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Intermolecular interactions of substituted benzenes on multi-walled carbon nanotubes grafted on HPLC silica microspheres and interaction study through artificial neural networks

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Abstract Purified multi-walled carbon nanotubes (MWCNTs) grafted onto silica microspheres by gamma-radiation were applied as a HPLC stationary phase for investigating the intermolecular interactions between MWCNTs and substituted benzenes. The synthetic route, simple and not requiring CNTs derivatization, involved no alteration of the nanotube original morphology and physical–chemical properties. The affinity of a set of substituted benzenes for the MWCNTs was studied by correlating the capacity factor (k') of each probe to its physico-chemical characteristics (calculated by Density Functional Theory). The correlation was found through a theoretical approach based on feedforward neural networks. This strategy was adopted because today these calculations are easily affordable for small molecules (like the analytes), and many critical parameters needed are not known. This might increase the applicability of the proposed method to other cases of study. Moreover, it was seen that the normal linear fit does not provide a good model. The interaction on the MWCNT phase was compared to that of an octadecyl (C18) reversed phase, under the same elution conditions. Results from trained neural networks indicated that the main role in the interactions between the analytes and the stationary phases is due to dipole moment, polarizability and LUMO energy. As expected for the C18 stationary phase correlation, is due to dipole moment and polarizability, while for the MWCNT stationary phase primarily to LUMO

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energy followed by polarizability, evidence for a specific interaction between MWCNTs and analytes. The CNT-based hybrid material proved to be not only a chromatographic phase but also a useful tool to investigate the MWCNT-molecular interactions with variously substituted benzenes. © 2015 The Authors. Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

The great interest of recent years for carbon nanotubes (CNTs) is due to their extraordinary properties that can be summarized in large surface area, high thermal and mechanical stability, capability to behave as conductors or semi-conductor materials depending on their preparation route (Herrera-Herrera et al., 2012), and to exert a number of different chemical interactions (as described below). The nanotubes unique properties, not comparable to those of traditional carbon materials (e.g. graphite, graphitized carbon, activated carbon) prompted the scientific community to undertake a number of studies in many research fields, highlighting their multidisciplinary potentiality. As a matter of fact, they are investigated in medicine as carrier for drug delivery (Chen et al., 2013; Merli et al., 2011), for nanoelectronic devices (Merli et al., 2009) in the electronic field, and in several branches of analytical chemistry. Specifically, in the latter area CNTs have been employed as adsorbents for solid-phase (micro)extraction (Herrera-Herrera et al., 2012; Speltini et al., 2014), to prepare electrochemical devices (Profumo et al., 2006; Suzuki et al., 2013), and for designing novel chromatographic stationary phases (Chambers et al., 2011; Li et al., 2005; Merli et al., 2010; Speltini et al., 2010, 2012, 2013a, 2013b).

In recent papers CNTs have been incorporated in polymeric phases to improve the chromatographic performance in the separation of aromatics (Aqel et al., 2012), or grafted on micrometric silica to prepare a stationary phase with satisfactory selectivity, retention capability and resolution for some alkylbenzenes and hypnotic drugs (Speltini et al., 2013a).

Derivatization can be a unique opportunity for many applications, but it has to be noted that strong treatments inevitably deteriorate the pristine properties of CNTs. In this regard, a recent paper highlighted the necessity to control the functionalization degree in order to avoid damaging of the nanotube structure (Wang, 2009). In order to evaluate the interaction between pristine CNTs and molecules is thus essential to preserve their original structure. The different approaches practiced so far to study the characteristic of the CNT-molecular interaction suffer from serious drawbacks; for instance fluorescence quenching induced by adsorption of the target probes on the CNTs is viable only if fluorescent probes are used (Debnath et al., 2010); Raman spectroscopy, to investigate the π - π interaction with aromatics, turned out to yield poor accuracy (Gotovac et al., 2007); indirect methods such as adsorption of a given analyte from standard solutions in a CNT dispersion are heavily affected by CNT-dispersant interaction and solvation of the dispersant-wrapped CNTs (Tomonari et al., 2006).

On the other hand, chromatography-based analysis using CNTs as stationary phase is a simple and accurate methodology for such intention (Menna et al., 2006; Yoo et al.,

2011). To date, only a few papers have been reported for this approach (André et al., 2009; Merli et al., 2010; Yoo et al., 2011), and the need for systematic studies to combine experimental data with computational models was there underlined. Correlating the retention time (or the retention factor) with the physico-chemical properties of the analyte can provide a good tool to evaluate the interplays established with the nanotubes, as reported for alcohols, separated and analyzed on CNT-columns by gas chromatography (Merli et al., 2010). This analytical technique was also exploited to explore by computational calculations the CNT-molecular interaction of aliphatic amines (Basiuk et al., 2002) and aminoacids (Basiuk and Bassiouk, 2008).

