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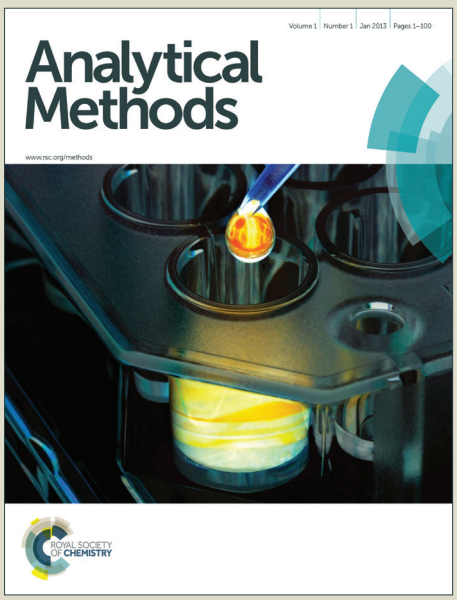

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ARTICLE

A carbosilane dendrimer bonded silica as stationary phase for capillary electrochromatography

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A novel carbosilane dendrimer bonded silica (G2-silica) was synthesized and evaluated as a potential chromatographic resin. The G2-silica was characterized by using various analytical techniques, such as infrared spectroscopy, elemental analysis and loss on ignition analysis. The G2-silica was packed into a capillary and used as the stationary phase for capillary electrochromatography (CEC). The packing characteristics such as the electric current vs. the applied voltage, the effect of the voltage, pH, and acetonitrile concentration on EOF, the hydrophobic selectivity, and the repeatability were all investigated. Seven model compounds including thiourea, benzene, toluene, ethyl benzene, butyl benzene, benzyl alcohol, toluene and naphthalene were well separated on the CEC column with the separation efficiencies ranged from 40,000 to 90,000 plates per meter. The repeatability was satisfactory with the RSD of the migration time (2.4%). These results indicate that carbosilane dendrimer bonded silica can be used as a novel packing material which has the characteristics of a typical reversed phase.

Key words:Carbosilane dendrimer; Silica; Capillary electrochromatography; Stationary phase

Introduction

Capillary electrochromatography (CEC) is recognized as a powerful micro-column separation technique due to its hybrid nature. It combines the characteristics of both high performance liquid chromatography (HPLC) and capillary electrophoresis (CE). Thus, it is feasible that CEC is well suited for the separation of a wide variety of compounds, where they are neutral or charged species. However, before it becomes widely accepted and finds its way to important applications, CEC must first improve in column technology, which is the heart of the separation process.[1-4] Fortunately, some major advances have already been made in the stationary phases specially designed for CEC. Typically, these stationary phases are based on silica micro-particles bounded with a mixed surface chemistry, such as octadecyl ligands (C18) with either low surface coverage in C18,[5,6] mixed C18-strong cation exchange[7,8] or mixed C18-strong anion exchange[9,10] in the aim of providing the nonpolar ligand for solute retention and the charged group to support the electroosmotic flow (EOF). These microparticulate stationary phases yielded a relatively strong EOF and exhibited the selectivity required for separation.

Dendrimers are synthetic, highly branched, nearly spherical and symmetrical macromolecules with well-defined sizes and compositions.[11] They are a class of polymers that have highly branched regular three-dimensional structure with many functional groups on the surface.[12,13] These polymers are unique “spherical macromolecules”, whose molecular architecture consists of a core and repeating units with branching and terminal groups. Each repeating unit contains a branching point to which two or several new repeating units are attached.[11] In recent years, applications of dendrimer in a large variety of fields have been explored, such as catalyses, functional materials and drug delivery.[14] Drug delivery scientists are especially enthusiastic about the possible use of dendrimers as drug delivery tools.[15] In the field of separation, dendrimers have been used in micellar electrokinetic capillary chromatography (MECC) and microemulsion electrokinetic chromatography (MEEKC) as a pseudostationary phase for protein separation.[16,17] In addition, poly(arylether) alcohols dendrimers are

used as the stationary phases in open-tubular and starburst polyamidoamine are used in monolithic column.[18,19] Besides that, dendrimers also have been modified by bound with β-cyclodextrin to coat the column in chiral capillary electrophoresis.[20] CEC resulting significantly increased surface and column capacity due to its highly branched structure.

