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Preparation and Evaluation of Carbon Coated Alumina as a High Surface Area Packing Material for High Performance Liquid Chromatography

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Abstract

The retention of polar compounds, the separation of structural isomers and thermal stability make carbonaceous materials very attractive stationary phases for Liquid Chromatography (LC). Carbon clad zirconia (C/ZrO₂), one of the most interesting, exhibits unparalleled chemical and thermal stability, but its characteristically low surface area (20 – 30 m²/g) limits broader application as a second dimension separation in two-dimensional liquid chromatography (2DLC) where high retentivity and therefore high stationary phase surface area are required. In this work, we used a high surface area commercial HPLC alumina (153 m²/g) as a support material to develop a carbon phase by chemical vapor deposition (CVD) at elevated temperature using hexane vapor as the carbon source. The loading of carbon was varied by changing the CVD time and temperature, and the carbon coated alumina (C/Al₂O₃) was characterized both physically and chromatographically. The resulting carbon phases behaved as a reversed phase similar to C/ZrO₂. At all carbon loadings, C/Al₂O₃ closely matched the unique chromatographic selectivity of carbon phases, and as expected the retentivity was increased over C/ZrO₂. Excess carbon – the amount equivalent to 5 monolayers - was required to fully cover the oxide support in C/Al₂O₃, but this was less excess than needed with C/ZrO₂. Plate counts were 60,000 – 76,000/meter for 5 μm particles. Spectroscopic studies (XPS and FT-IR) were also conducted; they showed that the two materials were chemically very similar.

1. Introduction

Carbonaceous materials are very attractive for stationary phases in liquid chromatography (LC) because of their chemical and thermal stability and chromatographically unique selectivity [1–8]. Even though carbon phases are reversed phases, they also retain certain polar solutes that show little retention on conventional silica based reversed phases. Their uniqueness is also exhibited in their ability to retain very polarizable anions [9] and in their exceptional selectivity for various classes of stereo isomers [4,6]. For instance, nitrobenzene is significantly more retained on carbon phases than is toluene; this is never observed on conventional alkyl bonded phases. The ability to separate structural isomers or closely

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related solutes is also a unique property of carbon phases. Jackson et al. showed that certain aromatic stereoisomers could be separated on carbon but could not be separated on an octadecyl silica (ODS) column [4]. All these characteristics have broadened the utility of the carbon phases to separation of biological samples including drug metabolites [10,11] and to carbohydrate analysis [12].

In 1990, Carr and coworkers successfully developed carbon clad zirconia as a HPLC support [13]. This study was motivated by the unparalleled chemical, mechanical and thermal stability of zirconia compared to silica under extreme conditions (i.e. pH 1–14 at >100 °C) [14]. Carbon was deposited on the surface of zirconia (C/ZrO₂) in a chemical vapor deposition (CVD) process by flowing organic vapor over a bed of zirconia at the elevated temperature at low pressure (< 60 torr). It was shown that the carbon load depends on the temperature, the reaction time and the carbon source. The physical and chemical properties of C/ZrO₂ were extensively explored [3,4,6–8,15], showing that C/ZrO₂ retains the unique selectivity of carbon materials as described above but with enhanced mechanical stability. Jackson et al. showed the similarity in chromatographic selectivity between C/ZrO₂ and porous graphitic carbon (Hypercarb), a commercial carbon phase [3,15]. These properties make C/ZrO₂ an excellent candidate for use as the second dimension column for fast 2DLC, which has tremendous potential in separation for complex bioanalytes [16].

However, the second dimension column in 2DLC is optimally highly retentive so as to provide a high degree of sample focusing. In 2DLC separations, an analyte that elutes from the first dimension column is sequentially injected onto a second column (termed the second dimension). It is very important that the separation in the second dimension be fast so that the analyte peak on the first dimension can be sampled more than once; ideally more than three or four times. Furthermore, the total analysis time is limited by the run time of the second dimension separation. Thus, there is great need to improve the speed of the separation in the second dimension; for instance, Carr et al. recently developed a fast 2DLC system that uses higher temperatures (> 100 °C) to accelerate the second dimension. An ideal column for the second dimension must have 1) high chemical and packed bed stability, 2) orthogonal selectivity compared to the first dimension column and 3) have high retentivity for the analyte. Although C/ZrO₂ meets the first two requirements, the intrinsically low surface area of commercial porous zirconia (20 – 30 m²/g) limits its retentivity. High retentivity is critical because analytes that are injected onto the second column from the first column are typically in a strong solvent environment. Furthermore, a large volume of sample is injected on a relatively low volume second dimension column (33 mm by 2.1 mm). This situation can lead to distorted peak shapes and thus, poor peak capacity and low analytical sensitivity if the second column fails to focus the analyte at the inlet of the column. Use of a stationary phase that is strongly hydrophobic helps to ameliorate this problem. Jackson et al. showed that carbon phases exhibit higher intrinsic hydrophobicity than ODS [15]. Thus, the use of a carbon phase is a partial solution to this problem due to its stronger retention of weakly retained analytes. An increase in phase ratio should also help as it will further increase retention factors.