For non-volatile compounds, however, liquid chromatography is mandatory. In this case, CNTs be directly packed in conventional analytical columns due to the high backpressure that would result from the eluent flowing through a nanometric phase (Speltini et al., 2013b). For this reason, CNTs require to be grafted onto chromatographic materials with micrometric dimensions, as described in the recent literature (Speltini et al., 2013b); in that paper several synthetic routes have been reviewed to prepare CNT-based stationary phases. However, in the framework of a CNT-molecular interaction study, some conditions must be satisfied; for instance, the contribute of the chromatographic support in the adsorption behavior of the test probes should be negligible, and the immobilization procedure should be as much possible preservative with regard to the CNT pristine structure. Indeed, as well highlighted in the recent paper by Yoo et al. (2011), hard conditions such as oxidizing treatments involve strong modification of the structure (i.e. carboxylic and/or hydroxyl groups are introduced on the carbon skeleton), thus hampering a rigorous evaluation of the interaction between the molecules and the CNT net surface. The development of non-destructive methods is under study; as an example, physical adsorption of CNTs onto silica gels (André et al., 2012; Fujigaya et al., 2011; Yoo et al., 2011) is more suitable for this purpose than those procedures involving oxidation and/or CNT derivatization (André et al., 2009; Menna et al., 2006; Speltini et al., 2012). Only few studies gave a systematic understanding of the affinity of selected compounds on CNTs in liquid chromatography (André et al., 2009; Kwon and Park, 2006; Yoo et al., 2011), while most of the papers interpreted the retention capability of the studied material mainly by focusing on π - π stacking and solute hydrophilicity as the key factors (Liang et al., 2010; Menna et al., 2006; Speltini et al., 2013a).

In this paper, we report the application of the multi-walled carbon nanotubes (MWCNTs)-modified stationary phase for HPLC separations and we carried out a neural network study to explain its retention behavior toward substituted benzenes. The MWCNTs were purified from the metallic impurities deriving from their synthesis, and then were firmly immobilized by gamma-radiation (from a ^{60}C source) onto silica microspheres

in presence of polybutadiene (PB) which has the sole function of linking agent, according to known methods (Merli et al., 2010; Speltini et al., 2013a). The CNT-based powder, easily packed in conventional chromatographic columns, acts as stationary phase for analytical applications and as a tool where the CNTs, permanently supported with no change in their physico-chemical properties, can interact with differently substituted benzenes.

The specificity of the binding affinity showed by the immobilized CNTs was investigated by comparison with the results obtained on a similar phase prepared by grafting activated carbon (AC) instead of CNTs on the same chromatographic support. The synthesized materials were characterized by scanning electron microscopy (SEM) and BET measurements.

To figure out the retention mechanism of MWCNTs, the chromatographic separation/retention of a set of analytes was compared to that observed on a typical C18 phase, and the role of the variables involved in the inter-molecular interaction was investigated by a computational model.

Since the interaction between CNTs and analytes can arise from many specific interactions and thus is dependent on the analyte properties, Quantitative Structure–Activity Relationship (QSAR) methodology was adopted for this study, currently used for drug design to estimate the properties of a chemical from its molecular structure (Sahlin et al., 2013; Dearden, 2004). In particular, when this approach refers to chromatographic data, the Quantitative Structure–Retention Relationship (QSRR) is used instead (Gianis and Tsantili-Kakoulidou, 2013; Héberger, 2007; Kaliszán, 2007).

For most of these applications, the final descriptive model was obtained with Multiple Linear Regression for simpler models or Principal Component Analysis, Partial Least Squares and derived methods when many descriptors are present. However, for the descriptors selected in this work, linear methods performed very poorly. In case of non-linearity, artificial neural networks (ANNs) are instead the best candidates (Borosy et al., 2000; González-Arjona et al., 2002; Manallack and Livingstone, 1999). In fact, ANNs are mostly used for finding models when numerous solutions can be suggested or the underlying model is unknown, such as pattern recognition, clustering and data reduction. This task exactly corresponds to the majority of QSAR studies. The modeling by ANNs is based on a learning or training step, which can be supervised or unsupervised and usually is an iterative method.

As previously reported by D'Archivio et al. (2012), linear solvation energy relationships (LSERs) can also be applied to model the effect of solute structure on the retention of analytes in reversed-phase HPLC. This approach is comparable or better than that of other multi-variable retention models for reversed-phase HPLC, at least when a sufficiently large training set is used (25 solutes and 11 columns).

Since our aim is to find a model for the interactions between analytes and CNTs, quantum chemical calculations are thus an attractive source of new molecular descriptors, which can, in principle, express all of the electronic and geometric properties of molecules and their interactions. The coupling of ANNs and quantum mechanical calculations is well known for drug design applications and indicated as Quantum QSAR (Karelson and Lobanov, 1996). In particular, ANNs trained with parameters calculated by Density Functional Theory (DFT) were used, and the most important parameters of the

correlation were extracted from the trained model (Blank and Brown, 1993; Karelson and Lobanov, 1996). Among the quantum descriptors used, E_{HOMO} , molecular dipole moment, and molecular polarizability were used as widely reported in literature (Azzaoui and Morinallory, 1995; Bodor et al., 1989; Clare and Supuran, 1994; Doichinova et al., 1994; Sotomatsu et al., 1989). Recently, a computational approach based on DFT calculations has been proposed also to study the interactions involved in the adsorption of acetic acid onto single-walled CNTs (Noei et al., 2013).

2. Experimental section

2.1. Materials and reagents

Pristine MWCNTs (o.d. 20–50 nm, i.d. 5–10 nm, length 10–20 μm , specific surface area 60 $\text{m}^2 \text{g}^{-1}$, purity > 95 wt%) were obtained from Cheap Tubes Inc. (Brattleboro, Vermont, USA). Silica microspheres (average particle size 5 μm , average pore diameter 100 \AA , surface area 200 $\text{m}^2 \text{g}^{-1}$) were purchased from Agilent Technologies Italia S.p.A (Cernusco sul Naviglio, Italy). Activated carbon (Norit, 40–140 mesh) was supplied by Norit Italia S.p.a. (Ravenna, Italy). HCl (37%, w/w, ultrapure) was from Merck Chemicals (Milan, Italy). Triton X-100, chloroform, HPLC grade acetonitrile (ACN), polybutadiene (PB, M_n 1530–2070) were supplied by Sigma–Aldrich (Milan, Italy). Ultrapure water (resistivity 18.2 $\text{M}\Omega \text{cm}^{-1}$) was produced by a Milli-Q system (Millipore, Milan, Italy). Analytical standards were purchased from Sigma–Aldrich and Carlo Erba Reagenti (Milan, Italy).

