibility and selectivity can be achieved through covalent binding between CNTs surface and modifier.

In a recent work, Amais et al. 2007 synthesized a nanocomposite based on alumina ($\text{Al}_2\text{O}_3$) supported on MWCNTs to be applied as a sorbent for nickel preconcentration prior to flame atomic absorption spectrometry (FAAS). Significant results mainly on the recovery studies (higher than 95%), stability of the column for more than 200 cycles without loss of adsorption capacity and a high analytical frequency due to the high flow used in the preconcentration step were achieved.

Zang et al. 2009, published an application concerning the use of ethylenediamine-MWCNTs as solid phase extractor for the preconcentration of $\text{Cr}^{3+}$, $\text{Fe}^{3+}$ and $\text{Pb}^{2+}$ at pH 4.0. A substantial increase on selectivity and sensitivity was observed for these metal ions determination by inductively coupled plasma optical emission spectrometry.

Pacheco et al. 2009 reported the immobilization of L-tyrosine on CNTs as a new adsorbent for cobalt ions preconcentration. The adsorption of other bivalent metal ions (Cu, Ni and Zn) was also investigated. It was observed that bigger cations are more adsorbed on L-tyrosine-CNTs, taking into account the formation of $\text{CuOH}^+$ and $\text{NiOH}^+$ at pH 9.0.

The preconcentration of $\text{Au}^{(II)}$ and $\text{Mn}^{(II)}$ in aqueous medium using MWCNTs modified with $\text{N,N'-bis(2-hydroxybenzylidene)-2,2'(aminophenylthio)ethane}$ with determination by flame atomic absorption spectrometry was described by Shamspur & Mostafavi, 2009. These metal ions were quantitatively recovered at pH 6.0. The proposed method was useful for $\text{Au}^{2+}$ and $\text{Mn}^{2+}$ determination with high sensitivity and low limits of detection, 0.01 and 0.03 μg L$^{-1}$, respectively.

The selectivity of MWCNTs towards $\text{Cd}^{2+}$ ions has also been reported by functionalization with L-cysteine (Liu et al. 2008). The preconcentration factor of $\text{Cd}^{2+}$ ions by using MWCNTs-cysteine packed into mini-column was found to be 33. Other chemical modifiers can be found in the literature, such as 1-(2-pyridylazo)-2-naphtol (PAN), $\text{TiO}_2$ and di-(2-ethylhexylphosphoricacid)(D2EHPA) and tri-n-octylphosphineoxide(TOPO) (Afzali & Mostafavi 2008, Zhao et al. 2010, Vellaichamy & Palanively, 2011).

The goal of this chapter comprises the modification of MWCNTs with histidine and demonstrates new analytical data obtained in our research group involving the development of a solid phase extraction system for the determination of cadmium ions by FAAS.

2. Materials and methods

2.1 Reagents and solutions

Analytical grade chemical reagents were used throughout and the water used was obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA). Before using, all laboratory glassware were kept overnight in a 10% (v/v) $\text{HNO}_3$ solution, in order to avoid any metal contamination. After that, they were rinsed with deionized water and submitted to a drying step. A standard stock solution of cadmium 1000.0 mg L$^{-1}$ (Merck, Darmstadt, Germany) was used to prepare standard solutions of concentration 30.0 μg L$^{-1}$. The latter was properly diluted from an intermediate solution of 10.0 mg L$^{-1}$ Cd$^{2+}$. Concentrated $\text{HNO}_3$
(Merck) and 30% (v/v) H$_2$O$_2$ (Merck) were used for the decomposition of the certified reference material in a block digester. Multi-wall carbon nanotubes (MWCNTs) were supplied by CNT Co., Ltd. (Yeonsu-Gu, Incheon, Korea) with purity > 93%, diameters between 10-40 nm and lengths of 5-20 μm. Histidine (98%) was purchased from Sigma-Aldrich (St. Louis, MO). Buffer solutions including Tris-HCl, ammoniacal, borate and phosphate (Merck) were prepared from their respective salts without further purification.