Organosilicon dendrimers have more significant diversity compared with other dendrimers for the high yields, and high selectivity. They not only have the characteristics of dendrimers but also have excellent properties of other silicon-containing polymers such as low glass transition temperature, low surface energy and etc. At present, there are more and more investigations about the carbosilane dendrimers.[21] Dendrimers, when bonded on silica gel as stationary phase, are imaged as bushy jungles. This is quite a different view from the relatively linear C8 and C18 moieties when solutes run through the surface and the inner pores of the packing materials. It is expected that the silica bonded with the bushy dendrimers will provide more effective surface shielding than that of a traditional stationary phase. Thus, dendrimers bonded surface will have more interaction with solutes and show better efficiency even at lower bonding density.

In this paper, we report the synthesis of a novel second generation carbosilane dendrimer bonded silica (G2-silica), which was packed into a capillary by the slurry packing method and evaluated for its performance in separation in CEC. It has been found that, for the first time, this dendrimer packing has the characteristics of a typical reverse stationary phase like C18. Further, comparable separation efficiency can be obtained even when the bonding density of the dendrimer is relatively lower than that of the C18 stationary phase.

Experimental

Chemicals and materials

The 5 μm sphere silica (148 Å pore size, 135 m^2/g specific area, 0.5 mL/g pore volume) and the 5 μm octadecyl bonded silica (ODS, 148 Å pore size) were purchased from Meigao Group Co., Ltd (Shandong, China). Tetramethoxysilane (TMOS), polyethylene glycol 10000 (PEG, MW=10,000), and $MeHSiCl_2$ were purchased from Tianjin Jiangtian Chemical Co., Ltd. (Tianjin, China). $H_2PtCl_6 \cdot 6H_2O$ was from Shanghai Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) and dissolved in isopropanol. Thiourea and aromatic compounds were purchased from Tianjin Chemical Plant (Tianjin, China). Double distilled water purified by a Barnstead Nanopure II system (USA) was utilized throughout the experiment. All of the reagents and chemicals were analytical grade. Capillary of 75 μm inner diameters (i.d.) and 375 μm outer diameters (o.d.) was purchased from the Yongnian Optic Fibre Plant (Hebei, China).

Instrumentation

Infrared spectra (KBr pellet) were recorded on a Nicolet FT-IR spectrometer from Germany Bruker. A Perkin-Elmer 240c elemental analyzer was used to determine the percentage of carbon on the silica gel. Nuclear magnetic resonance spectrum ($CDCl_3$ as regent) was recorded on Germany Bruker Avance 500 MHz instrument. Loss on ignition analysis was performed in a SX2-4-13 muffle furnace from Tianjin Tianyouli Technology Co., Ltd. (Tianjin, China).

A Bio-Rad HPE100 CE system (Hurcules, CA) was used for all of the CEC experiments. Data were collected and analyzed using the Chrome Perfect workstation developed by the Justice Innovations Inc (Mountain View, CA). An LDC Analytical Constametric 4100 HPLC pump (USA) was utilized to pack and flush the columns. A manual syringe pump (Unimicro Technologies Co., Ltd., China) was used to condition the CEC columns with mobile phases and dispel bubbles out of the capillary. A DL-180A ultrasonic cleaner (Zhixin Instrumental Co., Ltd., China) and a model XW-80A Vortex mixer (Kylin Medical Instrumental Plant, China) were used to mix the sol-gel solution thoroughly. A Motic XSZ-HS1 microscope was used to observe the microstructure formation in the sol-gel process.

Synthesis of second generation carbosilane dendrimer bonded silica

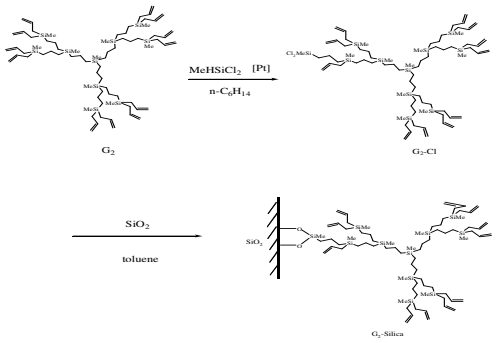


Fig. 1 Synthesis process of G2-C1 and G2-Silica

The second generation carbosilane dendrimer (G2) was synthesized according to some reviews which were described by Pijnenburg and Rasines *etal*.[22, 23] Then, G2 was boned to silica. Figure 1 showed the schematic diagram for the preparation of G2-Cl and G2 modified silica. The detail synthesis procedures were described in Fig. 1.

