



Comparison of hypercrosslinked polystyrene columns for the separation of nitrogen group-types in petroleum using High Performance Liquid Chromatography

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ABSTRACT

High performance liquid chromatography in a quasi-normal phase mode (QNP) is used to separate the nitrogen group-types (pyrrole and pyridine) that are found in petroleum. A new type of stationary phase, hypercrosslinked polystyrene, is used to achieve this separation. Three different hypercrosslinked polystyrene stationary phases are compared under quasi-normal phase mode; a commercial 5-HGN packing, and two hypercrosslinked phases on silica particles. The utility of the columns for petroleum-based separations was explored with the use of 21 analytical standards. Partial elucidation of adsorption retention mechanisms for the columns are shown, as well as a comparison of retention characteristics for the three columns. The silica particle column derived with toluene (HC-Tol) was found to have the best selectivity for nitrogen group-types and polycyclic aromatic hydrocarbons (PAHs), attaining a separation under gradient conditions in less than 30 min.

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1. Introduction

Petroleum plays a central and essential role in our economy and daily activities. Petroleum is a complex mixture of many hundreds of thousands of compounds. A better understanding of the composition of petroleum would aid research into production of cleaner and more efficient oil. In particular, compounds containing nitrogen cause refining problems, corrosion and catalyst fouling [1–4]. High pressure liquid chromatography (HPLC) enables the separation of the individual components of petroleum, allowing partial identification of which compounds or classes of compounds are present at different refining stages. The majority of the current published literature concerning petroleum-based separations focuses on polycyclic aromatic hydrocarbons (PAHs) and the different ways to separate them [5,6]. While this information is useful, more work is needed in the separation and analysis of the polar compounds, particularly nitrogen, in petroleum. Due to the complex nature of petroleum samples, the separation of individual compounds is not realistic. Therefore, the goal is to separate N-containing compounds by their group (pyrrole vs. pyridine). This class-specific separation will enhance the information content of analyses that are performed on the samples, such as high-resolution mass spectrometry (MS), as the sample itself will be quite simplified compared to the original petroleum sample [2].

HPLC separations of petroleum compounds are generally done in normal phase (NP) mode, due to the solubility of oil and petroleum samples in organic solvents. The published methods for polar compounds tend to be long, complicated, and involve multiple clean-up steps or columns. For instance, open column separations are used to concentrate and isolate nitrogen compounds from petroleum [7,8] but suffer from long separation times, poor control over sorbent activity and being labor intensive. Carlsson and Ostman achieved HPLC separation of pyrrole and pyridine groups on a dimethylaminopropyl silica stationary phase [9], but their procedure involves open column pre-separation, as well as back flushing of the column. Another experiment uses an alumina NP (HPLC) column to analyze nitrogen compounds in diesel oil without any sample clean-up, and is able to separate neutral nitrogen groups from hydrocarbons, but has a run time of over 100 min, and is unable to resolve basic nitrogen compounds from hydrocarbons [1].

Group classification of sulphur compounds has also been carried out. Polycyclic aromatic sulphur heterocycles (PASHs) have been oxidized to sulfones prior to HPLC separation, in order to separate them from the PAHs [10]. Alternately, a Pd-containing stationary phase can separate PASHs from PAHs. The PASHs can then be separated according to their number of aromatic double bonds on a β -cyclodextrin phase [3].

It is clear that a simple HPLC method to separate polar compounds in petroleum would be highly desirable. This paper focuses on the use of hypercrosslinked polystyrene phases for the group-type separation of polar compounds in petroleum.

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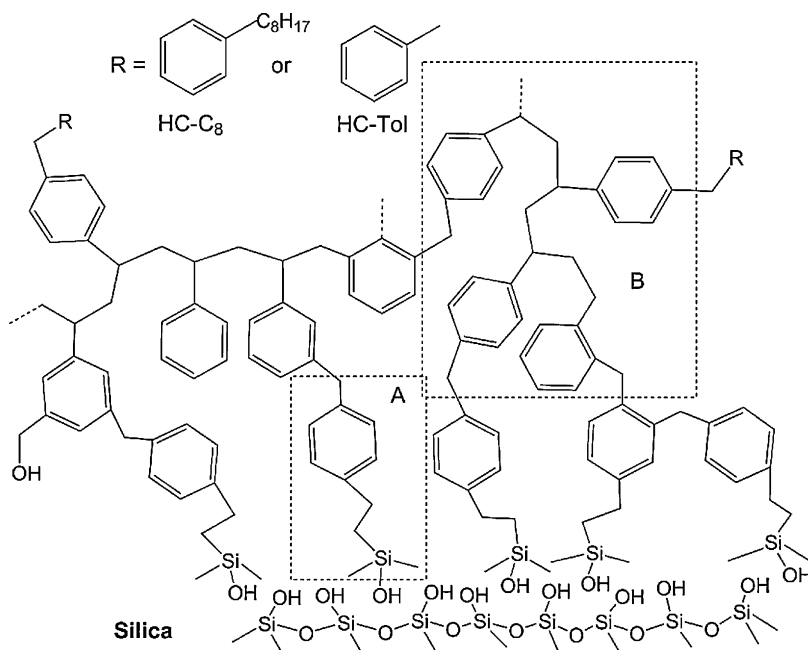


