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Multi-walled carbon nanotubes as the gas chromatographic stationary phase: Role of their functionalization in the analysis of aliphatic alcohols and esters

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ABSTRACT

Presented hereafter is the novel application of differently functionalized multi-walled carbon nanotubes (f-MWCNTs) as stationary phases for the GC separation of C_1–C_5 alcohols and esters. Low-cost packed columns, easily prepared in laboratory, were used with satisfactory results. Depending on the functional groups introduced on the pristine MWCNTs, remarkably different behaviours have been observed, thus indicating that derivatization is a key factor to achieve optimal resolution. The best performance was shown by the 2,2′-(ethylenedioxy)diethylamine derivatized MWCNTs, allowing the separation of alcohol isomers, for this reason chosen for the investigation of fermentation by-products in distilled spirits. The degree of derivatization has been assessed for each packing material by thermogravimetric analysis (TGA). A computational study has been performed to correlate the physico–chemical properties of alcohol probes with the retention behaviour.

1. Introduction

Carbon nanotubes (CNTs) are fullerene-related molecules that can be obtained as single-walled (SWCNTs) or multi-walled (MWCNTs) material. They have triggered the attention of scientists in view of their conducting or semiconducting properties [1] useful for the realization of electrochemical sensors for biomolecules [2,3] and metal ions [4]. They have also been employed as catalyst supports [5], probes for drug delivery [6] and packing material for solid-phase extraction (SPE) of organic contaminants [7–13], due to the good adsorption properties towards a large number of organic molecules.

The use of CNTs as stationary phase has been pursued in the last years for both liquid [14–16] and gas [17–23] chromatography. From an analytical standpoint, these carbon-made nanomaterials exhibit fascinating properties and, in particular, their capability to give π–π stacking with unsaturated and aromatic compounds, high thermal stability and enhanced adsorption properties with respect to a planar carbon surface [15], make them highly attractive for such purposes, especially if compared with traditional sorbents. Indeed, the inner hollow cavity of CNTs, the outside surface, and the interstitial spaces between the nanotube bundles are possible adsorption sites [17]. So far, purified non-derivatized CNTs (nd-CNTs) have been to some degree employed for the GC separation of various classes of analytes [17–22], but functionalized CNTs have not. The possibility to introduce chemically designed functionalities on the CNTs surface could be a valid strategy to modulate their affinity towards solutes. In this context, derivatization of MWCNTs has been shown to be crucial in the separation of linear and branched light alkanes and aromatics [24].

As we report in this article, alcohols too could be separated on derivatized CNTs: their determination is of great importance, as alcoholic compounds are widely present in foods, beverages and pharmaceuticals [25]. Furthermore, the determination of methanol and ethanol is of concern in the urban air, as they are often mixed with gasoline in the production of car fuels [26]. According to these considerations, a large number of analytical methods for their determination are currently available, and apart from suitable sample pre-treatment, good separation is essential for accurate determinations. Up today, analysis of alcohols is performed with various techniques, including enzymatic [26–28] and IR spectroscopy-based methods [29]. With respect to chromatographic techniques, the separation can be carried out by LC coupled with amperometric detection [30,31] and by capillary GC followed by flame ionization detection (FID) [25,26,32–34].

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Traditional reversed-phase polymer-based stationary phases [30], mixed mode mechanism polymeric [31] and polar [25,26,32–34] stationary phases, have been employed. Aliphatic alcohols have been recently separated on MWCNTs-based GC columns with better performance with respect to commercial carbon sorbents [18]. However, some shortcomings were found: resolution was not optimal and high temperature was necessary to prevent band broadening and tailing.

Starting from these interesting results, in this work we focused on the GC separation of light alcohols on functionalized MWCNTs (f-MWCNTs) stationary phases. Moreover, we evaluated the role of different derivatization processes on pristine MWCNTs (p-MWCNTs) in promoting the GC separation efficiency of C_1–C_5 alcohols. At this purpose, f-MWCNTs were employed as stationary phases for homemade-packed columns. The results obtained on f-MWCNTs and nd-MWCNTs were compared and critically commented, basing on the evaluation of the chromatographic parameters. Derivatized MWCNTs were thereafter employed for the analysis of *Grappa* flavor constituents (alcohols and esters).