G2 was added into dried hexane, then $MeHSiCl_2$ was dropwise added into the solution (mole ratio=1:1), and 1~2 drops of the $H_2PtCl_6 \cdot 6H_2O$ isopropanol solution were added into the solution, the mixture was refluxed for 5 h. The final product was washed with hexane to remove impurities. Then, solvent was distilled out of the mixture and a plain yellow product of chlorinated dendrimers (G2-Cl) was obtained. The G2-Cl was stored in dried condition for further use.

The 5 μm sphere silica gel was immersed in 6 mol/L hydrochloric acid for 24 h and then washed with distilled water to neutral, dried at 110 °C in an oven and continue to dried in a three-neck flask at 150-180 °C under a vacuum of 5 mmHg for 4~10 h. Then, dried toluene was added directly.G2-Cl with triethylamine as the catalyst was added directly. The suspension was mechanically stirred and refluxed for 24 h under nitrogen protection. Finally, the reaction solution was filtered and washed with dried toluene, acetone and methanol, respectively. A plain product of G2-Silica was obtained. The G2-Silica was stored in dried condition for further use.

Preparation of the packed capillary column

New capillaries were rinsed with 1 M NaOH, 0.1 M HCl for 1 h in turn and then with water thoroughly. After subsequent flushing with methanol for 10 min, it was dried by nitrogen under 120 °C. A simple preparation method for CEC packing was developed by the literature reported.[24]

The technique provided stationary phase with low thermal effect, which could reduce Joule heating. We quoted the method for the packing procedure of G2-Silica. A temporary frit as illustrated in Fig. 2 was made to hold the packing material. Simply, a thread of glass wool was filled into a stainless union. Then, the capillary was linked to the glass wool through a section of polyetheretherketone (PEEK) tubing with a finger tight screw. At the other end of the union, a similar finger tight screw was fixed. The glass wool functioned as the frit to prevent the silica gel from leaking into the other side when the packing material was packed into the capillary. The G2 modified silica (20 mg/mL) was dispersed in acetone. The suspension was sonicated for 15 min to obtain the slurry before it was poured into an empty HPLC column as slurry holder, which is connected to the capillary. The capillary was filled with the stationary phase under pressure (0-6000 psi) and flow (0.6 mL/min). Once the pressure reached the highest preset value of 6000 psi, it was released slowly. This process would take about 1 h and the columns were kept in an ultrasonic bath during the whole packing process to keep the stationary phase suspending in the slurry reagent. Finally, the capillary was taken away from the pump when the pressure dropped to atmospheric pressure.

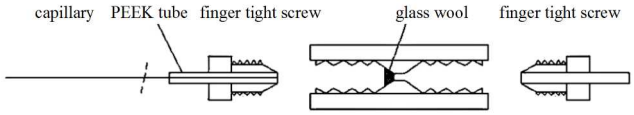


Fig. 2 Schematic diagram of temporary frits

Preparation of frits

The packing of particulate stationary phases in capillaries usually necessitate the fabrication of retaining frits at both ends of the capillary. The retaining frits are easy to be broken and form bubble, which disrupts the conduction of the electrical current so that the complete stop of the CEC process causes. To overcome these problems, frit less columns based on either porous polymers[4, 25] or porous silica-based monoliths[26-29] have been introduced. In our research work, the stationary phase was retained in the capillary by sol-gel frits. The sol-gel solution was prepared as the following:

A mixture containing of 0.5 mL TMOS, 1.25 mL 0.1 M HOAc, and 125 mg PEG was added into a vial. The mixture was shaken thoroughly till a homogenous solution was formed. Then, both the dried ends of the packed capillary were emerged in the sol-gel solution allowing 1~2 mm height of solution to be drawn up along the inside of the capillary by capillary action. After being dried at room temperature, the capillary with TMOS frit was linked to a 75 μm i.d. empty fused silica capillary through a polytetrafluoroethene (PTFE) tubing (0.3 mm i.d.). The i.d. of the PTFE tubing was slightly smaller than the o.d. of the capillary to obtain a tight fit. At last, a detection window was opened with a shaver close to jointing point on the empty capillary.