2.2. Liquid chromatography

MWCNT-silica and AC-silica were slurry packed into 3.9 \times 300 mm HPLC columns, as described elsewhere (Speltini et al., 2013a). The chromatographic system consisted of a Shimadzu (Milan, Italy) LC-20AT solvent delivery module equipped with a DGU-20A3 degasser and interfaced with a SPD-20A UV detector. Injection volume was 20 μL . For elution details see figures caption. For the intermolecular interaction study, all runs were performed in ACN–water (40:60), flow rate 1 mL min^{-1} and a commercial Hypersil C18 column (4.6 \times 250 mm, 5 μm) from Varian (Turin, Italy) was used for comparison under the same elution conditions. Injections of standard solutions in ACN were always performed in triplicate.

2.3. Characterization of the synthesized materials

SEM images of the powders were acquired in high vacuum using a Zeiss EVO®-MA10-HR microscope. Particle size determination was performed on the raw powders, while morphology was studied on gold sputtered conductive samples. Particle size distribution curves were obtained by the Digital Micrograph (™) 3.11.0 software. Specific surface area measurements on the samples were performed with a Sorptomatic 1990 by Thermo Fisher Scientific. About 0.350 g of powders was charged in the glass sample holder and degassed at 250 $^\circ\text{C}$ for 60 h. Subsequently, samples were cooled down at $-196\text{ }^\circ\text{C}$ and two adsorption–desorption cycles followed by a last adsorption run were performed (B.E.T. method, analyzing gas: N_2 , 50 points for run; blank done in He before the 1st adsorption run).

2.4. Intermolecular interaction study

At first glance, a linear model was chosen for the correlation between k' and analyte properties. We adopted a method based on the multiple linear regression having the formula:

$$\log k' = y_0 + \sum_{i=1}^I c_i X_i$$

where X_i is the i -th property of each compound, c_i is the unnormalized correlation coefficient of the i -th property and y_0 is the residual. Otherwise, in the case of ANN, the relationship between experimental k' and analyte properties was studied through three-layered ANNs. The neural networks used contain one linear input layer, one sigmoidal hidden layer and one linear output neuron (see figure in Supplementary data). Several topologies of networks were tested and no useful fittings were found with a linear hidden layer, this indicating a non-linear relationship of the input parameters with the retention factor. The neural networks were simulated by using the Joone freeware program (<http://sourceforge.net/projects/joone/>).

The theoretical capacity factor k' of each analyte is given by the overall fit function of our neural networks with H hidden sigmoidal neurons, I inputs:

$$\log k' = \sum_{h=1}^H \left[\sigma \left(\sum_{i=1}^I w_{ih} X_i - \beta_h \right) \right] w_h$$

where the sigmoidal function σ is defined as:

$$\sigma(a) = \frac{1}{1 + e^{-a}}$$

and β_h are the biases of hidden neurons; w_h are the weights between the input neurons (the descriptors) and the hidden neurons; w_{ih} are the weights between the hidden neurons and the output neurons ($\log k'$); X_i are the i -th properties of the analyte. All matrices and arrays present in the trained networks are listed in Supplementary Data. All inputs were chosen to be easily quantifiable by first-principle DFT calculations. The following properties are suitable for a good correlation with the experimental data: dipole moment (Debye), molecular mass (amu), rotational energy ($\text{cal mol}^{-1} \text{K}^{-1}$), HOMO energy (Ha), LUMO energy (Ha), zero-point energy (ZPE, Ha particle⁻¹), and isotropic polarizability (Bohr³). The training of neural networks was obtained by minimizing the root-mean-square error (RMSE). The number of neurons in the hidden layer was kept the lowest possible compatibly with a low RMSE in order to reduce the overfitting. The training procedure was divided into several steps decreasing gradually the learning rate (the variation of the neuron weights in each step) and momentum (the contribution of the last weight change in the previous iteration to the weight change in the current iteration).

We found that two different topologies of networks (different in the number of hidden and input neurons) best fit the behavior of CNT and octadecyl phases (see Section 3.3).

2.4.1. DFT calculations

All parameters used for the training of neural networks were obtained by DFT calculation with the B3LYP density functional with the 6-31G(d) basis set. Molecular geometries

were optimized *in vacuo* and frequency calculations were performed for all molecules in order to certify the minima (number of imaginary frequencies = 0). All calculations were made with the Gaussian09 program package.

3. Results and discussion

3.1. Characterization and properties of the obtained materials

The radiation-induced bonding of MWCNTs to the PB-coated silica gave a final composite material consisting of microspheres. These simply act as support/spacer for the CNT bundles, enabling them to interact with the solutes migrating in the eluent flow. The morphology of the CNT-based hybrid material can be appreciated from the SEM images reported in Fig. 1, where it is well evident that the CNTs “hanks” cover some silica 5 μm microspheres and the interstices among them. The AC-silica sample SEM analysis (not reported) shows only the spherical morphology of the silica particles. The particle size of the PB coated particles as obtained by the analysis of the SEM images (see Table 1) is similar for the two modified kinds of microspheres and 5% higher than the pristine silica particles. Concerning the surface area, there is a decrease due