Fig. 1. Structure of the HC-Tol and HC-C₈ stationary phases. Box A refers to the initial silanization step, and box B highlights the crosslinking step. Adapted from Refs. [21,22].

Hypercrosslinked polystyrene has been used in SPE cartridges, HPLC packings and as an adsorbant for the concentration of organic compounds [11–18]. Hypercrosslinked polystyrene is characterized by long polystyrene chains that have been crosslinked with methyl groups between the phenyl groups of polystyrene [12] (Fig. 1). The resultant structure is rigid and dense, with a high inner surface area and wide mobile phase compatibility. Also the retention mechanism (reversed phase vs. normal phase) may be changed simply by changing the mobile phase [11,12].

Recently, Peter Carr's group has synthesized a new family of highly pH and temperature stable stationary phases for reversed phase HPLC. These "HC" (hypercrosslinked) phases are silica based, and are synthesized as a thin monolayer on the surface of the silica [19–25] (Fig. 1). The motivation for their creation was to create a stationary phase that is stable under extremes of temperature and acid conditions, and they have been used to successfully separate basic drugs and non-electrolytes [22,25]. Of the HC phases, the two that are relevant to this work are designated HC-C₈ and HC-Tol (Fig. 1). The primary difference between the two being in the last step of synthesis; in the HC-C₈ phase, the hypercrosslinked polystyrene is derivatized with an octyl phenyl group, while in the HC-Tol phase it is derivatized with toluene [25]. The HC-Tol phase was shown to have equivalent stability, and better efficiency than the HC-C₈ phase. While these phases were designed for RPLC, they are compatible with NP conditions, but to the best of our knowledge have not been previously used for quasi-normal phase (QNP).

Our interest in hypercrosslinked polystyrene for petroleum separations arose from our previous work with the commercial 5-HGN packing for the separation of gas oil resins into fractions containing aromatics, sulphur compounds and nitrogen compounds [26]. The packing showed unique selectivity for compounds in petroleum. The work presented in this paper studies both purely polymer and silica based hypercrosslinked polystyrene. A suite of 21 model compounds found in petroleum were run on the commercial packing, an HC-Tol column, and two HC-C₈ columns. Discussion will focus primarily on the group-type separation of N-containing compounds, with some discussion of other polar functionalities based on sulphur and oxygen.