2.2 Apparatus
All measurements were carried out by using a Shimadzu AA-6601 flame atomic absorption spectrometer (Shimadzu, Tokyo, Japan), equipped with a hollow cathode lamp for cadmium and with a deuterium lamp for background correction. The hollow cathode lamp was operated at 8.0 mA and the wavelength was set at 228.8 nm. The flame composition was operated with an acetylene flow rate of 1.8 L min$^{-1}$ and air flow rate of 10.0 L min$^{-1}$. An Ismatec Model IPC peristaltic pump (Ismatec IPC-08, Glattbrugg, Switzerland) furnished with Tygon® tubes was used to propel sample and eluent solutions. The preconcentration/elution steps were selected by using a home-made injector-commutator made of Teflon® (PTFE, polytetrafluoroethylene). The pH of samples were measured by a digital pHmeter 826 pH mobile Metrohm (Herisau, Switzerland). The infrared spectra were obtained by using the conventional KBr pellet technique in a FT-IR Shimadzu 8300 spectrometer, operating in the transmission mode between 4000 and 400 cm$^{-1}$. The digestion of certified reference material was carried out by using block digestor (Marconi, Piracicaba, Brazil).

2.3 Preparation of MWCNTs functionalized with histidine
Oxidation MWCNTs procedure followed the literature (Tarley et al. 2006). Around 500 mg of MWCNTs were dispersed in 30 ml of concentrated HNO$_3$, following reflux for 1 h at 120°C. After this step, the sample was successively washed with deionized water in order to eliminate excess acid (pH of washing water was near 7). Finally, the material was dried at 100°C and stored at room temperature. This procedure opens the ends of carbon nanotubes and creates carboxyl group. Next, around 100 mg of oxidized MWCNTs were pulverized and dispersed in 50 ml of 1.3% (w/v) Triton X-100. The suspension was kept in ultrasound bath (40 KHz) during 10 min and stored for 12 h. After this procedure, the suspension was centrifuged (3600 rpm) during 10 min and the material was further dried and stored at room temperature (Liu et al. 2010, Rastogi et al. 2008). The functionalization of MWCNTs was performed in accordance to literature (Pacheco et al. 2009). Around 100 mg of oxidized and dispersed MWCNTs were added to 50 ml of phosphate buffer solution at pH 7.0 and 100 mg of histidine were further added. The suspension was heated at 45 °C in oil bath during 48 h. Finally, the resulting suspension was centrifuged and the fuctionalized MWCNTs was dried at room temperature. A polyethylene mini-column with 6 cm length and 1 cm internal diameter was filled with 15 mg functionalized MWCNTs and glass wool was introduced into the extremities of the mini-column in order to prevent possible losses of adsorbent during system operation.

2.4 On-line preconcentration system
A diagram of the on-line preconcentration system is shown in Figure 1. During the preconcentration step, a volume of 15.0 ml of sample buffered at pH 8.50 with 0.01 mol L$^{-1}$
ammoniacal buffer percolates through a mini-column filled with the adsorbent material (15 mg), at a flow rate of 4.0 ml min⁻¹. Then, by switching the central part of the injector, the elution step is accomplished at a flow rate of 5.0 ml min⁻¹ with 0.8 mol L⁻¹ HNO₃, at which Cd²⁺ is released towards FAAS detector. All absorbance signals were taken as peak height.

![Diagram](image)

**Fig. 1.** Diagram of on-line preconcentration system. (a) preconcentration step, (b) elution step. P= peristaltic pump, W= waste, S= sample, E= eluent 0.80 mol L⁻¹ HNO₃.