2. Experimental

2.1. Materials and reagents

All the chemicals were reagent or analytical grade and were used without any further purification. p-MWCNTs (O.D. 20–50 nm, I.D. 5–10 nm, length 10–20 μm, specific surface area 60 $m^2\,g^{-1}$, electrical conductivity > 100 $S\,cm^{-1}$, bulk density 0.28 $g\,cm^{-3}$, true density ~2.1 $g\,cm^{-3}$, purity > 95 wt%, ash < 1.5 wt%) were obtained from Cheap Tubes Inc. (Brattleboro, USA). Hexamethyldisilazane and toluene were purchased from Sigma–Aldrich (Milan, Italy). Merck Chemicals (Milan, Italy) ultrapure HNO_3 (65%, w/w), H_2SO_4 (98%, w/w) and HCl (37%, w/w) were used. Methanol, THF, thionyl chloride, 2,2′-(ethylenedioxy)diethylamine, PEG-200, 1,6-hexandiol and 1,10-decandiol were used for p-MWCNTs derivatization and were supplied by Sigma–Aldrich. PTFE 0.45 μm (Sigma–Aldrich) and paper (Schleicher & Schuell) filters were employed. Ultrapure water (resistivity 18.2 $M\Omega\,cm^{-1}$ at 25 °C) was produced by a Milli-Q system (Millipore, Milan, Italy). All the alcohols and esters were purchased from Sigma–Aldrich.

2.2. Apparatus

A Sigma 3B gas chromatograph (Perkin Elmer, Monza, Italy) equipped with splitless injector and flame ionization detector (FID), and interfaced with a C-R6A recorder (Shimadzu, Kyoto, Japan), was used. High purity nitrogen (99.9%) was employed as carrier gas at a flow rate of 20 $cm^3\,min^{-1}$. Injected volumes varied from 1 to 4 μL. The thermal program depended on the stationary phase used (see Figs. 2–4 captions). The injector temperature was 50 °C above the oven temperature, while detector temperature was fixed at 250 °C. The glass liner was sylanized before the analysis (see Section 2.5). A Vacuum/Pressure Station 400-3902 (Barnant Company, Barrington, USA) was employed to pack the glass columns.

An Orion 420A pH meter (Thermo Electron Corporation, Rodano, Italy) was used to control pH throughout CNTs derivatization and purification procedures.

ICP-OES analysis was performed with a Optima 3300 DV (Perkin Elmer, Monza, Italy). INAA (Instrumental Neutron Activation Analysis) was conducted with the TRIGA Mark II 250 Kw (General Atomic, USA) research nuclear reactor of the University of Pavia, with a neutron flux of $1.76 \times 10^{13}\,ns^{-1}\,cm^{-2}$ (further details are reported in Table S1). TGA scans were recorded with a 2950 TGA (TA Instruments, Vimodrone, Italy).

2.3. Derivatization of p-MWCNTs

Carboxylic derivatives of MWCNTs were synthesized according to a slight modification of a known procedure [4]. Briefly, commercially available pristine MWCNTs (35 g) were oxidized in 65% HNO_3–98% H_2SO_4 (1:3) and functionalized by conventional nucleophilic substitution on acylic chloride derivatized CNTs (MWCNTs-COCl), as previously described. A solution of $NH_2(CH_2CH_2O)_2$–$CH_2CH_2NH_2$ (20 mL) in anhydrous THF (70 mL) was poured in a round-bottom flask containing 6 g of MWCNTs-COCl and the mixture was refluxed for 24 h. The suspension was centrifuged and the solid repeatedly washed with methanol. Finally, the obtained product, called from now on MWCNTs-$CONH_2$, was dried at 80 °C for 4 h.

The same amount of MWCNTs-COCl was refluxed in PEG-200 (90 mL) for 4 h to give MWCNTs-PEG ester. The suspension was filtered on a paper filter, the solid repeatedly washed with methanol and finally dried at 80 °C for 4 h.

For the syntheses of MWCNTs-$COO(CH_2)_6OH$ and MWCNTs-$COO(CH_2)_{10}OH$ the acyl chloride derivatives were refluxed in anhydrous THF (50 mL) in which 6 g of either 1,6-hexandiol or 1,10-decandiol, respectively, were dissolved, following the same procedure described above.

2.4. Purification of p-MWCNTs

Since the content of impurities strongly affects the surface and adsorption properties of CNTs [24], a simple procedure was applied to remove metals from the raw material, similarly to that we previously showed [24]. Briefly, 5 g of p-MWCNTs were added to 100 mL of HCl solution (20%, w/w): the suspension was sonicated for 30 min at room temperature and thereafter refluxed for 4 h. Afterwards, the suspension was filtered and the solid was firstly rinsed with 500 mL HCl (1%, w/w), then with ultrapure water until neutrality and dried at 300 °C for 2 h. The metals content in pristine and purified MWCNTs was assessed by acidic bomb digestion, followed by ICP-OES determination—procedural details are reported elsewhere [24]. For some metals, the concentration has been also determined by INAA.

The thermal stability and the degree of derivatization of nd- and f-MWCNTs were assessed by TGA, by heating about 10 mg of each sample of at 5 °C min^{-1} up to 600 °C.