2. Experimental

2.1. Apparatus

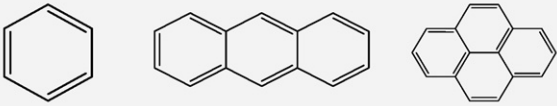
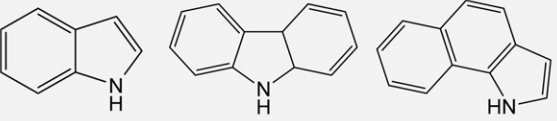
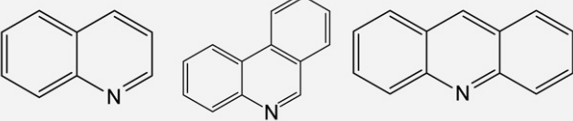
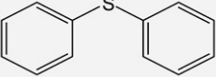
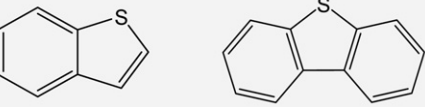
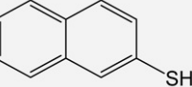
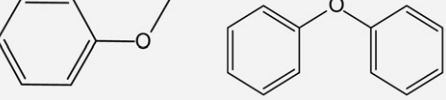
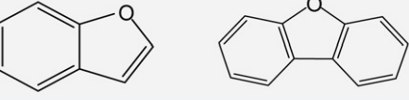
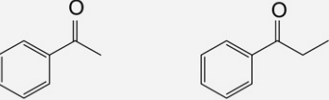
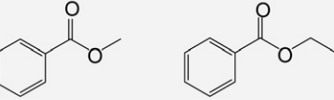
All experiments were performed on a Varian ProStar HPLC system (Varian, Palo Alto, CA, USA). Eluents were sparged with helium (<5 ppm water, Praxair, Mississauga, ON) before and during the experiments, and the helium was dried with an in-line gas dry filter trap (Chromatography Research Supplies, Louisville, KY, USA). Two Varian 210 ProStar pumps were used to pump at 1.0 mL/min. All tubing and fittings were stainless steel. Sample injections were performed with a Varian ProStar 410 autosampler equipped with a 1 μ L sample loop. To maintain a consistent 35 °C column temperature, an Eppendorf CH-30 column oven (Mississauga, ON, Canada) was used. Detection was performed at 254 nm with a 0.1 s rise time with a Knauer Smartline 2500 UV detector (Knauer-ASI, Franklin, MA, USA), fit with a 2 μ L flow cell connected via fibre optic cables. Data acquisition was performed using Varian Star Chromatography Workstation version 6.20 software, at a rate of 20 Hz.

2.2. Chemicals

Optima grade dichloromethane and hexanes (Fisher Chemicals, Fairlawn, NJ, USA) were pre-dried by adding 50 g of Type 3A, 8–12 mesh molecular sieves (EMD Chemicals, Gibbstown, NJ, USA) to a 4 L bottle 24 h before use. All chromatographic solutes were of >90% purity (Sigma-Aldrich, St. Louis, MO, USA), and were prepared at concentrations between 0.1 mg/mL and 10 mg/mL. Indole and carbazole were dissolved in dichloromethane, and the remaining solutes were dissolved in hexane and a minimum amount of dichloromethane to allow full dissolution. A list of these compounds along with their structures and group-types can be found in Table 1.

Chromalite 5-HGN hypercrosslinked polystyrene particles were a gift from Purolite (Purolite International Limited, Wales, UK and Bala Cynwyd, PA, USA). The particles have a mean diameter of 4.5–5.5 μ m, and their surface area is 1100–1500 m²/g. The HC-Tol phase was synthesized on Type-B Zorbax silica particles, with a par-

Table 1
Standards used in this work.

Group	Compounds	Structure
PAH	Benzene (1) Anthracene (2) Pyrene (3)	
N, pyrrole	Indole (4) Carbazole (5) 1H-benzo[g]indole (6)	
N, pyridine	Quinoline (7) Phenanthridine (8) Acridine (9)	
S, sulfide	Phenyl sulfide (10)	
S, thiophene	Benzothiophene (11) Dibenzothiophene (12)	
S, thiol	2-Naphthalenethiol (13)	
O, ether	Anisole (14) Phenyl ether (15)	
O, furan	2,3-Benzofuran (16) Dibenzofuran (17)	
O, ketone	Acetophenone (18) Propiophenone (20)	
O, ester	Methyl benzoate (19) Ethyl benzoate (21)	

ticle diameter of 5.0 μm and a surface area of 180 m^2/g [25]. The HC-C₈ stationary phase was synthesized on HiChrom silica particles, and the particle diameter is also 5.0 μm , and the surface area is 250 m^2/g [19,22]. The HC columns were a gift from Peter Carr (University of Minnesota).

2.3. Column packing

The 5-HGN particles were home-packed. The packing was performed using a Haskel air-driven liquid pump (DSF-122-87153, Haskel, Burbank, CA, USA). 2.2 g of particles were slurried in 40 mL of dichloromethane and sonicated for 15 min. This slurry was placed in an 80 mL reservoir (Lab Alliance, State College, PA, USA) and packed in the downward direction into a 5.0 cm \times 0.46 cm ID stainless steel column jacket (Grace Davison Discovery Science, Deerfield, IL, USA) using dichloromethane as the packing solvent. The pressure was increased from 0 psi to \sim 3200 psi over 1 min, and then maintained at 3200 psi until 300 mL of dichloromethane had passed through the column. The pressure was released and the column was fitted with stainless steel frits and flushed with the desired solvent mixture for 2 h prior to first use. The column efficiency was 1140, which corresponds to a plate height of 44 μm . This is superior to the 190 μm observed for a 25 cm column packed with 5-HGN [26].