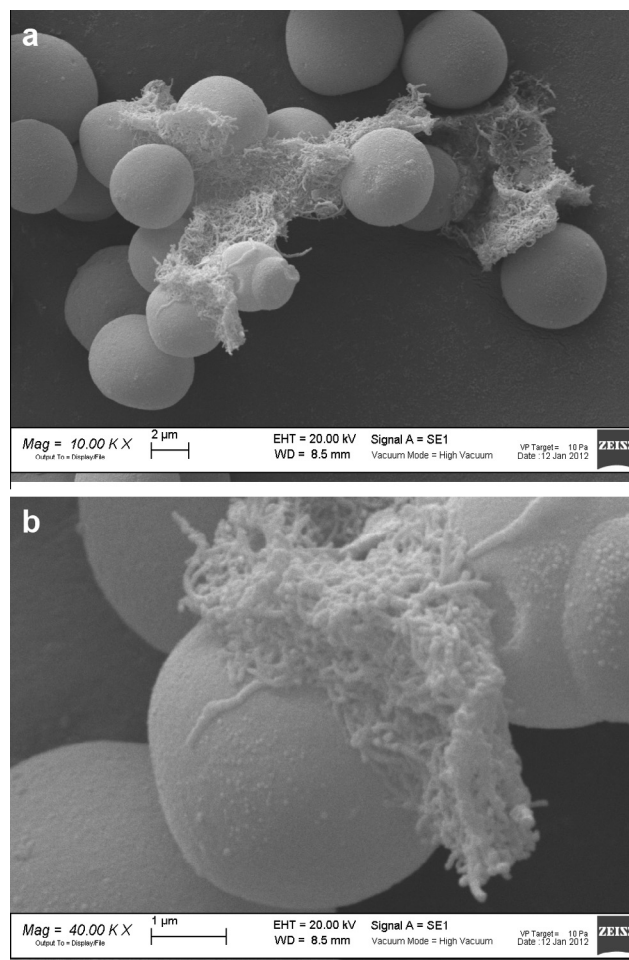


Figure 1 Scanning electron microscopy images of MWCNT-silica microspheres. The magnification reported in part b is to better show the morphology of the CNT structures.

Table 1 Surface area, pore size, and particle size of pristine and modified silica microspheres. The average particle size of the starting silica microspheres declared by the producer is 5 μm .

Sample	Surface area ($\text{m}^2 \text{g}^{-1}$)	Average pore diameter (nm)	Pore width (nm)	Particle size ^d (mm)	Average distribution width ^d
Silica (5 μm)	200 ^a	10 ^a	–	5.39(7)	0.20(1)
MWCNT silica (5 μm)	131 ^b	2.13 ^b	16.2 ^c	5.66(4)	0.16(1)
AC silica (5 μm)	156 ^b	2.08 ^b	14.3 ^c	5.64(6)	0.18(1)

^a Producer datum.^b Uncertainty: 3%.^c Uncertainty: 5%.^d From SEM.

to coating, with the MWCNT sample showing the lowest value (a 34.5% variation with respect to the pristine sample). This same sample presents a little higher pore width with respect to the AC sample (+12%), due to the different layering of the coating agent on the silica spheres.

In the new hybrid material the chemical bonding of MWCNTs preserved the nanotube structure after the grafting, as indicated by the moderate decrease of the relative intensity of the Raman G-band in MWCNT-functionalized powders with respect to the pristine sample – a full discussion is reported in Speltini et al. (2013a). It was experimentally found a value of *IG/ID* ratio equal to 0.70 for pristine powders and 0.63 for MWCNT-silica; a similar low defect ratio has been already observed in the case of physical adsorption of SWCNTs onto amino-silica particles (Fujigaya et al., 2011), testifying the mildness of the proposed immobilization method. The preservation of CNT pristine structure is fundamental to evaluate its real interaction with the solute. Actually, the nanotubes here immobilized by gamma-radiation can be regarded as MWCNTs whose carbonaceous structure is highly preserved; it is worth noting that the potential influence of the metallic impurities present in the commercial material on molecules retention times is minimized due to the purification procedure based on HCl washings. As already discussed (Merli et al., 2010; Speltini et al., 2010), this treatment enables a substantial removal of the metal fraction without altering the CNT morphology.

3.2. Intermolecular interactions of substituted benzenes

Separation in chromatography is generally driven by selective association with a specific solute with the stationary phase followed by dissociation, resulting in different retention times depending on the analyte nature; this evidence can be indirectly exploited to gain important information about the sorption process. Indeed, the markedly different capacity factors (mean values of three injections, relative standard deviation, RSD 0.1–3.8%) obtained for a set of substituted benzenes on the CNT-column (see Supplementary data) prove selective interactions between the nanotubes and each analyte. The role of the CNTs can be better appreciated comparing these results with those obtained on a commercial C18 stationary phase, that clearly indicate a different elution order on the two columns (see Supplementary data). This is expectable on the basis of the different base-structure and properties of each sorbent, and is in line with the recent literature (Liang et al., 2010;

Kwon and Park, 2006) where the different behavior among CNTs and more traditional reversed-phase materials was ascribed to the high number of interplays that CNTs are able to establish with the molecules with respect to the C18 alkyl strands. Indeed, while on the latter the adsorption affinity is recognized to be driven by mere hydrophobic interactions (Liang et al., 2010), on the contrary, π - π stacking, electrostatic (i.e. dipole–dipole) and dispersive forces, and molecular sieving also take place with CNTs: as a result, the combination of two or more interactions modulates the selectivity (André et al., 2009; Chambers et al., 2011). This is supported also by a recent paper proving that in the case of amino-acids, adsorption on SWCNTs is driven by a mechanism different from classical hydrophobic interaction, that only gives a limited contribution (Basiuk and Bassiouk, 2008).