Theoretically, the retentivity in adsorption chromatography is proportional to the total surface area of the stationary phase [17]. Since the retention mechanism of carbon phases is based on adsorption on the rigid carbon surface, we believe that use of a higher surface area substrate on which to deposit the carbon ought to enhance retentivity and thus improve the use of carbon based phases as the second dimension in 2DLC.

Many attempts have been made over the past three decades to prepare chromatographically useful carbon packing materials using a variety of substrates [18–20], but none enjoy the full combination of high surface area, mechanical strength and chemical homogeneity. Guiochon

et al. modified graphitized carbon black by depositing a thin layer of pyrocarbon to improve the mechanical strength of carbon black and use it for HPLC [21]. Knox et al. developed a new method to synthesize a porous graphitic carbon (PGC) [22]; they impregnated a phenol-formaldehyde polymer in the pores of a silica “template”, subsequently pyrolyzed the polymer to produce a carbonaceous material, then, removed the silica template by alkali treatment, heating the residual carbon skeleton at 2500 °C to produce a graphitic carbon. PGC is the most successful carbon packing material for HPLC. Although the PGC support has been widely used for various applications, it has some drawbacks including a costly manufacturing process and lesser mechanical strength than C/ZrO₂ [8,23]. Silica has also been used as a template to develop a graphitized carbon monolithic column [24]. Lebeda et al. developed a different method in which a metal catalyst was impregnated on silica to catalyze the chemical decomposition of a hydrocarbon [25,26]. Several other studies have shown that carbon can be deposited directly on silica either by chemical vapor deposition (CVD) without catalysts by using certain species as the carbon source [27,28] or by pyrolysis of pre-adsorbed oligomers [23]. Unfortunately, all the resulting materials suffered from low available carbon surface area, heterogeneous surface chemistries, and significantly tailed peaks and poor efficiencies in general.

In this study, we have developed a high surface area carbon phase on porous HPLC grade alumina (Al₂O₃) by depositing carbon using a CVD process related to that used to develop C/ZrO₂. The reaction took place at elevated temperatures (≥ 700 °C) and at atmospheric pressure. We subsequently evaluated its physical and chromatographic properties. We chose alumina for this work because it is a well known active catalyst for cracking hydrocarbons [29] and has comparable chemical stability to that of zirconia [30]. Because this material has a higher surface area compared to zirconia, we expected that the resulting material would have a higher retentivity while maintaining similar selectivity. Thus, in the work presented here, the C/ZrO₂ is used as a bench mark to compare retentivity and selectivity. We also examined its potential use for the 2DLC by using indolic metabolites which were of interest to us in our 2DLC studies.

2. Experimental

2.1. Chemicals

HPLC grade hexanes from Sigma-Aldrich (St. Louis, MO, USA) were used as the CVD carbon source. All chemicals, reagent grade or better, used for the chromatographic study were obtained from Sigma-Aldrich (St. Louis, MO, USA). Di(phenethyl)amide isomers were provided by Prof. T. R. Hoyer at the University of Minnesota. Standards of amphetamines drugs (1 mg/ml in methanol, were from Cerilliant (Round Rock, TX, USA)). Four indolic metabolites standards were prepared as described by Stoll [16]. They include Indole-5-hydroxy-typtamine (IHT), Indole-3-acetyl- ϵ -L-lysine (IAL), Indole-3-ethanol (IE) and Indole-3-butyric acid (IBA). HPLC eluents comprise HPLC grade acetonitrile from Burdick and Jackson (Muskegon, MI, USA) and HPLC grade water that was prepared in-house from a Barnstead Nanopure II deionizing system (Dubuque, IA, USA). This water was boiled to remove carbon dioxide and filtered through a 0.45 μ m nylon filtration apparatus (Lida Manufacturing Inc., Kenosha, WI, USA) prior to use.

2.2. Carbon phase preparation

Aluspher 5 μ m porous alumina (Al₂O₃) was a gift of Merck KGaA, (Darmstadt, Germany). 1 g of Al₂O₃ was placed in a baffled quartz heated reactor (Model HTR 11/75 Carbolite, Aston Lane, Hope, England) that oscillates between 0 and 180 ° to mix Al₂O₃ particles during the CVD process (see Fig. 1). Two gas controllers taken from a HP 5890 Gas

Chromatography (Agilent Technologies, Wilmington, DE, USA) were used to maintain gas flow at 200 ml/min for both directions.

Previous work on C/ZrO₂ in this laboratory showed that saturated hydrocarbons provide carbon phases with better efficiency and symmetrical peak shapes compared to unsaturated hydrocarbons [7]. Thus, we used hexane vapor as the carbon source in this study. The whole system is flushed with high purity nitrogen (99.99% purity) before elevating the temperature. Flowing hexane vapor is introduced by bubbling high purity nitrogen through a reservoir of the hexanes. The vapor is then passed over Al₂O₃ at 700 or 800 °C for 1.5 to 6 hours to deposit carbon. After deposition the oven temperature is allowed to drop slowly to room temperature while maintaining the makeup nitrogen flow to remove gaseous byproducts produced during the CVD process. Carbon coated alumina was sent for analysis of its carbon content (Atlantic Microlabs, Norcross, GA, USA).