### 3. Results

#### 3.1 Infrared spectra of raw and histidine functionalized MWCNTs

Fig. 2 shows the infrared spectra of raw and histidine functionalized MWCNTs and histidine functionalized MWCNTs. The band observed at 3445 cm⁻¹ corresponds to the

![Infrared Spectra](image)

**Fig. 2.** Infrared spectra of the raw and histidine functionalized MWCNTs.
stretching vibrations of $\nu$(OH), indicating the existence of carboxyl groups on the external surface of the MWCNTs due to oxidation with HNO$_3$. In addition, the band can be attributed to hydroxyl groups present in water physically adsorbed. The signal at 1097 cm$^{-1}$ is assigned to the $\nu$(C-O) stretching vibration (Gaoa, et al. 2009, Vukovic et al. 2009). The band at 2924 cm$^{-1}$ is assigned to asymmetric stretching vibrations of $\nu$(CH$_2$). The functionalization with histidine can be confirmed from the formation of secondary amide on the MWCNTs at 1545 cm$^{-1}$ (Liu et al. 2008). In addition, the band at 1641 cm$^{-1}$ is attributed to stretching of amide carbonyl (C=O). Another indication of the functionalization is the band at 1425 cm$^{-1}$ corresponding to N-H bending of secondary amide (Pompeo & Resasco, 2002). The stretching mode of aromatic amine group is observed at 873 cm$^{-1}$ and the stretching mode of C-OH is assigned at 672 cm$^{-1}$ (Shen et al. 2007, Jin et al. 2007).

### 3.2 Influence of sample pH

The influence of the sample pH was investigated in the range 3.0-9.5 (Figure 3). The concentration of cadmium used in this study was 40.0 µg L$^{-1}$.

![Absorbance/peak height vs. Sample pH](image)

**Fig. 3.** Influence of sample pH on the adsorption of cadmium ions onto raw and functionalized MWCNTs. Conditions: HNO$_3$, as eluent, at 0.5 mol L$^{-1}$ concentration and preconcentration flow rate of 4.0 ml min$^{-1}$.

As observed, the analytical signal increases with increasing of the pH from 3.0 up to 8.5. The very low adsorption of cadmium ions in acid medium is due to protonation of nitrogen atoms of histidine leading to an electrostatic repulsion with cadmium ions. A slight decrease on analytical signal probably occurs above pH 8.5, due to the cadmium precipitation as hydroxides. The pH dependence was also evaluated for raw MWCNTs. As verified, a highly improved adsorption of cadmium ions towards functionalized MWCNTs was observed after comparison with raw MWCNTs, confirming the chemical immobilization of histidine onto MWCNTs surface. Thus, all further works were carried out at pH 8.5.
3.3 Influence of buffer concentration
The sample pH at 8.5 was buffered with ammoniacal buffer. In order to evaluate the effect of sample buffer concentration, the concentration of ammoniacal buffer was studied from 0.01 up to 0.25 mol L\(^{-1}\) (Figure 4). Higher responses were clearly noted when low buffer concentrations are employed. Such behavior shows that the use of high buffer concentrations, probably by a competitive effect for the adsorbent sites of functionalized MWCNTs, significantly decreases the analytical signal. Despite these findings, the use of low buffer concentrations results in the reduction of the buffering capacity. So, as a compromise between sensitivity and buffering capacity, we have chosen the buffer concentration of 0.05 mol L\(^{-1}\).

![Graph showing influence of buffer concentration](image)

Fig. 4. Influence of buffer concentration on the adsorption of cadmium ions onto functionalized MWCNTs. Conditions: pH 8.5, HNO\(_3\) as eluent at 0.5 mol L\(^{-1}\) concentration and preconcentration flow rate of 4.0 ml min\(^{-1}\).

3.4 Influence of eluent concentration
It is well known that eluent concentration plays an important role on the efficiency of solid phase preconcentration systems. Thus, the influence of HNO\(_3\) concentration, as eluent, was investigated in the range 0.5–1.0 mol L\(^{-1}\). An improvement at ca. 15% on the analytical response was observed by changing the eluent concentration from 0.5 to 0.8 mol L\(^{-1}\). On the other hand, no significant difference was observed by increasing of eluent concentration from 0.8 up to 1.0 mol L\(^{-1}\). Thus, due to absence of memory effect after each preconcentration/elution step and, in order to preserve the life-time of functionalized MWCNTs, an eluent concentration of 0.8 mol L\(^{-1}\) was chosen for subsequent studies.