It is well known that in liquid chromatography the role and the nature of the eluent are basilar (Basiuk and Bassiouk, 2008; Kwon and Park, 2006) and the retention time of a solute results from the combination of the repartition in the liquid eluent (dependent on solute physical–chemical properties) and the affinity for the sorbent, generally governed by hydrophobicity in the case of usual reversed-phase materials (Liang et al., 2010). For this reason, elution was performed maintaining the same mobile phase for the two columns, so that the different adsorption behavior of the solutes on the two sorbents was ascribable to the neat interaction with either the nanotubes or the C18 strands, and a direct comparison between the two data set of capacity factors was possible.

The key role of the nanotubes in the adsorption process was demonstrated by comparison on a blank control column filled with PB-silica (prepared by the same procedure omitting CNTs) and on a column packed with AC-silica particles. As verified for alkylbenzenes and aromatic acids (Speltini et al., 2013a), the contribution of the PB-silica was negligible, so that separation on the CNT phase can be ascribed essentially to the intermolecular interplays between solutes and nanotubes.

The role of the MWCNTs anchored by gamma-radiation has been further proved by substituting the nanotubes with the same amount amorphous carbon (5 wt%) grafted onto the silica microspheres following the same gamma-radiation procedure. Results unquestionably evidenced the different morphology and physical–chemical properties of the two carbon-made materials, as in the case of BTX, so strongly adsorbed on the carbon to require very high percentage of ACN for the desorption (up to 90% v/v) that occurred with a broadening band (Fig. 2). These results further prove the goodness of the method used for the preparation of a

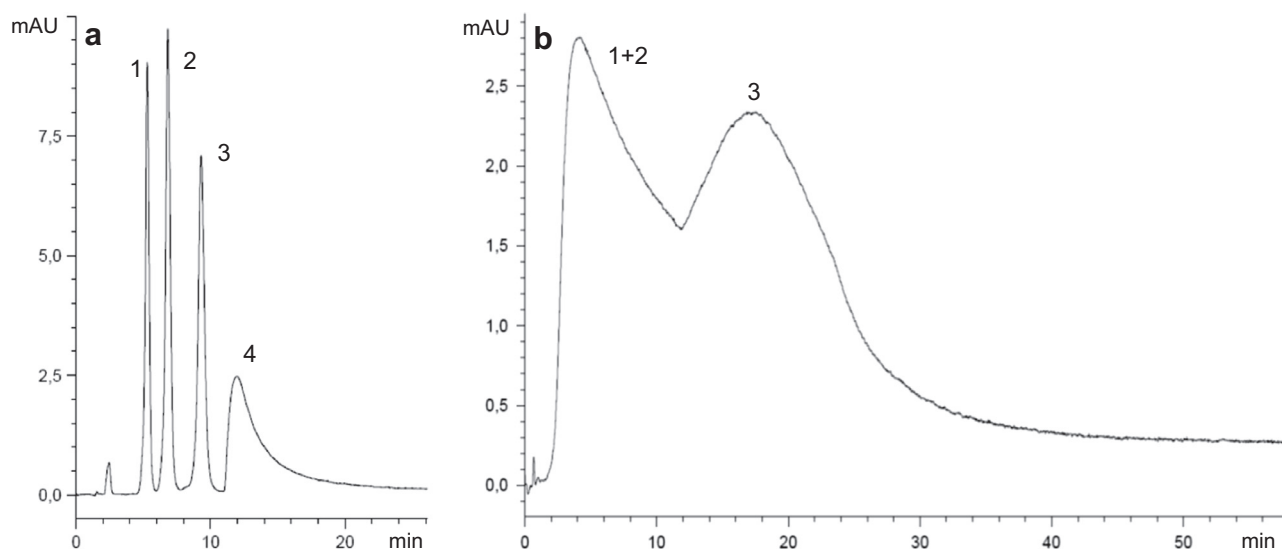


Figure 2 Elution profiles obtained on (a) the MWCNT-column (3.9×300 mm) and on (b) the AC-column (3.9×300 mm). Peak identification: benzene (1), toluene (2), *p*-xylene (3), naphthalene (4). Mobile phase: (a) ACN–water (40:60), flow rate 1 mL min^{-1} ; (b) isocratic ACN 50% for 9 min, linear gradient to ACN 90% in 11 min, isocratic ACN 90% for 40 min. Detection: 254 nm.

CNT-containing hybrid material *really* basing its properties and binding affinity on carbon nanotubes. This is very important in the framework of an intermolecular interaction study.

Indeed, the preparation method is a crucial step, as not all the materials and methods proposed in the last years are suitable to selectively study the neat interaction between CNTs and solutes. For instance, in polymeric matrices, consisting of CNTs embedded in the polymer phase, this last plays a major role in the separation/adsorption process (Aqel et al., 2012; Chambers et al., 2011; Li et al., 2005), thus shading the actual function of the nanomaterial.

To verify which variables were significantly involved in the retention process on the CNT stationary phase and to provide a predictive model, a computational approach has been elaborated. The correlation was made by using the $\log k'$ instead of k' , as reported in the literature (Gianis and Tsantili-Kakoulidou, 2013; Héberger, 2007; Kaliszan, 2007). The $\log k'$ values for each column are reported in Table 2. With this method, two analytes were excluded for each stationary phase due to their low k' values (phenol and 2,4-dinitrophenol for CNT; 2,4-dinitrophenol and 4,6-dinitro-ortho-cresol for C18).

All the descriptors were normalized between 0 and 1, in order to have a better understanding of the weights calculated by the model (see Supplementary data). Even if the elution order was different on the two studied stationary phases, a strong correlation exists between the $\log k'$ values observed on the two columns (see Supplementary data), indeed only compounds having entries 3, 10 and 25 differ significantly.