2.3. Column packing

The columns were packed by procedures very similar to what have been reported elsewhere [16]. C/ZrO₂ (3 μm, carbon loading = 8 %), obtained from ZirChrom Separations Inc. (Anoka, MN, USA), was packed by the same procedure as described above.

2.4. Chromatographic studies

All chromatographic data were collected by a HP 1090 LC system controlled by Chemstation software version A.10.01 (Agilent Technologies, Wilmington, DE, USA). The instrument is equipped with an autosampler, thermostating column compartment and photodiode array UV detector (DAD). All solutes were detected at 210 nm unless otherwise noted. Column dead times were measured from the retention time of acetone. All retention data are an average of triplicate runs.

2.5. Conductivity measurement

The electrical conductivity of the pyrolyzed carbon on alumina was measured using the circuit shown in Fig. 2. A hole was drilled through both sides of a plastic tube to create a cavity. The particles were placed in the cavity and screws were used to densely pack the materials for maximum interparticle contact. The reference resistance (R_s) was adjusted until the reading voltage (V_{AB}) dropped to half of the reference voltage (V_s) reading. Then, the sample resistance (R_x) was calculated with Eq. (1).

$$V_{AB} = \frac{V_s \times R_x}{R_x + R_s} \quad (1)$$

Voltages were measured by an EMF 16 potentiometer (Lawson Labs Inc., Malvern, PA) controlled with EMF Suite 1.02 software (Fluorous Innovations, Arden Hills, MN) at room temperature (25 °C). The circuit was tested using the resistance of bare Al₂O₃ and graphite (≥ 99.0 % C, ≤ 20 μm) obtained from Sigma-Aldrich (St. Louis, MO, USA). The resistance of the bare alumina was greater than the maximum measurable resistance (10¹⁰ Ω).

2.6. N₂ adsorption

The pore structure of the alumina before and after carbon deposition was characterized by nitrogen sorption performed on a Micromeritics ASAP 2000 sorptometer (Micromeritics, Norcross, GA). The specific surface area of the particles was computed using the BET method [31]. Approximate pore size distributions were computed using the BJH method [32].

2.7. X-ray photoelectron spectroscopy (XPS) and transmission FT-IR spectroscopy

The samples (24 % C/Al₂O₃ and C/ZrO₂) were sent for XPS analysis (Characterization Facility of the University of Minnesota, MN, USA). The XPS measurements were performed on an SSX-100 system (Surface Science Instruments) equipped with a monochromated Al K_α X-ray source, a hemispherical sector analyzer (HSA) and a resistive anode detector. FT-IR spectra in the mid-IR range (4000 – 400 cm⁻¹) were obtained on a Nicolet Magna-IR 760 spectrometer using potassium bromide pellets of the samples (0.1 % w/w) including 24% C/Al₂O₃ and C/ZrO₂ in a nitrogen atmosphere. The same weight percent of decanophenone in the pellet was used as a control.

3. Results and discussion

3.1. Reproducibility of carbon deposition on Al₂O₃

Table 1 compares the batch-to-batch reproducibility and chromatographic properties of several preparations of carbon coated alumina (C/Al₂O₃). Three replicate coatings gave an average of 23.3 % of carbon load with 6 % standard deviation. We evaluated the resulting materials chromatographically by measuring the efficiency and retention of nitrohexane, toluene and nitrobenzene. As is shown in the table, this carbon stationary phase gave reproducible efficiency (12 % RSD) and retention (6 – 10 % RSD). Fig. 3 shows that we can obtain reasonably symmetric peak shapes of nitroalkanes on 24 % C/Al₂O₃. Nitroalkanes are used to evaluate the column because they provide maximum efficiency and the least peak tailing. Given the chromatographic data and the reasonably reproducible coating process, it is clear that these materials are potentially useful as packing materials for HPLC.

3.2. Effect of CVD conditions on the property of the material

3.2.1. Physical and chemical characteristics

Surface coverage: Carbon load increased with deposition time and reactor temperature. We obtained 40 % (w/w) carbon at 800 °C in 6 h as compared to 24 % (w/w) at 700 °C for the same time. For chromatographic application it is necessary to fully cover the Al₂O₃. The amount of carbon needed to fully cover the Al₂O₃ was ascertained by using benzoic acid as a probe as per the method of Trammell [33]. Effective blockage of benzoic acid-binding sites on the oxide is critical as such sites strongly interact with any Lewis base analytes such as carboxylic acids, resulting in low analyte recovery or broadening and tailing peaks. Benzoic acid did not elute after adsorption on the 6 and 14 % carbon indicating poor coverage of alumina, but it was fully eluted on 24 % and 40 % carbon (data not shown). This suggests that 24 % C/Al₂O₃ may have the optimum amount of carbon on Al₂O₃ to fully cover the oxide surface without excessively reducing surface area. This will be borne out by other techniques.