2.5. Columns setup

MWCNTs were packed in glass columns (3 mm I.D., 90 cm length, Tecnovetro, Monza, Italy), previously silanized with hexamethyldisilazane in toluene (10%, v/v), for 12 h [35]. Columns were rinsed with dry toluene and anhydrous methanol and heated at 80 °C for 1 h. Silylated columns were filled with 4 g of adsorbent materials – previously grounded and sieved at 20–140 mesh – under 70 kPa vacuum and mechanical vibration; the columns extremities were sealed with silanized quartz wool and settled overnight at 300 °C (nd-MWCNTs) and 140 °C (f-MWCNTs) under 20 $cm^3\,min^{-1}$ nitrogen flow rate. Stabilized columns pressure was around 220 kPa. Notice that this step is crucial for reproducible sorbent packing and elimination of volatile impurities eventually adsorbed on the stationary phases.

2.6. Computational study

The retention behaviour of a set of selected alcohols was correlated with their physico–chemical properties through a computational study. Quantum chemical calculations were carried out by the hybrid functional B3LYP using the 6-311G(d,p) basis set. Hybrid functionals incorporate a portion of exact exchange energy

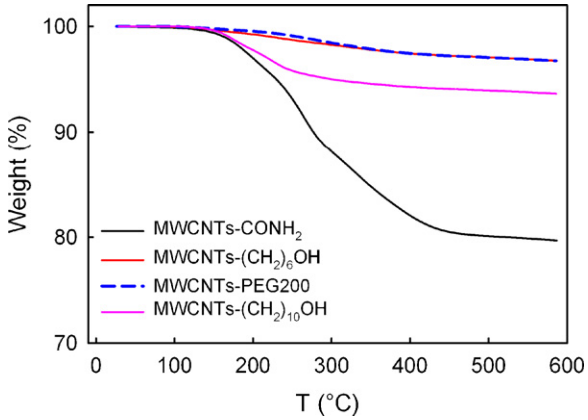

Fig. 1. TGA plots for the investigated f-MWCNTs: MWCNTs-CONH$_2$ (black); MWCNTs-(CH$_2$)$_6$OH (red); MWCNTs(CH$_2$)$_{10}$OH (pink); MWCNTs-PEG200 (blue and dotted). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

from Hartree–Fock theory to the exchange pure density functional theory (DFT) energy [36]. In the case of B3LYP, the exchange energy by Becke's functional is mixed with the energy from Hartree–Fock theory by means of three parameters, while the correlation energy derives from the Lee–Yang–Parr functional [37]. B3LYP is one of the most used functionals and this is due to the superior capability of hybrid functionals to simulate many molecular properties, including bond lengths and vibration frequencies. The basis set adopted is of triple-Z quality and makes use of polarization functions also on light atoms. Ground state optimizations were conducted in the gas phase by using the Gaussian 03 package [38]. Frequencies were evaluated at the same level of theory, so that the structures reported were certified as minima. The most stable conformation of each molecule was considered. All the bond lengths are automatically given by the software at the end of the optimization procedure. The same is true for the dipole moment of the molecule, which is also expressed in terms of its component with respect to the cartesian axes. Finally, the volume of the molecule has been evaluated by means of the "volume" keyword, as demanded by the above mentioned software. The parameters (in terms of *Z*-matrix) of the structures optimized at the B3LYP/6-311G(d,p) level are available in Table S2.

3. Results and discussion

3.1. Purification and functionalization

The HCl treatment allowed the elimination from p-MWCNTs of high amounts of metal catalysts residue coming from CNTs synthesis. Purification efficacy has been substantially confirmed by INAA (see Table S3). In particular, refluxing p-MWCNTs with HCl led to a better efficiency in metals removal with respect to sonication with HCl [24].

The functionalization degree (*FD*), expressed in terms of moles of derivative unit *per* gram of f-MWCNTs, was determined by TGA scans. Fig. 1 reports the thermograms of all the investigated f-MWCNTs. For each sample, weight losses in the temperature range between 100 and 500 °C are observed, which may be reasonably related to the decomposition of the anchoring units. In the case of purified nd-MWCNTs, weight losses lower than 1% are generally detected up to 600 °C [24]. Different derivatization levels are observed depending on the functionalizing groups. In the particular case of the MWCNTs-CONH$_2$, a very high *FD* (4.5 mmol g^{-1}) is obtained. Despite this, from the TGA plots, *FD*s of 0.30, 0.39 and 0.16 mmol g^{-1} are determined for MWCNTs-COO(CH$_2$)$_6$OH, MWCNTs-COO(CH$_2$)$_{10}$OH and MWCNTs-PEG, respectively.

3.2. Gas chromatographic separations

The chromatographic results obtained on the various sorbents tested are reported and commented in the following. C$_1$–C$_4$ alcohols (methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, 2-butanol) were chosen as chemical probes for the investigation on the retention behaviour. GC thermal conditions were optimized for each stationary phase to gain the best resolution and reasonable retention times for all the considered compounds. Alcohols mixtures were prepared in aqueous solution (1%, v/v) and the injected volume was fixed at 3 μL. Since alcohols have been usually separated on polar phases, we introduced polar functional groups on pristine MWCNTs, with the aim to establish different interactions (polar, π stacking, adsorption) between the analytes and the packing surface.