2.4. Calculations

Retention times and peak widths at half-height were determined using Varian's Star Chromatography Workstation software, version 6.20. The dead time was determined by the refractive index peak caused by injection of hexane in dichloromethane and confirmed by injection of dichloromethane in a hexane mobile phase. The column efficiency (N) was calculated based on the width-at-half-height.

3. Results and discussion

The stationary phases used in this work are all hypercrosslinked polystyrene. The hypercrosslinked phase is rigid, and has many $-\text{CH}_2-$ bridges linking the phenyl groups of polystyrene [12,21,22]. The absence of polar groups on the stationary phase means that there are no specific adsorption sites on the surface. For this reason, we use Davankov's term of *quasi-normal phase chromatography*, to describe the adsorption of solutes on a largely non-polar stationary phase under normal phase conditions [11,12,26]. There are two types of hypercrosslinked polystyrene studied in this work; the HGN phase is a bed of the hypercrosslinked material (phenyl groups of polystyrene linked with methyl groups) [12], while the HC phases have hypercrosslinked polystyrene bonded to the silica surface of the stationary phase (Fig. 1). The HC phases are synthesized in a multi-step process, and their somewhat more complicated structure bears further explanation. Silica is silanized with an agent such as dimethylchloromethylphenylethylchlorosilane (DM-CMPES) ("A" in Fig. 1), which is then polymerized and crosslinked with a styrene heptamer ("B" in Fig. 1). Some self-condensation also occurs during this process. The phase is derivatized with the desired moiety (R in Fig. 1), and is finally acid washed for increased stability [21,22]. The resultant polymer phase is a thin layer, rather than a bed of material. The siloxane bonds with the surface of the silica are broken to eliminate any acid-labile bonds on the stationary phase. The hypercrosslinked polystyrene surrounds the silica particles and is not washed off, despite the lack of bonds holding the polystyrene to the silica.

The HGN polymeric phase is a gel-type polymer, meaning it has to swell to be used in chromatography [27]. One unique property of

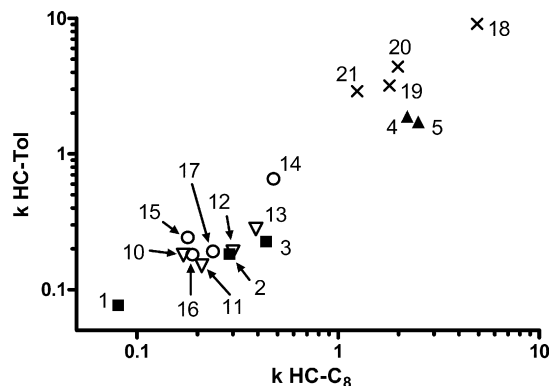


Fig. 2. Retention factors of standards on the HC-Tol column versus the HC-C₈ column, at a solvent strength of 25% DCM in hexane. Numbering corresponds to the compounds listed in Table 1. The size of the data points includes error. *k* values are the average of three runs. ■ PAHs, ▲ pyrroles, ▽ S compounds, ○ ethers and furans, × ketones and esters.

hypercrosslinked polymers is that they are able to swell in any solvent, and once they are swollen, they are not sensitive to changes in eluent [11,12,27]. The changes in pressure observed with our HGN column with different solvents are consistent with what we would expect from viscosity changes. No additional pressure change indicates that the phase is not swelling or shrinking.

3.1. Retention behavior on hypercrosslinked phases

3.1.1. Retention of standards

The HC-Tol and HC-C₈ phases are very similar in terms of their structure. At the derivatization step of the synthesis, the HC-Tol is derivatized with toluene, while the C₈ phase uses octyl benzene (Fig. 1). Fig. 2 compares the QNP retention of model compounds these two columns, with *k* for HC-C₈ plotted on the x-axis, and *k* for HC-Tol plotted on the y-axis. As expected, the retention behavior of model compounds on these phases is quite similar. Most remarkably, PAH standards show very weak retention on both of the columns (■ in Fig. 2), with *k* < 1 on both columns. Fig. 2 also shows that as retention increases on one column, it generally increases on the other, and that different polar groups are generally grouped together in terms of retention. On the two HC columns, the order of elution for the N groups is the same, with the pyrrole compounds (▲ in Fig. 2) eluting after the PAHs (■ in Fig. 2), followed by the pyridine compounds (not shown in Fig. 2 due to very strong retention on the C₈ phase). The retention of groups is overall higher on the C₈ column than the Tol column, but the groups occupy comparable "windows" in the chromatograms between the two columns. The most notable difference between HC-Tol and HC-C₈ is the retention of the pyridine groups, in that the retention on the C₈ phase is so strong for this group that they essentially are not eluted. This stronger retention on the C₈ phase will be discussed in more detail in Section 3.2.