Pore size distribution: Table 2 summarizes the amount of carbon deposited under different conditions and the BET pore characteristics. Clearly, the surface area and the pore volume decrease with increasing carbon load. A carbon load of 40 % (w/w) removes about 80 % of the initial surface area and leaves only 13 % of the original pore volume. Thus, it is critical to find the optimal carbon load that maximizes retentivity before the loss of the pore area and volume cause a decrease.

On the basis of the BET data, assuming a uniform coating process and that the carbon has graphite-density, we estimate the % C that is theoretically required to form one hypothetical monolayer of carbon (see Table 2). As a result, 6 % (w/w) of carbon load obtained by 700 °C for 1.5 h is expected to provide full coverage of Al₂O₃. However, as mentioned above, 24 % carbon, which corresponds to about 5 monolayers of carbon, was required to fully block access to the Al₂O₃ substrate. This suggests that the carbon coating by our CVD

method is not in fact uniform. However, it should be noted that 8 % carbon must be loaded on ZrO_2 to fully block access to the substrate and, at its lower surface area, this is equivalent to about 11 monolayers. A carbon load of 24 % resulted in the loss of 40 % of the surface area in $\text{C}/\text{Al}_2\text{O}_3$; this is a rather reasonable loss when compared to the loss of 88 % of the surface area after 3 % carbon is loaded on zirconia [26].

To better understand how nonuniform the carbon coating is, we plotted pore area and pore volume distributions from nitrogen adsorption and desorption data in Fig. 4. The adsorption and desorption curves are considered to reflect the size distribution of pore bodies and pore throat, respectively [34]. Low carbon loads (6 %) had little effect on the pore structures as both small and large pore sizes were only slightly decreased. However, high carbon loads considerably changed both the area and volume distributions. Noticeable shifts of the distribution peaks towards smaller pore sizes are observed indicating a decrease of the average pore diameter. Uniform coating of carbon would not cause a shift in this manner. Thus, the observed shift confirms that carbon deposition on Al_2O_3 is not uniform.

Nonuniform deposition of pyrolytic carbon is not surprising; several review papers explain nonuniform coating thickness and even patchiness at early stages of coating [35,36]. However, the rather large excess carbon (equivalent of 5 hypothetical monolayers) required to fully block access of species which adsorb irreversibly on Al_2O_3 could cause pore plugging, which would be quite detrimental to the performance of a chromatographic support. Thus, we used Reeder's models [34] to test the geometry of carbon deposition. Though these models were developed to understand coatings resulting from the solution deposition of a polymer in a porous body, we believe that the models are geometrically applicable to explain whether in our process excess carbon well beyond a hypothetical monolayer is needed and yet does not block the narrowest pores. Reeder's first two models are 1) uniform thickness of layers through all pores (model 1) and 2) nonuniform thickness of layers for different size of pores (model 2), but with constant volume fraction deposition in the pores - thicker in larger pores, but with constant proportion of coating thickness to pore diameter. The differential pore distribution plots for pore volume and surface area based on the models are shown in Fig. 5. As compared in Fig. 4 – 5, the actual changes in the area and volume distributions with increasing carbon load is closer to model 2. Apparently in our samples carbon deposited nonuniformly in the pores with thicker layers in larger pores; this confirms that the excess carbon is not necessarily blocking small pores. This also helps explain why it requires excess carbon to make sure the smaller pores are adequately covered – consistent with our observation that we must use as much carbon as would be required for 5 hypothetical monolayers.

Electrical resistivity: Fig. 6 presents a comparison of the resistivity of the different carbon coated particles with C/ZrO_2 and graphite. Since the carbon is a conductor and alumina is not, we expect those materials that have a lower fraction of the alumina covered by carbon will have higher resistivity as the carbon layer will be less continuous. The resistance clearly decreased upon increasing the amount of carbon deposited. The 14 % carbon material showed higher resistivity than did 24 % carbon, but little additional change in resistance was seen above a load of 24 %. This again suggests that 24 % carbon is adequate to fully cover Al_2O_3 . Olesik et al. showed that lower resistance of carbon phases exhibits higher polarizability leading to increased retentivity [23]. As 24 % $\text{C}/\text{Al}_2\text{O}_3$ shows comparable resistivity to graphite, this material retains considerable sp^2 hybridization. It also has a lower resistance than C/ZrO_2 , which may imply a higher degree of sp^2 hybridization and thus a higher degree of polarizability of the carbon on $\text{C}/\text{Al}_2\text{O}_3$.