Good results were achieved with the MWCNTs-CONH$_2$ column, which allowed a good separation for the six analytes, though a partial overlapping was observed for ethanol and 2-propanol as well as for 1-propanol and 2-butanol, as depicted in Fig. 2a. Notice that symmetrical and sharp peaks were obtained also for the highly retained compounds, and this is really remarkable considering that peak tailing is more favored on packed than on capillary columns.

On MWCNTs-PEG stationary phase, linear and branched alcohols were discerned, but secondary C$_n$ co-eluted with primary C$_{n-1}$ probes. This behaviour was observed independently of the oven temperature, in isothermal conditions and with a temperature gradient program. A typical chromatogram obtained on this column is shown in Fig. 2b.

Completely different performance was exhibited by MWCNTs-COO(CH$_2$)$_6$OH and MWCNTs-COO(CH$_2$)$_{10}$OH (see Fig. 3): despite separation occurred, chromatograms showed broaden peaks, thus evidencing a stronger sorbate–sorbent interaction, probably due to the presence of the longer aliphatic chains on the MWCNTs surface. On these stationary phases, alcohols isomers separation was not possible.

Despite peak tailing, a quite good resolution was also exhibited by nd-MWCNTs (Fig. 2c) for the six compounds considered, but, as below described, performances are not satisfactory if more complexes mixtures are analyzed. As it is evident from the above, MWCNTs-CONH$_2$ gave the best performance in terms of peak shape, thus indicating faster mass transfer properties, minimizing the potential source of band broadening [23].

In view of the experimental results just reported, and as expected, the separation process is strictly dependent on different types of interaction taking place between analytes and functional groups inserted on CNTs skeleton. In this context, the affinity of these compounds towards the stationary phase can be thought to be modulated by the combination of two different contributions [39]: a *specific* interaction (acid–base or donor–acceptor) between the alcoholic functionality and the polar groups on the packing, and a *dispersive* interaction – principally due to Van der Waals forces – among the lipophilic portion of the analytes and the apolar structure of CNTs, including the aliphatic chains chemically introduced. The combination of these contributions played a predominant role in the separation efficiency, thus highlighting the role of derivatization in the chromatographic performance.

nd-MWCNTs and MWCNTs-CONH$_2$ were further tested for the simultaneous determination of methanol and by-products deriving from alcoholic fermentation, that can be found in wine and distillates [40–43]: 1-propanol, 1-butanol, 2-butanol, 2-methyl-1-propanol, 3-methyl-1-butanol, ethyl acetate and ethyl lactate (see Section 5).

The nd-MWCNTs allowed good resolution only for some compounds (see Fig. 4a), as ethyl acetate, 2-methyl-1-propanol and 2-butanol co-eluted. Broaden peaks were observed for the most adsorbed analytes, even with a gradient up to 280 °C. Contrariwise,

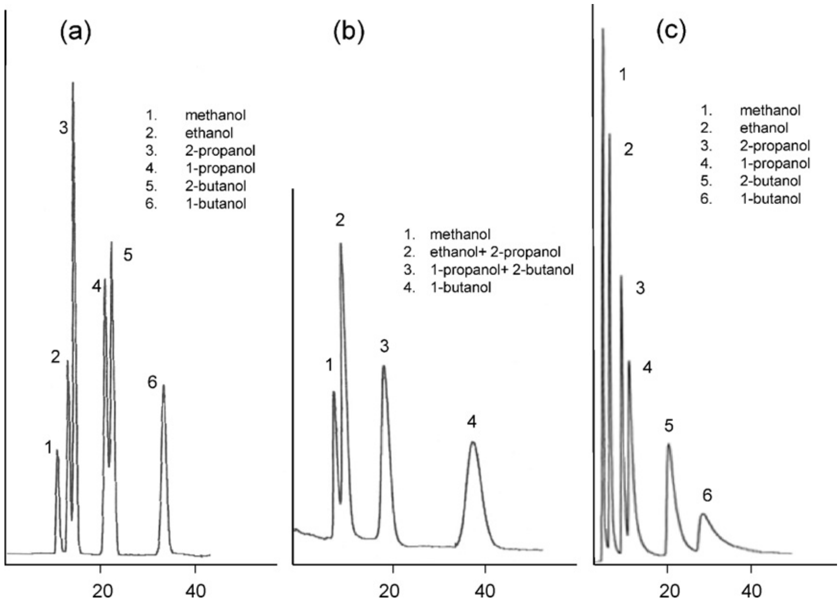

Fig. 2. Chromatograms of a standard mixture of alcohols (1%, v/v) on the different stationary phases: (a) MWCNTs-$CONH_2$: from 80 to 115 °C, 1 °C min^{-1}; (b) MWCNTs-PEG: 90 °C; (c) nd-MWCNTs: 145 °C for 17 min, gradient to 155 °C, 3 °C min^{-1}. Abscissa: retention time [min]; ordinate: FID response [area].