Chromatography conditions

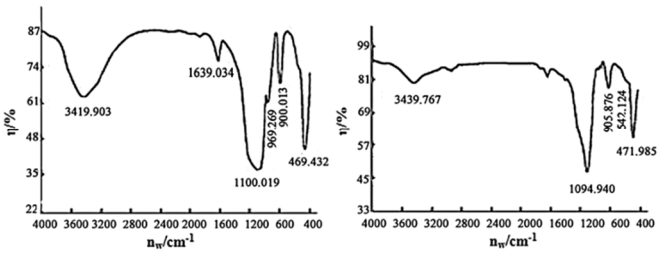
The mobile phase was filtered through a 0.22 μm membrane and degassed under vacuum before using. Sample solutions were dissolved in the mobile phase at proper concentrations before injection.

A steady electroosmotic flow (EOF) introduced by the hydroxyl groups in silica was determined at 214 nm by using thiourea as the neutral marker. Prior to the EOF determination, the column was conditioned with mobile phase for about 30 min by a manual syringe pump, and then electrokinetically conditioned from low to high electric field strength. No bubbles were observed near the joint of the packed column and the coupled empty capillary during this period.

Results and discussion

Characterization of G2-Silica

NMR and infrared spectroscopy (IR) are useful tools to detect functional groups in organic molecules. NMR was used to characterize the G2. δH (300 MHz; CDCl3; G2) -0.145 (3H, s, CH3Si-G0), -0.084 (18H, s, CH3Si2G1), 0.523 (36H, m, SiCH2CH2CH2), 1.210 (18H, m, SiCH2CH2CH2), 1.493 (24H, d, SiCH2CH=CH2), 4.788 (24H, t, CH=CH2), 5.726 (12H, m, CH=CH2). For detecting the existence of G2-Silica, an IR scope was used to characterize the G2-Silica and regular silica stationary phases. Obvious differences were observed in the spectra of bare silica and G2-Silica (Fig. 3).



(A) (B)

Fig. 3 IR spectrum of bare silica gel (A) and G2 bonded silica gel (B)

A broad peak of Si-OH group at 3439 cm^{-1} was much smaller after the modification of the bare silica with G2. This result indicated that many hydroxyl groups in silica gel reacted with the Si-Cl group during the bonding process. In the spectra of modified silica, the peaks at 1095 cm^{-1} is assigned to the asymmetrical stretching vibration of Si-O-Si. The both of 906 cm^{-1} and 472 cm^{-1} are ascribed to the stretching vibration of Si-O. The peaks at 2800~3000 cm^{-1} are assigned to the free organic solvent vibration of C-H ($Si-CH_3$).

Elemental analysis and loss on ignition analysis were carried out to determine the bonding density and the C content of the G2-Silica. The result of elemental analysis found: C, 6.05; H, 1.23%. The bonding density was about 0.52 μmol/ m^2 and 0.63 μmol/ m^2 according to the C content and according to the H content, respectively. The weight of the G2-Silica, which was burned 2 h at 600 ℃,changed from 0.130 g to 0.110 g. The data found C, 10% and the bonding density was about 0.87 μmol/ m^2. These results indicated that the bonded density and the C content were lower than the common ODS (2~3.5 μmol/m^2, 17%). Perhaps it is attributed to the bulkiness of the dendrimer.

Characterization of capillary electrochromatography

Preparing frit and its stability One of the frequently reported problems associated with the packed CEC columns is the frits. In general, frits must be mechanically strong enough to withstand the high pressure, yet they must also have a high permeability so as not to significantly impede the EOF. Many research groups have employed alternative means of retaining the stationary phase to avoid making frits, such as in-line filters,[31] drawing the capillary out to a fine taper,[32,33] sol-gel technique,[34,35] forming macroporous photopolymers, etc.[36, 37] The sol-gel frits have sufficient mechanical stability and good permeability. Their pore sizes can be easily controlled by selecting proper reagents. All sol-gel frits used for this study were prepared by the above-mentioned method.

The stability of the sol-gel frits was investigated further and founded to be quite grateful. No particles were leaked out during the whole experiments. The frits had enough mechanical stability to withstand high pressure at least 3000 psi. Moreover, frits, made by this sol-gel technique, don't increase the back pressure due to the porous structure.