Spectroscopic characterization: XPS was conducted on the 24 % $\text{C}/\text{Al}_2\text{O}_3$ and C/ZrO_2 . Both samples are sufficiently conductive and no charge neutralization was applied. Due to

the thinness of the carbon coating, oxygen from both the alumina and zirconia dominated the spectra. As a result, the O 1s spectra from both samples were attributed to the metal oxides. Fig. 7 compares the C 1s spectra for the 24 % C/Al₂O₃ and C/ZrO₂; it shows rather sharp (FWHM = 2.3 eV) and strong peaks at 284.6 and 284.4 eV, respectively. On the whole the C 1s spectra predominantly have the same character as a combination of graphite and diamond like carbon [37]. The peaks are asymmetric and are comparable to the spectra reported for pyrolytic carbon [38]. The broad and tailed region of the C 1s spectra at around 286 – 289 eV could be due to minor C-O and C=O components [39]. However, overall the C 1s spectra show great similarity in the chemical environment of these carbons and an insignificant amount of oxygen incorporated in both carbon surfaces. We further ran these carbon samples by FT-IR by preparing pellets with potassium bromide. However, we could not see any peaks of carbon-oxygen functional groups whereas the same weight % of decanophenone in the pellet showed a very significant carbonyl signal.

3.2.2. Chromatographic characteristics—Carbon phases are known to be reversed phases. The nature of the surface can be examined by measuring the hydrophobic selectivity, which is the slope of log *k'* vs. the number of methylene groups (*n*_{CH₂}) for a homolog series of solutes.

$$\log k' = A + Bn_{\text{CH}_2} \quad (2)$$

Positive slopes and a linear relationship are expected for all reversed phases. The slope (B) can be used to calculate the free energy of transfer per methylene group from the mobile phase to the stationary phase ($\Delta G_{\text{CH}_2} = -2.3BRT$; R is gas constant and T is the temperature) [40]. Thus, bigger slopes indicate stronger affinity of stationary phases for a methylene group.

The log *k'* of two different homolog series was plotted against *n*_{CH₂} for C/Al₂O₃ and C/ZrO₂ (see Fig. 8). The slopes, intercepts and free energies of transfer per methylene group with their standard deviations are listed in Table 3. All the carbon phases including the lowest carbon load obviously behave as a reversed phase. A slight deviation from linearity for the alkylbenzenes, which is not observed on the conventional octadecyl bonded silica phase (ODS), is one of the unique properties of carbon phases attributed to the different retention mechanisms of carbon and ODS materials [6,15]. The intercepts and slopes show that all carbon loadings, C/Al₂O₃ have similar methylene affinity as C/ZrO₂. Retentivity for both homolog series increases with carbon load up to 14 % carbon, stays about the same at 24 %, and then decreases from 24 to 40 %.

The slopes of log *k'* vs. homolog number plots on ODS phases for different homologs are typically almost indistinguishable [8], but they are clearly different on the various C/Al₂O₃ materials and C/ZrO₂. We believe that this has more to do with the aromaticity of the alkylbenzenes as compared to the nitroalkanes as how the phenyl ring interacts with the carbon surface. However, the difference in slope between the nitroalkanes and the alkylbenzenes is significantly smaller on the C/Al₂O₃ materials than on C/ZrO₂. Since the slope of such plots is independent of the specific surface area, this strongly suggests that the chemical and/or physical nature of the carbon surfaces is different although we could hardly detect any chemical difference by XPS. For the alkylbenzenes the difference in the slopes for the three C/Al₂O₃ phases is smaller than the difference in slope of the C/Al₂O₃ relative to the C/ZrO₂.

Although there was no further increase in retentivity above 14 % carbon, we further increased the carbon load to block solute secondary interactions with Lewis acid sites on Al_2O_3 .

Comparison of the 24 % $\text{C}/\text{Al}_2\text{O}_3$ and C/ZrO_2 , both of which show full blockage of substrate, indicates that it takes fewer monolayers of carbon to fully cover Al_2O_3 (about 5 monolayers required) than ZrO_2 (about 11 required). However, the 24 % carbon phase provides much higher retentivity than does C/ZrO_2 under the same elution conditions. This improvement is not due to the higher % carbon on Al_2O_3 . The carbon load per unit surface area is higher on C/ZrO_2 ($290 \mu\text{mol}/\text{m}^2$) than that on the $\text{C}/\text{Al}_2\text{O}_3$ ($133 \mu\text{mol}/\text{m}^2$). Rather we attribute it to the difference in total surface area. We calculated the total surface area for both materials in the same size column based on the BET surface area and the density of Al_2O_3 and ZrO_2 [30]. A column packed with 24 % $\text{C}/\text{Al}_2\text{O}_3$ has 2.4 folds more surface area than a column packed with C/ZrO_2 . In fact the increase in the retentivity of the $\text{C}/\text{Al}_2\text{O}_3$ relative to C/ZrO_2 is higher than the ratio of the surface areas. This could be attributed to the higher affinity of $\text{C}/\text{Al}_2\text{O}_3$ for methylene group as indicated by the higher free energy of transfer which implies a greater hydrophobicity for $\text{C}/\text{Al}_2\text{O}_3$ as compared to C/ZrO_2 . We infer from the lower resistivity of 24 % $\text{C}/\text{Al}_2\text{O}_3$ as compared to C/ZrO_2 that the $\text{C}/\text{Al}_2\text{O}_3$ has a higher polarizability which we believe may contribute to the increased retentivity.