MWCNTs-$CONH_2$ proved to be capable of separating all these compounds at relatively low temperatures, with good chromatographic profile (Fig. 4b). This suggests an optimal physico–chemical affinity between sorbates and sorbent [19]. Separation at low temperatures is essential for these stationary phases, the MWCNTs-$CONH_2$ one in particular, to prevent any loss of functionalization, as shown in Fig. 1.

Functionalization was proven therefore to be valuable, indicating in CNTs highly selective sorbents. At this regard, chemical modification helps to prevent tight bundles of CNTs from forming, almost completely separating CNTs from each other [44], thus allowing to better exploit their excellent properties.

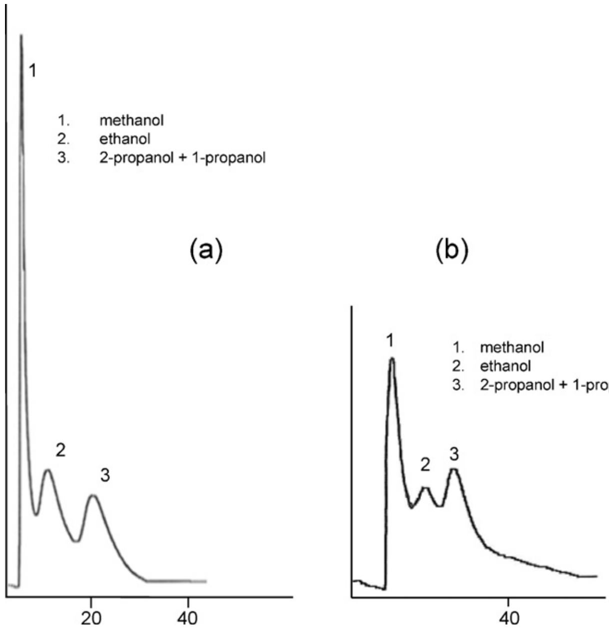

Fig. 3. Chromatograms of a standard mixture of alcohols (1%, v/v) on (a) MWCNTs-$COO(CH_2)_6OH$: 130 °C; (b) MWCNTs-$COO(CH_2)_{10}OH$: 140 °C. Abscissa: retention time [min]; ordinate: FID response [area].

3.3. Chromatographic parameters

The evaluation of the investigated stationary phases as separation media was done by calculating the characteristic parameters of chromatographic columns: resolution (*Rs*), selectivity factor (α), capacity factor (K') and the number of theoretical plates (N). All the experiments were carried out on each packing material by injecting 3 μL of a 1-propanol–methanol mixture (5%, v/v). The temperatures of the oven, injector and detector were 120 (isothermal program), 150 and 250 °C, respectively.

Both the resolution (*Rs*) and the selectivity factor (α) were calculated by considering methanol and 1-propanol, whereas N and K' were obtained from 1-propanol. In all cases, methane was used as dead time marker. As can be appreciated from Table 1, the best chromatographic profiles of the selected analytes were obtained with MWCNTs-$CONH_2$ as stationary phase.

4. Computational study

With the aim to better understand and elucidate the mechanism at the basis of the separation process, nd-MWCNTs and MWCNTs-$CONH_2$ stationary phases were tested for the analysis of various alcohols, differing in molecular weight, polarity and degree of branching.

Injections (3 μL, 1%, v/v) were performed in isothermal conditions, at 95 and 150 °C, for functionalized and nd-MWCNTs, respectively. In reason of the different capacity factors of the two columns, the temperatures above reported are appropriate to get reasonable retention times for the highly retained analytes. A detailed summary of retention times and physico–chemical properties of the chemical probes here tested is available in Table S4.

A computational study has been carried out and, among the parameters obtained from the output of the simulation, volume and dipole were selected for the correlation with retention times. We chose to only focus on these two variables to simplify as much as possible the relationship between retention times and alcohols characteristics, basing on the statistical evaluation further described. Fig. 5 clearly evidences a good correlation between the product of volume *per* dipole and

