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 retention times of each probe to its physico-chemical characteristics (calculated by Density Functional Theory methods). 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 moreover 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 a octadecyl 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, as expected, to dipole moment and polarizability. As a significative difference, the MWCNT stationary phase presents correlations with HOMO energies and a restricted favourable range of polarizability, evidence for a specific interaction between MWCNTs and analytes. The 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.

Keywords: carbon nanotubes; feedforward neural networks; intermolecular interaction; liquid chromatography

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-conductors 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 charcoal) 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 a., 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. At 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 degree 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 is 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 (Speltini 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 cannot be directly packed in conventional analytical columns due to the high back-pressure 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 recent literature (Speltini et al., 2013b); in that paper several synthetic routes have been proposed 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 behaviour 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 explained its retention behaviour towards substituted benzenes. The MWCNTs were purified from the metallic impurities deriving from their synthesis, and then were firmly immobilized by gamma-radiation onto silica microspheres in presence of polybutadiene (PB) with 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 also 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 charcoal (AC) instead of CNTs on the same chromatographic support. The synthesized materials were characterized by scanning electron microscopy (SEM) and by BET measurements.

To figure out the retention mechanism of MWCNTs, the chromatographic separation/retention of a set of analytes were compared to those observed on a typical octadecyl-silica 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. QSAR methods are often used for drug design for estimating the properties of a chemical from its molecular structure. Moreover QSAR can be useful in many other applications such as hazard assessment, risk assessment (Sahlin et al., 2013) and the assessment of persistent, bio-accumulating and toxic products in the chemical industry (Dearden, 2004). 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. If the problem to solve is highly non-linear, like our case, 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 where 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 modelling by ANNs is based on a learning or training step, which can be supervised or unsupervised and usually is a iterative method.

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 feedforward neural networks (FFNNs) and quantum mechanical calculations is well known for drug design applications and indicated as Quantum QSAR (Karelson and Lobanov, 1996). In particular, FFNNs 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, 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 $m^2\ g^{-1}$, purity > 95 wt%) were obtained from Cheap Tubes Inc. (Brattleboro, Vermont, USA). Silica microspheres (average particle size 5 μm, average pore diameter 100 Å, surface area 200 $m^2\ g^{-1}$) 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_n1530-2070) were supplied by Sigma-Aldrich (Milan, Italy). Ultrapure water (resistivity 18.2 MΩ 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 × 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 also a commercial Hypersil C18 column (4.6 × 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 ($^{\text{TM}}$) 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 were charged in the glass sample holder and degassed at 250°C for 60 h. Subsequently, samples were cooled down at -196°C and 2 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 hyperplane having the formula:

$$k'_j = y_0 + \sum_{i=1}^{I} c_i X_{ij}$$

Where X_{ij} is the *i*-th property of the *j*-th 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 FFNNs. The neural networks used contain one linear input layer, one sigmoidal hidden layer and one linear output neuron. 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.

The theoretical retention time *k*' of the *j*-th analyte is given by the overall fit function of our neural networks with *H* hidden sigmoidal neurons, *I* inputs and *J* analytes:

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

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 and the hidden neurons; w_{ih} are the weights between the hidden neurons and the output neurons; X_{ij} are the *i*-th properties of the *j*-th analyte. All matrices and arrays present in the trained networks are listed in the Supplementary Data. All inputs were chosen as easily quantifiable by first-principle DFT calculations. We found that the following properties are suitable for a good correlation with experimental data: dipole moment (Debye), molecular mass (amu), rotational energy (cal mol^{-1} K^{-1}), translational energy (cal mol^{-1} K^{-1}), vibrational energy (cal mol^{-1} K^{-1}), zero-point energy (ZPE, Ha $particle^{-1}$), isotropic polarizability ($Bohr^3$), HOMO energy (Ha). 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 in 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 behaviour of CNT and octadecyl phases. In particular, for the CNT-stationary phase, we found that molecular mass and ZPE give a negligible contribute for the prediction of *k*'; the number of neurons in the hidden layer was 7. For the interpolation of *k*' from C18-stationary phase the complete input set with 5 neurons in the hidden layer was instead used.

The goodness of modelization given by neural networks was assessed by reducing the training set down to 15 analytes. However the choice of the training set heavily affects the prediction capability of the network. In fact, due to the non-linear behaviour of the fitting, it is necessary to select representative analytes for each category. In our case this was not a drawback since our aim is to extract the most important parameters that affect *k*', viz. the nature of interaction between analytes and stationary phase.

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 is well shown 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 a 5% higher than the pristine silica particles. Concerning the surface area, there is a decrease due 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 %), probably due to the different layering of the coating agent on the silica spheres.

In this 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. As a matter of fact, 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.