Since the relative retentivity depends on the type of compound used, it may be also due to different degree of surface oxidation although both metal oxide based materials are prepared by very similar methods. It is well known that surface of carbon generally contain hetero atoms like oxygen, which induce various types of surface oxides [41,42]. However, as shown by the XPS data above and very similar selectivity of these carbon (see Fig. 9), the difference in oxygen content is likely not large.

We chose a series of monosubstituted (polar and nonpolar) benzene derivatives to compare the chemical selectivity of the carbon phases. Various benzene derivatives had been used to show the unique selectivity of C/ZrO_2 compared to the octadecyl bonded silica phase (ODS) [3]. We obtained the retention factor of 18 benzene derivatives on all of the carbon phases and on the ODS under the same elution conditions and calculated the retention factor of the derivatives relative to that of benzene. This normalization eliminates the phase ratio (see Fig. 9). The derivatives are arranged in the order of increasing retention on ODS. As shown in Fig. 9, the elution order of these solutes on all carbon phases is the same but differs radically from that on ODS. This indicates a dramatic difference in the chemical selectivity of the carbon phases from that of ODS. All the $\text{C}/\text{Al}_2\text{O}_3$ phases provide very similar chemical selectivity to that of C/ZrO_2 although there are small differences among the $\text{C}/\text{Al}_2\text{O}_3$ materials and C/ZrO_2 . In particular 24 % $\text{C}/\text{Al}_2\text{O}_3$ behaves more like the C/ZrO_2 phase than do the 6 and 14 % $\text{C}/\text{Al}_2\text{O}_3$ phases. We believe this is likely due to the incomplete coverage of Al_2O_3 at the low carbon loads.

In addition, we wanted to see if $\text{C}/\text{Al}_2\text{O}_3$ matches C/ZrO_2 in superior resolving power towards structural isomers [4]. We selected three different pairs of isomers (see Table 4). ODS shows poor separation of these compounds as seen by the selectivity (α), however, the 24 % $\text{C}/\text{Al}_2\text{O}_3$ can easily separate these isomers under the same conditions, which is consistent with the C/ZrO_2 .

$\text{C}/\text{Al}_2\text{O}_3$ also shows a subtle enhancement of retentivity for polar compounds that is highly desirable for the application of the carbon phases to drug metabolites. Amphetamines, a weakly retained family of drugs on conventional reversed phases, were injected on both the 24 % $\text{C}/\text{Al}_2\text{O}_3$ and C/ZrO_2 under the same gradient condition. As shown in Fig. 10, the $\text{C}/$

Al₂O₃ provides at most about a 3-fold increase in retention although the increment of retentivity depends on the analyte.

We hypothesized that improved retentivity would help analytes to be focused at the inlet of the second dimension column in the fast 2DLC, thus alleviating the detrimental effect of strong injected solvent on peak distortion. Accordingly, we ran conditions similar to those used in the second dimension of 2DLC on both carbon phases (see Fig. 11) to examine the potential use of C/Al₂O₃ in 2DLC. Mixtures of 4 indolic metabolites were prepared in progressively stronger eluents from 20 to 80 % (v/v) acetonitrile in water, and a relatively large volume (25 μ l) of each mixture was injected on both columns. The gradient conditions on C/Al₂O₃ were adjusted to have the first (IHT) and the last (IBA) indoles eluted in the similar time window as on the C/ZrO₂. As a result, the C/Al₂O₃ column required much stronger initial and final eluent conditions indicating its higher retentivity for the indoles than that of the C/ZrO₂ column. The overlays of chromatograms from samples made up in different solvent compositions compare the effect of the strong injected solvent on the peak shapes on both carbon phases. The considerable peak distortion of the early eluting indoles was observed on both phases. However, there was almost no effect of the strong injected solvent on the later eluting peaks from the C/Al₂O₃ column whereas the effect was apparent in all of the peaks on the C/ZrO₂. This comparison clearly demonstrates not only the need for high retentivity of column on the second dimension in the 2DLC but also the improvement made by the C/Al₂O₃. As the C/Al₂O₃ exhibits similar selectivity but enhanced retentivity compared to C/ZrO₂, we believe that the C/Al₂O₃ will be an excellent alternative for the second dimension column in the 2DLC.

4. Conclusions

A new CVD carbon phase based on high surface area Al₂O₃ using hexanes vapor as a carbon source has been developed. This carbon packing material offers reasonable chromatographic efficiency and can be prepared reproducibly. We obtained various carbon loads on Al₂O₃ by varying the CVD conditions, and all carbon loadings gave both hydrophobic and polar selectivity similar to C/ZrO₂. About 24 % (w/w) carbon (5 monolayers) was necessary to obtain maximum retentivity, balancing full coverage of the underlying Al₂O₃ against excessive loss of surface area, but it should be noted that commercial 8 % (w/w) C/ZrO₂ has the equivalent of 11 monolayers of carbon.