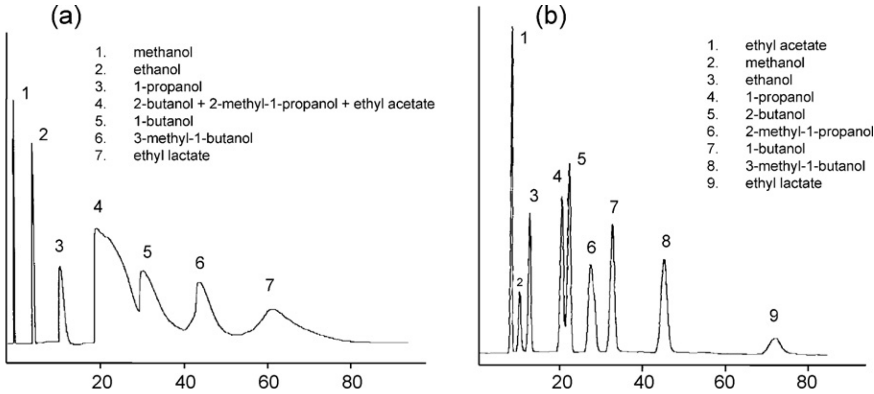

Fig. 4. Chromatograms of a standard mixture of alcohols and esters (1%, v/v) on (a) nd-MWCNTs: 145 °C for 17 min, gradient to 280 °C, 3 °C min^{-1}; (b) MWCNTs-CONH$_2$: from 80 to 115 °C, 1 °C min^{-1}. Abscissa: retention time [min]; ordinate: FID response [area].

Table 1
Chromatographic parameters calculated for each MWCNTs stationary phase. In parentheses the standard deviations (n = 3).

Parameter	nd-MWCNTs	MWCNTs-CONH$_2$	MWCNTs-PEG	MWCNTs-COO(CH$_2$)$_6$OH	MWCNTs-COO(CH$_2$)$_{10}$OH
Rs	1.65 (3)	1.66 (1)	0.87 (6)	1.16 (8)	1.62 (9)
α	14 (1)	2.08 (1)	1.96 (1)	13.2 (5)	14.6 (9)
K'	9.4 (3)	6.0 (1)	13.3 (1)	8.2 (3)	7.2 (1)
N	22.6 (9)	153 (1)	63.1 (8)	15 (1)	24 (3)

retention times. This relationship is described by the exponential model $y = a\,e^{bx}$, linearizable in $\ln y = \ln a + bx$. The equations obtained through linearization for MWCNTs-CONH$_2$ and non-derivatized CNTs are: $\ln y = 0.7(3) + 0.021(2)x$, r^2 0.83, F 67.9, and $\ln y = -1.4(5) + 0.035(4)x$, r^2 0.85, F 77.2. The hypothesis that the correlation between independent (product volume per dipole, x) and dependent (retention time, y) variables is casual can be unquestionably discarded on the basis of the F test. Indeed, the F statistics obtained by the output of OLLSR (ordinary linear lower squares regression) is much greater than the F critic value (α 0.05, $\nu_1 = 1$, $\nu_2 = 14$), equal to 4.6. These findings are in good agreement with the studies based on computational models (i.e. configurational-bias Monte Carlo, CBMC) for the GC separation of light alkanes, reporting that the higher retention times of linear compounds with respect to the related isomers indicate that slender molecules have stronger interactions with the stationary phase, due to the higher packing efficiency [45,46]. A further role of molecular sieve in the process may exclude large compounds from the interstices of the CNTs bundles or from the space between the walls of adjacent MWCNTs [19,46]. This is well substantiated by the higher retention time observed for 1-butanol in comparison with 2-methyl-2-propanol (see Supplementary Table S4), attributable to the difference in steric hindrance. However, in the case of alcohols, separation is not exclusively dependent on the degree of branching, but other factors, such as dipole, are involved in. This may explain, for example, why 2-butanol eluted faster than 1-butanol. The value of dipole could be also determinant to justify the retention of C$_6$ isomers, whose elution order is different on the two stationary phases considered, hence underling the influence of functionalization (see Supplementary data).

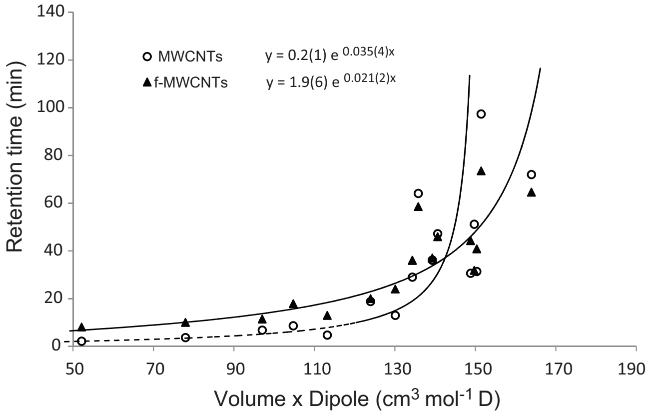

Fig. 5. Variation in alcohols retention time with volume *per* dipole for nd-MWCNTs (dotted line) and MWCNTs-CONH$_2$ (continuous line) stationary phases. In parentheses coefficients uncertainty.

A statistical evaluation of the available – though limited – dataset was performed through a factorial discriminant analysis (FDA) by using the program XLSTAT (Version 2010.3.01, Addinsoft^{TM}) to investigate whether the retention behaviour was dependent on the variables taken into account.