3.5 Influence of preconcentration flow rate
The preconcentration flow rate in solid phase extraction is an important factor, particularly when high flow rates are used and usually accompanied by decreasing the analytical
responses. As expected, the analytical signal decreases with increasing flow rate in range 3.0-5.0 ml min$^{-1}$. This behavior indicates that the kinetics of mass transfer of cadmium ions towards functionalized MWCNTs surface depends on the flow rate. So, in order to improve the sample throughput of the proposed method, the preconcentration flow rate of 4.0 ml min$^{-1}$ was adopted for further experiments. Under this condition, the required time to preconcentrate 15.0 ml of sample was found to be 3.75 min, which is a usual and satisfactory condition in a flow system.

3.6 Influence of buffer solution
The preconcentration of cadmium ions using different buffers (Tris-HCl, ammoniacal, borate and phosphate) at pH 8.5 and concentration of 0.05 mol L$^{-1}$ was performed. The investigation of influence of the different buffers solutions pointed out to phosphate as the worst buffer. This result was somewhat expected, once even at low cadmium concentrations, phosphate can form insoluble salts with cadmium. Thus, the subsequent experiments were carried out using ammoniacal buffer, in which the signal was slightly higher.

3.7 Interference studies
The preconcentration of cadmium ions was carried out in the presence of co-existing ions in order to assess the feasibility of the proposed method for cadmium monitoring in real and complex samples. Binary solutions containing cadmium ions with Ni$^{2+}$, U$^{4+}$, Ca$^{2+}$, Mn$^{2+}$ or Ba$^{2+}$ in the following proportion (cadmium:co-existing ion) 1:1, 1:10 and 1:100 (w/v) were preconcentrated under optimized conditions. Generally, an error of ±10%, by comparing the analytical signal of cadmium alone with that obtained by preconcentrating a cadmium solution in the presence of co-existing ions, is tolerable. The results obtained indicated that the method can tolerate the co-existing ions in the following amount: Ni$^{2+}$ (2 mg L$^{-1}$), U$^{4+}$ (2 mg L$^{-1}$), Ca$^{2+}$ (4 mg L$^{-1}$), Mn$^{2+}$ (2 mg L$^{-1}$) and Ba$^{2+}$ (2 mg L$^{-1}$). Therefore, the proposed method is not vulnerable to these co-existing ions at high concentrations. Undoubtedly, the analysis of real samples can be susceptible to the interferent ions, however, the determination of cadmium ions by proposed method in water and biological samples is free-interferent, as will be demonstrated later, thus attesting the good selectivity of preconcentration system.

3.8 Breakthrough curve
The maximum adsorption capacity of functionalized MWCNTs towards cadmium was determined from breakthrough experiment. The assay was performed using a mini-column packed with 15 mg of functionalized MWCNTs, where a solution of 0.25 mg L$^{-1}$ Cd$^{2+}$ ions at pH 8.5 in 0.01 mol L$^{-1}$ ammoniacal buffer was continuously percolated at 4.0 ml min$^{-1}$ flow rate. Aliquots of 5 ml of the mini-column effluent were further collected and the amount of Cd$^{2+}$ ions was determined by FAAS. A graph of C/C$_o$ as a function of effluent volume (ml) was built, as shown in Figure 5. The dynamic adsorption capacity of the material was found to be 2.34 mg g$^{-1}$ after loading through mini-column with 250 ml of sample solution.