A carbon load of 24% on Al₂O₃ gives more retention than 8 % C/ZrO₂. We obtained even more retention than would be expected based on the increase in the ratio of the total surface area. We attribute this to stronger interactions of the new carbon phase with solutes possibly due to its higher polarizability and polarity. Although the increment of retention varied depending on the test solute, C/Al₂O₃ overall exhibited higher retentivity for both polar and nonpolar solutes compared to C/ZrO₂. This improvement helped to lessen peak distortion caused by injection of the large volume of sample solvent. Comparison of the relative retention factor of various solutes including stereoisomeric compounds between C/Al₂O₃ and C/ZrO₂ showed great similarity in selectivity patterns. The new carbon packing material is very promising as a packing material for HPLC and should be quite suitable for use as the second dimension in 2DLC.

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Figure 1. Schematic of chemical vapor deposition (CVD) apparatus. 1 and 2 are gas controllers for each direction (200 cc/min.)

Figure 2. Schematic of the device used to measure the resistivity of various carbon materials including C/ZrO₂, C/Al₂O₃, and graphite. The circuit consists of a reference voltage (V_S); a measured voltage (V_{AB}); the reference resistance (R_S); the sample resistance (R_X); A and B are connected to the potentiometer that has input impedance of $> 100 \text{ G}\Omega$.

Figure 3.

Chromatogram for a homolog series of nitroalkanes. LC conditions: 35/65 MeCN/water, T = 40 °C, F = 0.4 ml/min. 50 × 2.1 mm id. column, solutes: nitropropane, nitrobutane, nitropentane and nitrohexane (100 µg/ml), 1 µl injection.

Figure 4.

Differential pore size distribution for pore volume and surface area for various carbon load computed by the BJH method from nitrogen adsorption (upper) and desorption (lower) data. (*) bare Al_2O_3 ; (◇) 6 %; (□) 24 %; (▲) 40 % C/ Al_2O_3

Figure 5.

Differential pore size distribution for pore volume (upper) and surface area (lower) for various carbon load on Al_2O_3 using the models: A, model 1 of smooth coating of uniform thickness; B, model 2 of smooth coating thicker in larger pores (*i.e.* not uniform thickness, but instead with uniform volume fraction of carbon in pores; carbon thickness/pore diameter constant for all values of pore diameter). See Fig. 4 for symbols.

Figure 6.

Plot of log (resistance, ohms) for various carbon materials. See Fig. 2 and Eq. 1 for the calculation of the resistance. Bars indicate a standard deviation obtained from triplicate measurement (no error bar if not measured at least three times).

Figure 7.
XPS C 1s spectra for (a) 24 % C/Al₂O₃ and (b) C/ZrO₂

Figure 8.

Plot of $\log k'$ vs. number of methylene groups for (a) nitroalkane homologs (nitropropane, nitrobutane, nitropentane and nitrohexane); (b) alkylbenzene homologs (benzene, toluene, ethylbenzene, propylbenzene and butylbenzene). LC conditions: $F = 0.4$ ml/min., $T = 40$ °C and (a) 35/65 MeCN/water; (b) 50/50 MeCN/water. (○) C/ZrO₂ (33 × 2.1 mm id. column); (◇) 6 %; (□) 14 %; (□) 24 %; (▲) 40 % C/Al₂O₃ (50 × 2.1 mm id. column)

Figure 9.

Plot of $\log(k'/k'_{\text{benzene}})$ vs. benzene substituted compounds. LC conditions: $F = 0.4$ ml/min., $T = 40$ °C, 50/50 MeCN/water. (○) C/ZrO₂ (33 × 2.1 mm id. column); (◇) 6 %; (□) 14 %; (☆) ODS (50 × 2.1 mm id. column); (*) ODS (50 × 2.1 mm id. column)

Figure 10.

Ratio of the retention time of basic drugs on 24 % C/Al₂O₃ and C/ZrO₂. LC conditions: A, 20 mM perchloric acid; B, MeCN; 10 – 80 % MeCN, in 0–2.5 min. F = 1 ml/min. T = 40 °C, 33 × 2.1 mm id. column for both. PMA, p-Methoxyamphetamine; MDA, 3,4-Methylenedioxyamphetamine; PMMA, p-Methoxy-methamphetamine; MDMA, Methylenedioxy-N-methylamphetamine; MDEA, 3,4-Methylenedioxy-N-ethylamphetamine; MBDB, 3,4-Methylenedioxy- α -ethyl-N-methylphenethylamine

Figure 11.