Relationship between applied voltage and electric current The relationship between the current and the applied voltage was investigated at the following conditions, the total column length was 30 cm, packed bed length was 10 cm, buffer was consist of 10 mM phosphate buffer at pH 7 containing 60% acetonitrile. It was found that, even without cooling, the current was proportional to the

applied voltage in the range of 1~12 kV (r=0.9994). The detail data was shown in Fig. 4A. The 12 kV is the highest limit of voltage on this Bio-Rad 100 instrument. The results showed that Joule heating was not obvious and could be ignored under the experimental conditions. At the same time, the good linearity showed that the synthesized G2-Silica is stable under the tested strength of electric field.

Effect of applied voltage on EOF EOF is a function of the applied electric field (E), the dielectric constant of the mobile phase (ε), the zeta potential (ζ), and the viscosity (η) of the mobile phase, as shown in Equation 1.

$$u_{eof} = \mu_{eof} E = \frac{\varepsilon \zeta E}{\eta} \quad (1)$$

Where μ_{eof} is the electroosmotic mobility, u_{eof} is the electrooosmotic velocity.

When 10 mM pH 7.0 phosphate buffer containing 60% acetonitrile was used as the mobile phase and thiourea as EOF marker was injected 5 s at 5 kV, the effect of applied voltage on linear flow velocity of EOF was investigated. A linear relationship between applied voltage and EOF was obtained as predicted in Eq.1. The result (Fig. 4B) indicated that the linear flow rate increased linearly with the increase of the applied voltage in the range of 1~12 kV (r=0.9967), while other modified silica's correlation modulus usually reach to 0.99-0.9978.[30, 31]

Effect of pH value on EOF The pH value of the mobile phase not only can affect the effective mobility of weak electrolyte sample, but also control EOF through changing the surface property of the stationary phase. Thus, it must be to select the optimal value through optimization experiments.

For silica based packing materials, EOF was produced by the residual Si-OH groups on the surface of the stationary phase. The effect of different pH value 3 to 7 on EOF was investigated through fixing running voltage at 4 kV and other conditions. Dating was shown in Fig. 4C. It can be seen that the EOF increased with the increase of pH value. At high pH, the degree of ionization for the Si-OH increased and thus the charge density on the surface of the stationary phase increased accordingly. Therefore, higher EOF was obtained. The tendency of the EOF-pH curve was similar to traditional silica based stationary phase.[38]

Effect of acetonitrile concentration on EOF EOF is related to the dielectric constant of the mobile phase (ε), the zeta potential (ζ), and the viscosity (η) of the mobile phase according to Eq. 1. Usually, the three factors will be affected by organic modifier content in the mobile phase. Thus, EOF also changes with the variation of the organic modifier. The effects of three factors on EOF are not always consistent. Many researchers reported that EOF increased with the increase of organic modifier content,[39-42] while some others obtained the opposite conclusion.[42] The effect of acetonitrile content on EOF of G2-Silica packed capillary column was investigated with the fixing all condition except the acetonitrile content varied between 20~60% (as shown in Figure 4D). It can be seen that EOF decreases with the increase of acetonitrile from 20% to 40%, and even then increases to 50% acetonitrile. EOF decreases slightly when acetonitrile content is over 50%. Therefore the EOF mobility slightly varies between 0.012~0.014 $cm^2/V \cdot min$.

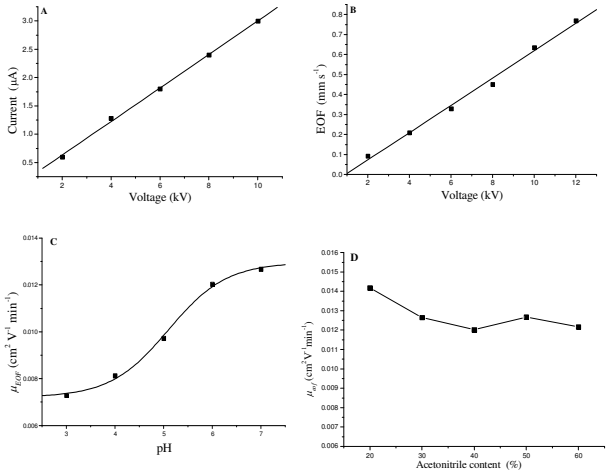


Fig. 4 Effect of applied voltage on current and effect of applied voltage, pH value and acetonitrile content on EOF