3.9 Characteristics of the analytical method, application and validation
The analytical characteristic data under optimized conditions were considered very satisfactory taking into account the application of the proposed method for the
determination of cadmium in water and biological samples. The analytical curve presented a linear range from 2.0 up to 140 \( \mu \text{g L}^{-1} \). The repeatability of the method, expressed as the relative standard deviation, for the determination of 40 \( \mu \text{g L}^{-1} \) Cd\(^{2+}\), was 3.11\% \((n =10)\). By preconcentrating 15 ml of sample, a preconcentration factor of 17.7 fold, defined as the ratio of slopes of linear section of the analytical curves before and after the preconcentration, was obtained. So, low limits of detection and quantification, assessed as the preconcentration of analyte giving an equivalent signal to three and ten times the S.D. of the blank signal plus the net blank intensity (Long and Winefordner 1983), were found to be 0.20 and 0.63 \( \mu \text{g L}^{-1} \), respectively. The sample throughput, consumptive index and concentration efficient were 16 samples per hour, 0.85 ml and 4.70 min\(^{-1} \), respectively. The favorable characteristics observed by the proposed preconcentration method allowed its application for the determination of cadmium in different types of water samples and biological materials using external calibration. Mineral and tap waters were spiked with 30 \( \mu \text{g L}^{-1} \) Cd\(^{2+}\) ions and, as observed from Table 1, recoveries ranging from 95.8 to 104.1\% indicate that Cd\(^{2+}\) ions are preconcentrated without matrix effect. Igapó lake water was collected in polypropylene bottles from a lake located at Londrina city. The sample was filtered through 0.45 \( \mu \text{m} \) cellulose acetate membranes under vacuum and analyzed as soon as possible after pH adjusting with 0.05 mol L\(^{-1} \) ammoniac buffer solution. Certified reference material weighting 150 mg [Tort-2 (Lobster Hepatopancreas)] was decomposed in block digestor with 15.0 ml of concentrated HNO\(_3\) and 5.0 ml 30\% (v/v) of H\(_2\)O\(_2\) during 4 h. After digestion, the sample was heated on a hot plate to near dryness and then cooled at room temperature with further buffering at pH 8.5, using ammoniacal buffer. As shown in Table 2, the amount of cadmium determined in this certified reference material (26.7 ± 0.6 mg Kg\(^{-1}\)), employing external calibration, is in good agreement with certified value (27.7 ± 0.7 mg Kg\(^{-1}\)) with confidence interval of 95\% (test \( t \)-student).
4. Conclusions

The present work demonstrates the functionalization of MWCNTs with histidine as a simple, rapid and reliable approach for improving the selectivity and adsorption capacity of MWCNTs towards Cd\(^{2+}\) ions. Moreover, the use of histidine as substrate associated with the inherent features of MWCNTs avoids the incompatibility of problems between MWCNTs and aqueous medium in flow injection analysis. The developed method provides satisfactory figures of merit in comparison with previously published methods. The successful application of method was attested by analysis of water samples of different sources as well as certified reference material with high power of detection. The efficient functionalization with histidine was confirmed from 100 preconcentration/elution steps without losses of adsorption capacity towards Cd\(^{2+}\) ions.

5. Acknowledgment

The authors would like to acknowledge the assistance of CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), FAPEMIG (Fundação de Amparo à Pesquisa do Estado de Minas Gerais), CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior)
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Superior) and INCT of Bioanalytical (Instituto Nacional de Ciência e Tecnologia de Bioanalítica) for the financial support and fellowships.

6. References


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Salam, M.A. & Burk, R. (2008). Novel application of modified multiwalled carbon nanotubes as a solid phase extraction adsorbent for the determination of polyhalogenated...


Since their discovery in 1991, carbon nanotubes have been considered as one of the most promising materials for a wide range of applications, in virtue of their outstanding properties. During the last two decades, both single-walled and multi-walled CNTs probably represented the hottest research topic concerning materials science, equally from a fundamental and from an applicative point of view. There is a prevailing opinion among the research community that CNTs are now ready for application in everyday world. This book provides an (obviously not exhaustive) overview on some of the amazing possible applications of CNT-based materials in the near future.

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