For the ANN modeling, the total set was divided into two sets, the first was used as training set while the remaining data were used as validation set. At this purpose, some criteria were tested in order to choose the proper training set. The first criterion was to select the most retained compounds (highest k'), as it was supposed that for these molecules the interactions with the stationary phase are maximized. This criterion was abandoned because the validation set showed a bad match with the experimental results, thus indicating that the model

Table 2 Log k' values for CNT and C18 stationary phases.

ID	Compound	Log k' CNT	Log k' C18
1	Benzene	0.30	0.79
2	Toluene	0.50	1.04
3	<i>p</i> -Xylene	0.72	1.31
4	Chlorobenzene	0.66	1.04
5	Iodobenzene	0.90	1.28
6	Bromobenzene	0.74	1.15
7	Naphthalene	0.84	1.31
8	Phenol	–	0.23
9	Nitrobenzene	0.25	0.64
10	Ethylbenzene	0.74	1.28
11	Ethylbenzoate	0.48	0.93
12	<i>m</i> -Cresol	0.03	0.44
13	<i>o</i> -Dichlorobenzene	0.88	1.26
14	<i>p</i> -Dichlorobenzene	0.94	1.32
15	1,2,4-Trichlorobenzene	1.26	1.66
16	<i>o</i> -Chlorophenol	0.17	0.50
17	<i>p</i> -Chlorophenol	0.10	0.55
18	Benzylbromide	0.64	0.81
19	Benzonitrile	0.18	0.54
20	Benzaldehyde	0.16	0.46
21	2-Chlorotoluene	0.83	1.35
22	4-Chlorotoluene	0.84	1.34
23	2,4-Dichlorotoluene	1.20	1.70
24	2,6-Dichlorotoluene	1.19	1.69
25	2,4-Dichlorophenol	0.43	1.10
26	2,5-Dimethylphenol	0.22	0.66
27	3,5-Dimethylphenol	0.14	0.62
28	2,4-Dinitrophenol	–	–
29	4,6-Dinitro-ortocresol	0.08	–
30	3-Methyl-4-nitrophenol	0.19	0.44

created did not match the experimental data. The second criterion for the training set choice was to select only few compounds covering the total span of $\log k'$ values. The trained networks, for both stationary phases, were created with 5

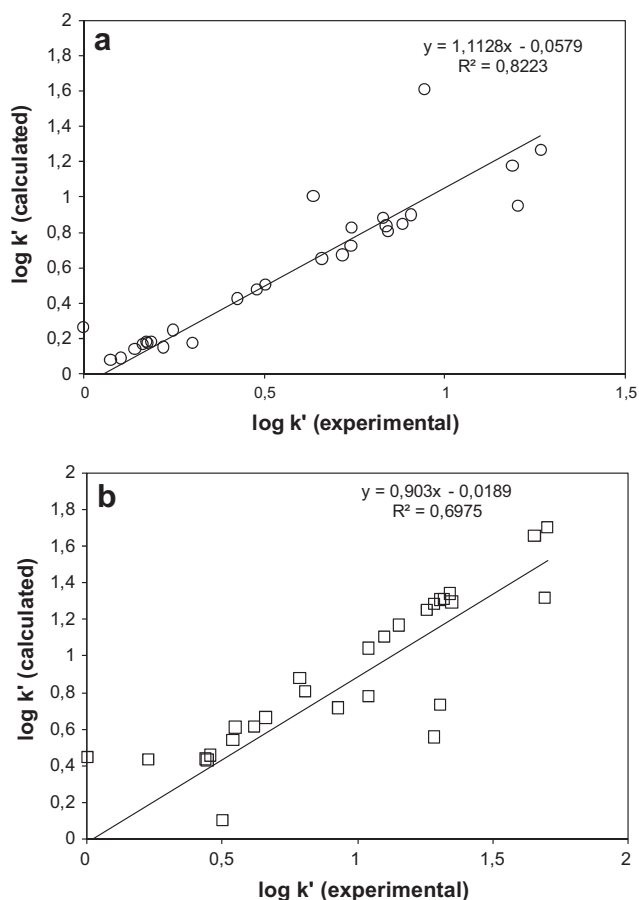


Figure 3 Calculated vs. experimental $\log k'$ values for (a) CNT and (b) C18 ANNs (see text for details).

Table 3 Optimized values obtained for CNT and C18 modeling by multiple linear regression.

	CNT	C18
Offset (y_0)	0.617684	0.811781
Dipole	-1.03372	-0.93446
Molecular weight	0.245752	0.167107
Rotational energy	-0.61173	-0.78897
HOMO energy	-0.82631	-1.04132
LUMO energy	0.663399	0.933196
Zero-point energy	-0.80059	-0.81893
Polarizability	1.700572	2.284468

sigmoidal neurons in the hidden layer and showed interesting results with the fitting of the validation set.

In order to simplify the model and to avoid (or reduce) overfittings, a simplification of the network was achieved. The first simplification was made by decreasing the number of descriptors. The method used to find out the less relevant descriptor was based on the sum of absolute values of network multipliers (Despagne and Massart, 1998; Nord and Jacobsson, 1998; Olden and Jackson, 2002). For CNT it was clear that the least important descriptors were molecular weight and zero point energy, while for the C18 stationary phase the HOMO and LUMO parameters had low weights.

The networks were trained again without those descriptors, and the results showed few differences with the model based on all the descriptors. For the CNT model the agreement with the validation set was good except for some compounds that were not well represented in their chemical classes, such as benzonitrile, benzaldehyde and ethyl benzoate. In fact, these compounds were the only representative molecules for their chemical class tested in our experiments. The training set was thus modified by adding these compounds. At the end, the trained network showed a good correlation with experimental data (see Fig. 3a), as indicated by the good correlation coefficient (R^2). Moreover, the number of hidden neurons was reduced from 5 to 3, without affecting the correlation. The CNT stationary phase model resulting from neural networks showed how LUMO position and polarizability are the most important factors affecting the retention time.