Hydrophobic selectivity The capacity factors (k') of a series of alkyl benzenes, which have different methylene numbers, are related only to the hydrophobic interaction between the solutes and the stationary phase. This interaction is called the hydrophobic selectivity (α) (also called methylene selectivity).[44] The relationship between the capacity factor k' and the hydrophobic selectivity α is shown in Equation 2 and 3.

$$\ln k' = p\,n + q \quad (2)$$

$$\alpha = \frac{k'_{n+1}}{k'_n} = e^p \quad (3)$$

Where n is the methylene numbers of alkyl benzenes, p is the slope and q is the intercept. Capacity factor can be calculated based on the following formula (Equation 4 and 5):

$$k' = \frac{t_r - t_0}{t_0} \quad (4)$$

$$k = k' + k' \frac{\mu_{ep}}{\mu_{eof}} + \frac{\mu_{ep}}{\mu_{eof}} \quad (5)$$

Where k is the capacity factor for CEC, k' is the capacity factor which was determined by chromatography, μ_{eof} is the electroosmotic mobility of mobile phase, and the μ_{ep} is the electrophoresis mobility of solute, t_r is the retention time of solute, and t_0 is the dead time.

The hydrophobic selectivity of the stationary phase was investigated using benzene, toluene, ethyl benzene, and butyl benzene as probe solutes and thiourea as the void volume marker. The separation chromatography was shown in Fig. 5. The retention time, capacity factor and column efficiency of these aromatic hydrocarbons were listed in Table 1. Thiourea was eluted firstly due to no retention with the stationary phase, and then benzene, toluene, ethyl benzene and butyl benzene were eluted sequentially. All of these aromatic hydrocarbons were neutral and their separation depended on the hydrophobic difference of the stationary phase. The eluted sequence indicated that the carbosilane dendrimer modified silica has the characteristics of a typical reversed phase packing material. Moreover, Table 1 also indicated that the column efficiencies for the tested analysts were between 40,000~90,000 plates/meter, which is better than 25 cm HPLC column packed by the G2-Silica, less 10,000 plates/meter. In our experiment, the average pore size of the bare silica is 148 Å, the specific area is 135 m^2/ g, the pore volume is 0.5 mL/g. Furthermore, the packed length was only 10 cm. Better column efficiency would be obtained if better bare silica and longer packed column was used.

Table 1 Retention time, capacity factor and efficiency of aromatic hydrocarbons

	Retention time t_r (min)	Capacity factor k'	Column efficiency N ($N \cdot m^{-1}$)
Thiourea	7.12		86681
Benzene	7.99	0.12	73073
Toluene	8.44	0.19	58377
Ethyl benzene	9.05	0.27	53952
Butyl benzene	11.03	0.55	40095

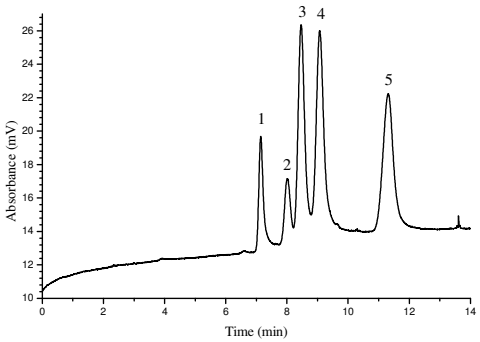


Fig. 5 Chromatograms of thiourea and four aromatic hydrocarbons Conditions: effective/total length=10/30 cm; mobile phase: 5 mM pH=7 phosphate containing 60% ACN; injection: 0.5 psi for 5 s; applied voltage: 8 kV; solutes: 1 thiourea 2 benzene 3 toluene 4 ethyl benzene 5 butyl benzene

In Eq. 2, the slope p can be obtained through the plot of $\ln k'$ and n. At the condition of 60% (v/v) acetonitrile, a good linear relationship between $\ln k'$ and n was obtained (r=0.9999) with the slop p at 0.38. Through calculation, the hydrophobic selectivity α of the carbosilane bonded silica was 1.46, which was comparable to the most commonly used ODS packing material.[44] Therefore, comparing hydrophobic selectivity between the new dendrimer packing and the ODS packing, it was indicated that the carbosilane dendrimer could provide effective coverage of the surface of silica. The solutes have more adequate interaction with this stationary phase than traditional ODS.