Ellipticity of alcohols, a parameter ranging between 0 and 1 as defined below, stretching frequency and length of the –OH bond, were added to the initially considered volume and dipole. Ellipticity was determined by considering the Cartesian components of dipole: assuming the relation $D_{\max} > D_{\text{middle}} > D_{\min}$ among the dipole components (D_i with i either x, y or z), ellipticity was defined as $(D_{\text{middle}} - D_{\min})/(D_{\max} - D_{\min})$. Thus, a higher value is indicative of a sphere-like shape, whereas a lower one accounts for a non-homogeneous distribution in the space.

A fairly good differentiation was possible among primary, secondary and tertiary and between linear and branched alcohols. By considering further variables, specifically molecular weight, number of carbon atoms and boiling points, a more accurate and reliable differentiation between linear and branched alcohols was achieved (see Supplementary data). However, as the three new parameters introduced were well correlated each other and with volume and since stretching frequency/length of the –OH bond and ellipticity turned out to have a minor weight, volume and dipole were chosen in the simplified model, as above reported.

5. Application to real samples

Since the MWCNTs-CONH$_2$ stationary phase was found to be better performing in comparison with the other columns and with the foresight to work at temperatures not greater than 150 °C, it

Table 2
Analytical results obtained on the MWCNTs-CONH$_2$ column for the compounds investigated. In parentheses errors on slope and intercepts, calculated by ordinary linear lower squares regression (OLLSR).

Analyte	Slope	Intercept	r^2	Reproducibility ($n = 10$)		Peak area precision ($n = 3$)
				RSD%	Retention time (min) α 0.05	RSD%
Methanol	302219 (886)	−923 (28)	0.999	1.5	10.5 ± 0.1	4.8
Ethanol	452320 (7914)	−781 (218)	0.999	1.0	12.81 ± 0.09	5.4
1-Propanol	865541 (29429)	−627 (809)	0.998	1.0	20.7 ± 0.2	5.1
2-Propanol	601592 (8106)	−1203 (223)	0.999	1.4	14.4 ± 0.1	4.3
1-Butanol	2023400 (254265)	15626 (8041)	0.984	1.5	33.2 ± 0.4	5.2
2-Butanol	972365 (26329)	−43 (724)	0.999	1.0	22.5 ± 0.2	5.2
2-Methyl-1-propanol	640735 (29768)	−7705 (3414)	0.996	1.0	27.2 ± 0.2	8.0
3-Methyl-1-butanol	3456342 (103863)	9334 (3284)	0.999	1.2	46.4 ± 0.4	4.3
2-Methyl-2-butanol	941582 (42349)	−14667 (4870)	0.996	1.0	24.7 ± 0.2	8.0
Ethyl acetate	418090 (21795)	−826 (599)	0.995	1.1	8.11 ± 0.06	7.5
Ethyl lactate	3126948 (96921)	10499 (3026)	0.999	0.9	75.5 ± 0.5	2.8

was chosen for the analysis of the famous Italian *Grappa*—a spirit obtained by distillation of grape marc, sometimes after addition of wine remains. Alcoholic fermentation promoted by yeast species leads to the formation of sugars' sub-products, contributing to wine's flavor and organoleptic properties, detectable in alcoholic beverages at the milligrams *per* liter level [40–43]. In addition to methanol, 1-propanol, 1-butanol, 2-butanol, 3-methyl-1-propanol, 3-methyl-1-butanol, ethyl acetate and ethyl lactate were investigated.

More complex matrices, i.e. wines and other non-distilled products, cannot directly be analyzed on this column, owing to their content of higher boiling points constituents that would require higher cleaning temperature of the column. The analytical parameters were calculated for all the analytes considered. Linearity was determined by OLLSR: five points calibration curves were generated ($n = 3$) by injection (4 μL) of standard solutions at concentrations in the range 0.005–0.5% (v/v), depending on the sensitivity of the analyte. Reproducibility in retention time and peak area precision were measured as relative standard deviation (RSD%). Details are gathered in Table 2.