The neural network model for the C18 stationary phase is acceptable (R^2 0.6975, see Fig. 3b) with dipole and polarizability as the most important factors. In this case, the decrease of the network from 5 to 3 neurons reduced significantly the correlation with the validation set.

The models obtained by neural networks were compared with those aroused from multiple linear regression (with respect to neural network model, the multiple linear regression

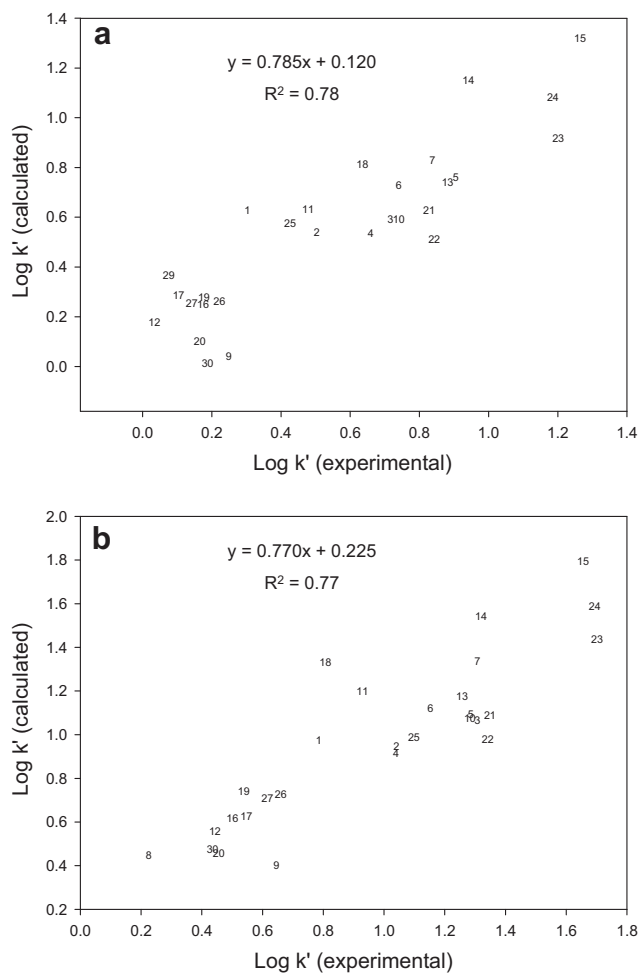


Figure 4 Calculated vs. experimental $\log k'$ values for (a) CNT and (b) C18 obtained by multiple linear regression.

is comparable to the behavior of one neuron having linear response). The parameters (c_i) obtained from multiple linear fitting are gathered in Table 3. As it can be seen from Fig. 4, the R^2 resulted to be 0.78 for the CNT column and 0.77 for the C18 one, indicating a poor modeling (see Supplementary data for a complete list of parameters).

The linear and ANN models showed some similarities and differences. Starting from the model obtained for the CNT stationary phase, both models showed the importance of the polarizability, while the less important descriptor was molecular weight.

The principal difference for the ANN model is the importance given to the frontier orbitals, in particular LUMO. The importance of frontier orbitals for the modeling of interactions seems reasonable because CNT orbitals might interact with the analytes. On the contrary, linear models cannot take into account interactions having an energy range like

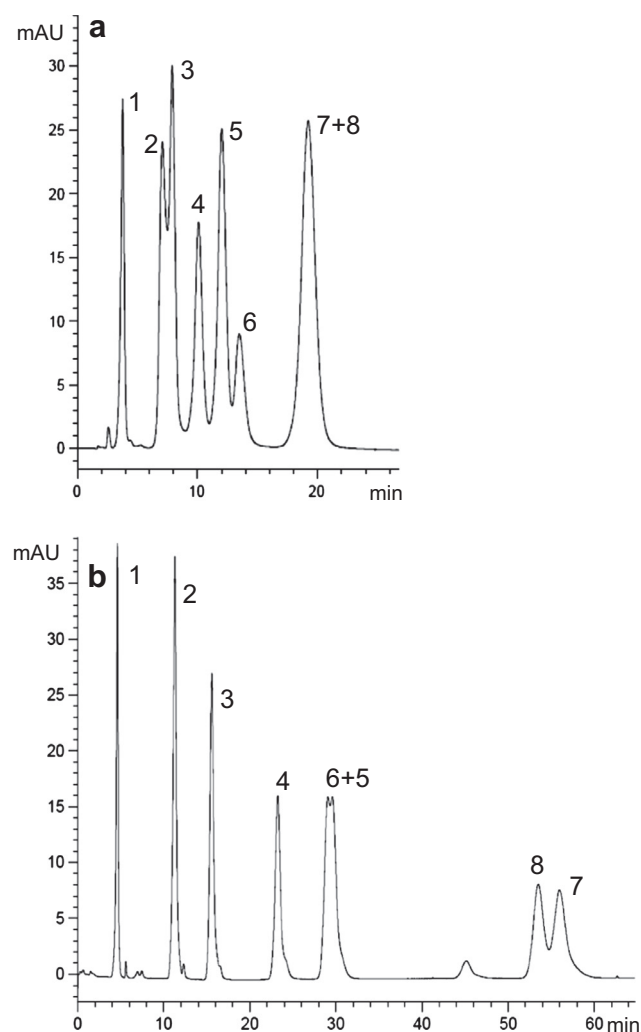


Figure 5 Chromatograms obtained for a mixture of benzene compounds ($20\text{--}100\text{ mg L}^{-1}$) on (a) the MWCNT-column ($3.9 \times 300\text{ mm}$) and on (b) the C18 column ($4.6 \times 250\text{ mm}$). Peak identification: phenol (1), nitrobenzene (2), benzene (3), ethylbenzoate (4), toluene (5), chlorobenzene (6), *p*-xylene (7), ethylbenzene (8). Mobile phase: ACN–water (35:65), flow rate 1 mL min^{-1} . Detection: 254 nm .

those arising from electron donor and acceptor properties. In the case of the C18 material, the ANN model did not show particular improvements with respect to the multiple linear model.