3.3. Intermolecular interactions of substituted benzenes

Separation in chromatography is generally driven by selective association of 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 Table 2) 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 octadecyl silica stationary phase, that clearly indicate a different elution order on the two columns, as apparent from Table 2. This is expectable on the basis of the different base-structure and properties of each sorbent, and is in line with recent literature (Liang et al., 2010; Kwon and Park, 2006) where the different behaviour 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), π–π stacking, electrostatic (i.e.

dipole-dipole) and dispersive forces, and molecular sieving also take place with CNTs, and the combination of two or more interactions modulate 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 (dependant on solute physical-chemical properties, *viz.* solubility, hydrophilicity, possibility to give hydrogen bond, etc.) 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 behaviour 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 contribute 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. Charcoal (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 charcoal to require very high percentage of ACN for the desorption (up to 90% v/v) that occurs with a broadening band (Fig. 2). These results further prove the goodness of the method used for the preparation of a 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, in which CNTs were 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 of the CNT stationary phase and to provide a provisional model, a computational model has been elaborated. At first glance, the linear model was adopted (see Section 2.4). The squared correlation coefficients (R^2) resulted to be 0.67 for the CNT column and 0.62 for the C18 one, indicating a poor modelization. Furthermore, the residuals (i.e. differences between the predicted and the experimental values) showed a non-random distribution with respect to *k*’, further supporting the inconsistence of the linear model. With these results, there was two possibilities, the first was to change the descriptors used in the model, while the second was to adopt a non-linear model with the same descriptors. Since the variables used are in our opinion sufficient for the description of the interactions between the stationary phase and analytes, we decided to use non-linear methods. In fact, the selected descriptors are some common properties such as polarity, polarizability, HOMO energies and molecular weight together with properties related to entropy and molecular rigidity (rotational energy, translational energy, Zero-Point Energy and vibrational energy).

Due to the high non-linearity of the working functions, ANN with one hidden layer were used. The choice of the number and type of neurons and network topology is critical for ANNs, in fact, due to the high number of variables contained in a network (the so-called neuron weights and biases) it is easy to find good fits by increasing the number of neurons, but in that case the final model is a network with too many connections is useless. So, even if the training procedure (backpropagation algorithm) is automatic, the choice of the number, type of neurons and topology of the network depends on the user and cannot be automated. In particular, several networks must be tested excluding some data from the training procedure and checking if the missing data fits with the known results (model check) and this procedure should be repeated decreasing the number of neurons in the hidden layer (overfitting check) in order to find a compromise between the fit parameters and the number of neurons. In our case, the results of trained neural networks are in good accordance with experimental data, as shown by the correlation coefficients higher than 0.98 (see Fig. 3). The coefficients of the trained networks were crucial to extract the meaningful parameters that affect retention times. ANNs usually gave good fittings but in the past they have also been labelled as black boxes because they provide little explanatory insight into the relative influence of the independent variables in the prediction process. However, many methods for the extraction of information from ANNs are known in literature (Olden and Jackson, 2002). In our case, in order to extract the main parameters that increase the *k*' (and thus the interaction between analytes and stationary phase) we studied the changes of activation of hidden neurons after a normalization of weights by using the sensitivity analysis (Olden and Jackson, 2002).

For both stationary phases the most important parameters resulted to be the dipole moment, followed by polarizability of the molecule, but the CNT column showed a strong interaction in a shorter range of polarizability, i.e. low and high polarizable molecules interact less with nanotubes. This could be related to specific interactions between analytes and CNTs. Another difference lies in the molecular weight that, in contrast to the C18 material, had no influence to explain interactions with the CNT sorbent, while HOMO energy enhanced the fitting only for CNTs.

3.2. 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 previously assessed on independently synthesized batches (Speltini et al., 2013a).

The chromatographic traces obtained for variously substituted benzenes, are reported in Figs. 4 and 5a. In particular, Fig. 4 proves that the MWCNT-column is 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.

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 nanotubes for ethylbenzene and *p*-xylene, C_8H_{10} isomers (see Table 2), prevented their chromatographic resolution. The two structural isomers resulted hard to be separated also on the C18 sorbent, as shown in Figs 5b with retention times up to 60 min under the same elution conditions applied to the CNT column. Notice that, while on CNTs separation was not possible for the two isomers, the C18 sorbent was instead 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, 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 as gamma radiation were applied as HPLC stationary phase for studying interactions between nanotubes and various substituted benzenes. The intrinsic characteristics of MWCNTs are preserved after the chemical bonding on the silica support and impart 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 such compounds 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. In order to build the model an automatic learning through ANNs was used. This investigation gave good correlations for both the columns and for each column 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.

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Figure captions

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.

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.

Figure 3 Experimental vs. calculated capacity factors for (a) C18 and (b) CNT stationary phases.

Figure 4 Chromatogram obtained on the MWCNT-column (3.9 × 300 mm) for a mixture of benzene compounds (20-100 mg L^{-1}). Peak identification: phenol (1), nitrobenzene (2), chlorobenzene (3), ethylbenzene (4). Mobile phase: ACN-water (50:50), flow rate 1 mL min^{-1}. Detection: 254 nm.

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

Figure 6 Chromatograms obtained for a mixture of (poly)chlorobenzenes (50 mg L^{-1}) on (a) the MWCNT-column (3.9 × 300 mm) and on (b) the C18 column (4.6 × 250 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.

Table titles

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

Table 2 Mean capacity factors (*n=3*) and elution order of the test probes on the MWCNT column and on the C18 column under same elution conditions: ACN-water (40:60), 1 mL min^{-1}.

Supplementary Data Full parameters list of trained neural networks discussed in the text.