Separation of neutral compounds The separation mechanism for neutral solutes on CEC were the same with HPLC. In the ODS column, which were prepared as the technique provided in literature 45, the polarity of the stationary phase surface is weak, and several neutral compounds with strong polarity, including the thiourea, benzyl alcohol, toluene and naphthalene, could not combine well with the surface of the stationary phase. Depending on the migration rate of different neutral solvents and the interaction between analytes and stationary phase, the neutral solutes were eluted sequentially (Fig. 6). The column efficiencies for the tested analysts were between 40,000-60,000 plates/meter, and the resolutions were between 0.93-1.77.

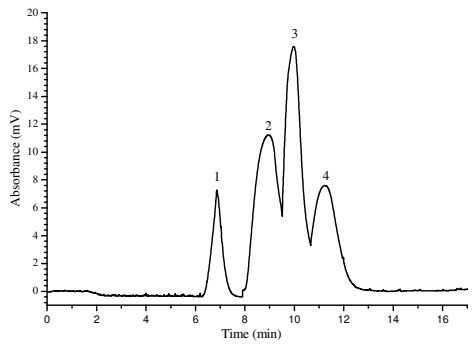


Fig. 6 Chromatograms of some neutral compounds by ODS capillary column Conditions: mobile phase: 20 mM pH=7.4 phosphate containing 60% ACN; effective/total length=10/30 cm; injection: 0.5 psi for 5 s; applied voltage: 8 kV. Solutes: 1 thiourea 2 benzyl alcohol 3 toluene 4 naphthalene.

In order to compare with the ODS column; we used the same compounds to characterize the packing column with G2-Silicon dendrimer. Several neutral compounds, thiourea, benzyl alcohol, toluene and naphthalene were separated on this capillary column (Fig. 7). The migration sequence of those neutral solvents were the same with the result separated by ODS capillary column. The weaker hydrophobic benzyl alcohol was eluted following on the heels of unretained thiourea, while the more hydrophobic toluene and naphthalene were eluted latter.

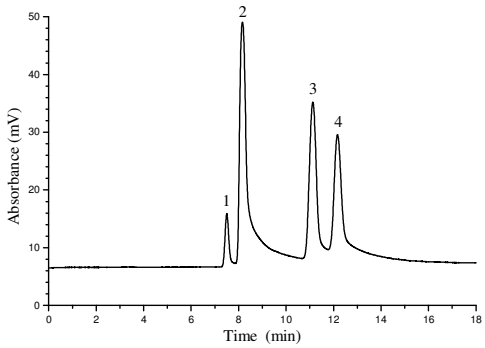


Fig. 7 Electrophoretogram of four neutral compounds

Conditions: mobile phase: 10 mM pH=7 phosphate containing 60% ACN; effective/total length=10/30 cm; injection: 0.5 psi for 5 s; applied voltage: 8 kV. Solutes: 1 thiourea 2 benzyl alcohol 3 toluene 4 naphthalene; the other conditions were the same as Fig.5.

The repeatability The repeatability of capillary column packed by G2-Silica was assessed through the percent relative standard deviation (%RSD) for the retention time of thiourea. The repeatability of six continuous injections was 2.4% (RSD) on this column, which is in close agreement with and even more than those recently reported in the literature[46].

Conclusions

The novel carbosilane dendrimer G2 bonded silica was synthesized and used as stationary phase for CEC. The G2-Silica was packed into a capillary column, and evaluated in the CEC mode. Several neutral aromatic hydrocarbons were successfully separated on this dendrimer stationary phase with satisfactory efficiency. Results indicated that the novel stationary phase has the characteristics of a typical reverse phase stationary phase, and it has more adequate interaction with this stationary phase than traditional packing. There were a number of double bonds on the surface of the dendrimer packing material. Therefore, it is still possible to develop more dendrimer based stationary phase for different modes of chromatography, such as ion-exchange. Meanwhile, the further application of the new solid phase in pharmaceutical analysis is currently underway.

Acknowledgements

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