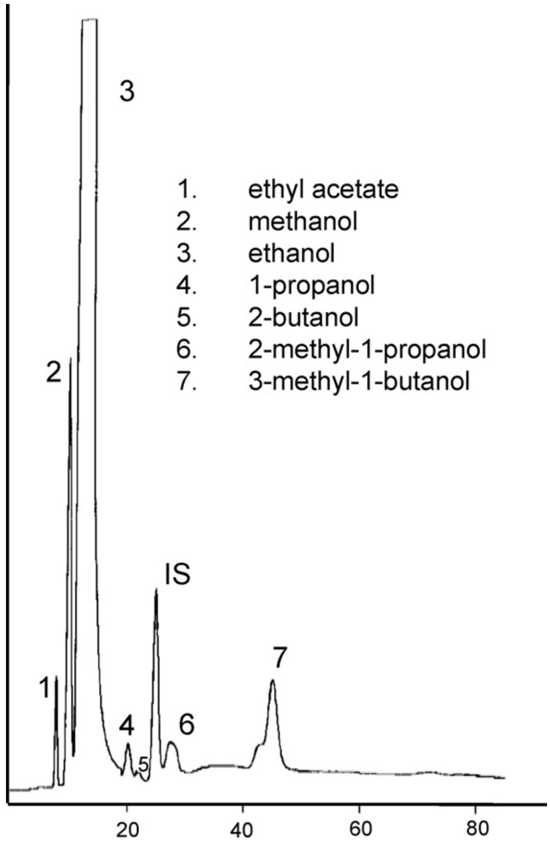

Fig. 6. Typical chromatogram obtained on the MWCNTs-CONH$_2$ column by analysis of *Grappa*. Abscissa: retention time [min]; ordinate: FID response [area].

As can be seen in Table 2, good linearity was observed for all the analytes. Reproducibility in retention times (RSD ≤ 1.5%) was comparable to that of commercial GC columns and precision in peak area was as much satisfying: RSD ≤ 8% and ≤20% for 0.5 and 0.01% (v/v) concentration levels, respectively.

The column–column within-lab reproducibility was evaluated by calculating the capacity factor (K') for 1-propanol on three different MWCNTs-CONH$_2$ columns set up under the same conditions. A RSD of 2.8% was observed, thus indicating that the preparation procedure is reliable and reproducible. Moreover, working at temperature not greater than 150 °C, the functionalized stationary phase did not undergo damages during an 8-month analysis period, evidencing that it can be used for a prolonged time with optimal performance.

Two different Italian spirits were thereafter analyzed: the first distillate (A) is an aromatic G*rappa*, *viz.*, it shows the characteristic aroma of vine, whereas the second one (B) is an aged distillate, *viz.*, the wood barrel it has been stored in – for a minimum of 12 months – contributes to its final aromatic properties. Nominal alcoholic grades were 40 and 42% (v/v), respectively. A typical chromatogram is reported in Fig. 6.

Methanol, 2-methyl-1-propanol and 3-methyl-1-butanol spikes gave satisfactory recovery (80–114%). Quantification was performed by using 2-methyl-2-butanol as I.S., chosen for several reasons: it is not an alcoholic fermentation sub-product, its retention time was halfway among all the compounds to be determined and its peak was perfectly resolved. Blanks were prepared in ultrapure water containing 42% (v/v) ethanol.

Table 3
Mean concentrations of the analytes detected in the two *Grappa* samples ($n = 4$, α 0.05).

Analyte	Grappa A (mL/100 mL ethanol)	Grappa B (mL/100 mL ethanol)
Methanol	0.6 ± 0.1	0.75 ± 0.09
1-Propanol	0.049 ± 0.007	0.03 ± 0.01
2-Butanol	0.02 ± 0.01	0.007 ± 0.001
1-Butanol	n.d.	n.d.
2-Methyl-1-propanol	0.11 ± 0.02	0.070 ± 0.003
3-Methyl-1-butanol	0.17 ± 0.03	0.12 ± 0.02
Ethyl acetate	0.009 ± 0.002	0.03 ± 0.01
Ethyl lactate	n.d.	n.d.

n.d.: not detectable.

The investigation, whose results are shown in Table 3, revealed the presence of methanol and typical compounds constituting the “fermentation bouquet”.

6. Conclusions

In the present study we demonstrated how the chromatographic behaviour of MWCNTs is heavily influenced by the nature of derivatization. The introduction of various functionalities on the carbon skeleton proved to be a powerful way for modulating the interaction between solute and stationary phase to obtain optimal resolution. In this context, in spite of relatively high retention times, the MWCNTs-$CONH_2$ stationary phase gave the best resolution and excellent peak shape, allowing the separation of alcohol isomers and esters, or else a mixture of alcoholic fermentation subproducts. At this regard, the analysis of commercial Italian *Grappa* has been performed with satisfying results. Likewise, the columns here proposed are remarkable for simplicity of preparation and low cost (5$ *per* gram, price of MWCNTs in May 2010), in comparison with commercial stationary phases (some hundreds $, price in May 2010 for capillary or wide bore GC columns): they could become really attractive stationary phases for a wide range of applications.

Albeit usage of f-MWCNTs as GC stationary phases presents some crucial aspects, such as pH sensitivity and possibility of removing functional groups at high temperature (>200 °C), the employment of conveniently f-MWCNTs as separation media for low molecular weight polar compounds was here found to be advantageous in terms of chromatographic resolution. Derivatized MWCNTs therefore showed to have great potentialities as high-performance separation media for practical applications.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2010.09.024.

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