3.3. Chromatographic tests

As above anticipated, the CNT-based material turned out to be useful also for analytical applications due to its chromatographic properties. Indeed, the material was tested as stationary phase for separation of various aromatics among those above selected for the computational study. The column-to-column reproducibility was assessed on independently synthesized batches (Speltini et al., 2013a).

The MWCNT-column was able to selectively retain mono-substituted benzenes bearing different chemical groups on the aromatic ring, providing symmetrical peaks and baseline resolution in about 13 min run times (see chromatogram in Supplementary data).

The chromatographic profile in Fig. 5 refers to a sample containing eight aromatics; specifically, separation of toluene and *p*-xylene accounts for methyl group selectivity, while the methylene group is the discriminating factor in the separation of toluene and ethylbenzene. The overall interaction with the

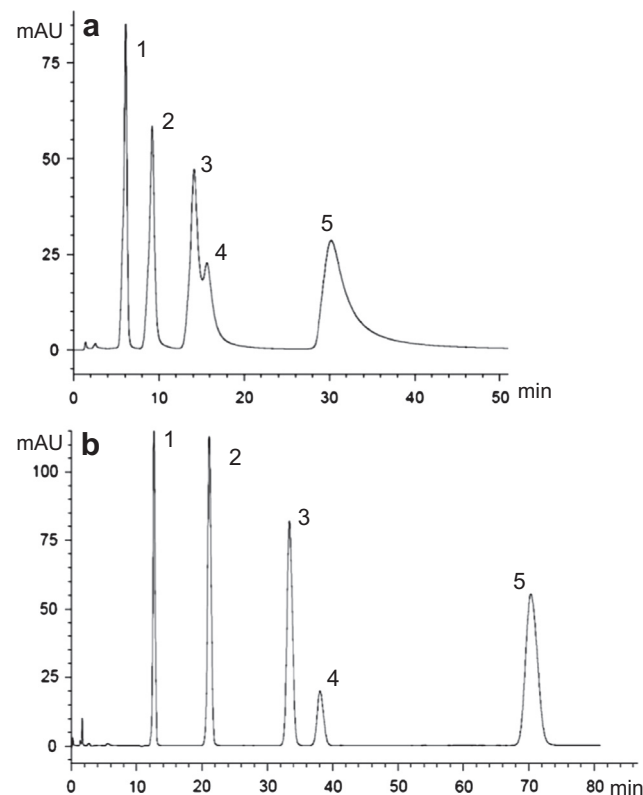


Figure 6 Chromatograms obtained for a mixture of (poly)chlorobenzenes (50 mg L^{-1}) on (a) the MWCNT-column ($3.9 \times 300\text{ mm}$) and on (b) the C18 column ($4.6 \times 250\text{ mm}$). Peak identification: benzene (1), chlorobenzene (2), *o*-dichlorobenzene (3), *p*-dichlorobenzene (4), 1,2,4-trichlorobenzene (5). Mobile phase: ACN–water (40:60), flow rate 1 mL min^{-1} . Detection: 210 nm .

nanotubes for ethylbenzene and *p*-xylene, that are C₈H₁₀ isomers (see [Supplementary data](#)), prevented their chromatographic resolution. These two structural isomers resulted hard to be separated also on the C18 sorbent, as shown in [Fig. 5 b](#) with retention times up to 60 min under the same elution conditions applied to the CNT column. Notice that the C18 sorbent was unable to separate toluene and chlorobenzene; moreover, the elution order of the analytes was different ([Fig. 5b](#)). These facts well evidence the different retention process taking place on the two chromatographic materials.

As shown in [Fig. 6](#), on CNTs chlorinated benzenes were separated in isocratic elution, although with a certain peak tailing for compound 5, as well the two positional isomers, that is *o*-dichlorobenzene and *p*-dichlorobenzene (resolution, *R*_s 0.7, selectivity factor, α 1.12).

4. Conclusions

Silica microspheres derivatized with MWCNTs by a simple and mild method (employing gamma radiation) were applied as HPLC stationary phase for studying interactions between nanotubes and various substituted benzenes. The intrinsic characteristics of MWCNTs were preserved after the chemical bonding on the silica support and imparted specific properties to the stationary phase, as gamma irradiation did not significantly modify the CNT structure. Being the adsorption affinity of the material strictly related to the CNT properties, this stationary phase resulted as a double-purpose material, suitable for the chromatographic applications, and at the same time a valid tool to study the intermolecular interactions of substituted benzenes with the CNTs. In this sense, a model based on analyte properties (descriptors) determined through DFT that can correlate with experimental results was studied. The model here applied evidenced a non-linear relationship between calculated parameters and retention time (or capacity factor). In order to build the model, an automatic learning through ANNs was used. This investigation gave good correlations for the CNT column and the key descriptors involved in the interaction were figured out. Summing up, these results prove the potentiality of CNTs for practical applications in chromatography and highlight possible theoretical modelizations of liquid phase chromatography with ANNs.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.arabjc.2015.02.019>.

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