Chromatograms of mixture of 4 indolic metabolites on (a) C/ZrO₂ and (b) 24% C/Al₂O₃. LC conditions: A, 20 mM perchloric acid in water; B, MeCN; 8–35 % B in 0–3.5 min for (a); 15–60 % B in 0–3.5 min for (b); F = 1 ml/min, T = 80 °C, 220 nm, 25 µl injection, 33 × 2.1 mm id. column for both. The analyte diluents (B/A) are 20/80 (black solid line), 40/60 (red dashed line), 80/20 (blue dotted line)

Table 1

Reproducibility of carbon deposition process

	Batch 1	Batch 2	Batch 3	Average	SD	% RSD
% C w/w ^d	24.4	23.8	21.7	23.3	1.4	6
Plate count/m ^b	69,580	59,860	76,480	68,640	8450	12
<i>k'</i> of nitrohexane ^c	17.7	20.7	17.4	18.6	1.8	10
<i>k'</i> of toluene ^d	5.2	5.7	5.2	5.4	0.3	6
<i>k'</i> of nitrobenzene ^d	19.5	23.8	22.2	21.8	2.2	10

^a 6 h CVD at 700 °C.^b Plate count for nitrohexane. LC conditions: *F* = 0.4 ml/min, *T* = 40 °C, 50 mm × 2.1 mm i.d. column.^c 35/65 MeCN/water.^d 50/50 MeCN/water.

Table 2

Characteristics of different carbon load on Al₂O₃

CVD conditions	% C (w/w)	Carbon ^a (μmol/m ²)	Hypothetical Carbon thickness ^b (monolayer)	S _{BET} ^c (m ² /g)	Pore volume ^d (cm ³ /g)	Nominal BET pore diameter ^e (nm)	log (resistance, Ω)
Bare	na	na	na	153	0.38	10.0	>10
700 °C 1.5 h	6	30	1	119	0.29	9.7	9.4
700 °C 6 h	24	133	5	92	0.18	7.8	2.1
800 °C 6 h	40	218	8	31	0.05	5.9	2.6

^a (% C) × (106) / ((100) × (S_{BET} of Bare) × (12.01)).

^b $N_A \times \pi (1.42 \times 10^{-10})^2 \times (\text{result from } a) / (10^6)$ [8]. This is the number of monolayers in a hypothetical uniform-thickness coating, with no pore size effect. Assumption: graphitic carbon, 1.42 Å for carbon bond length and homogeneous coating process.

^c Surface area (S_{BET}).

^d Pore volume obtained from single total pore volume less than 140, 198, 251, 125 nm diameter at P/P_0 of 0.986, 0.990, 0.992 and 0.984, respectively (from top to bottom).

^e Nominal pore diameter of an equivalent single cylinder, calculated by $4 \times (\text{pore volume}) / S_{\text{BET}}$. The pore size distributions (Fig. 4) are much more meaningful, but we include this figure for comparison with other materials (for which this figure is often cited).

Table 3

The slopes, intercept and ΔG_{CH_2} obtained from different carbon phases. See Fig. 8 for LC conditions

	Nitroalkane ^a			Alkylbenzene ^b				
	Slope (B)	Intercept	R ²	$\Delta G_{CH_2}^c$	Slope (B)	Intercept	R ²	$\Delta G_{CH_2}^c$
C/ZrO ₂	0.322 ± 0.002	-1.349 ± 0.003	0.99995	-461 ± 3	0.235 ± 0.002	-0.412 ± 0.007	0.99992	-336 ± 3
6% C	0.359 ± 0.003	-1.38 ± 0.01	0.99990	-514 ± 4	0.306 ± 0.002	-0.20 ± 0.01	0.99997	-438 ± 2
14% C	0.368 ± 0.003	-0.99 ± 0.01	0.99987	-526 ± 4	0.327 ± 0.006	0.19 ± 0.02	0.99971	-468 ± 8
24% C	0.376 ± 0.003	-1.02 ± 0.01	0.99986	-538 ± 4	0.304 ± 0.001	0.230 ± 0.004	0.99998	-435 ± 2
40% C	0.375 ± 0.003	-1.39 ± 0.01	0.99989	-536 ± 4	0.312 ± 0.008	-0.13 ± 0.02	0.99937	-446 ± 11

^aThe slope and intercept of the linear regression of log *k'* vs. nCH₂ based on the data in Fig. 8(a).

^bThe slope and intercept of the linear regression of log *k'* vs. nCH₂ based on the data from ethylbenzene to butylbenzene in Fig. 8(b).

^cThe free energy of transfer per methylene group calculated from the slope.

Table 4

Separation of structural isomers on C/Al₂O₃ and ODS.^a

<i>d^b</i>	ODS	C/Al ₂ O ₃ ^c
1-Phenyl-3-propanol/1-phenyl-1-propanol ^d	1.0	1.5
Di(phenethyl)amides ^e	1.0	1.2
<i>cis-/trans</i> -stilbene ^e	1.0	15

^a *F* = 0.4 ml/min, *T* = 40 °C.

^b The ratio of two retention factor.

^c 24% carbon load.

^d 50/50 MeCN/water.

^e 60/40 MeCN/water, 50 mm × 2.1 mm i.d. for both columns.