On both HC columns, the model sulphur compounds also experience very weak retention. (▽ in Fig. 2), clustering below a *k* of 0.4, and overlapping with the PAHs. The ether and furan oxygen groups are weakly retained (*k* < 1) at all solvent strengths (ranging from 100% DCM in hexane to 5% DCM in hexane) on the HC columns (○ in Fig. 2), but the ketone and ester oxygen groups (×) experience much more retention and are well separated from the rest of the oxygen and sulphur compounds, with retention comparable to the pyrroles. In summary, both HC columns show the same retention trend for all oxygen and sulphur standards. This is contrary to what is observed for the nitrogen standards.

Fig. 3 compares retention for the HC-Tol and HGN columns. The retention of standards on the HGN column is similar to the HC

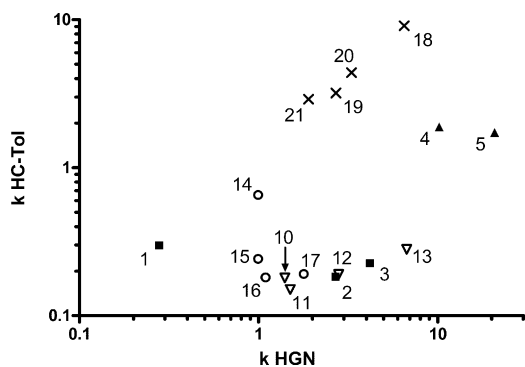


Fig. 3. Retention factors of standards on the HC-Tol column versus k on the HGN column, at a solvent strength of 25% DCM in hexane. Numbering corresponds to the compounds listed in Table 1. The size of the data points includes error in the point. k values are the average of three runs. ■ PAHs, ▲ pyrroles, ▽ S compounds, ○ ethers and furans, × ketones and esters.

phases in that the PAHs are eluted before the pyrroles. Like the HC-C₈ phase, the pyridines are also irreversibly retained at all solvent strengths. Fig. 3 also shows that there is no distinct group-type separation of the compounds on the HGN, and that groups will overlap in the chromatograms. While different groups are separated on the HC-Tol column, the HGN column has a scattering of groups across the range of k values. This type of phase shows notable retention for the PAHs, as well as the sulphur and oxygen standards, which can be seen with the spread of points along the x -axis of Fig. 3. The pyrroles are slightly separated from the rest of the compounds, but not sufficiently to be considered a group-type separation. The detailed retention mechanism for this phase is discussed in Section 3.1.2.

3.1.2. Mechanism of retention

Hemström and Irgum present an excellent discussion on the use of simple equations to describe retention behavior [28]. When a partitioning mechanism is occurring, the linear solvent strength model can be used [29]:

$$\log k = \log k_w - S\varphi \quad (1)$$

where k is the retention factor in the chosen eluent, k_w is the retention factor in pure weak eluent, φ is the volume fraction of the strong eluent, and S is the slope of a plot of $\log k$ vs. φ derived from a linear regression.

Similarly, a competitive adsorption mechanism can be described by the Snyder–Soczewiński equation [30,31]:

$$\log k = \log k_B - n \log X_B \quad (2)$$

where k_B is the retention factor of the solute in pure eluent B, n is the number of molecules of solvent B displaced by a single solute molecule, and X_B is the mole fraction of solvent B, the stronger eluent. These two models can be used to determine whether or not retention is behaving under an adsorption mechanism or a partition mechanism. Plots can be made of $\log k$ vs. % DCM (partition) and $\log k$ vs. \log (% DCM) (adsorption), and linear behavior for either of the plots will indicate which mechanism is favored [28].

To gain more insight into the retention mechanism of the columns, these plots were generated for both HC columns, as well as the HGN column. Figs. 4 and 5 show these plots for the HC-Tol column and the HGN column. The plot corresponding to partitioning behavior (Eq. (1), Figs. 4B and 5B) shows clear curvature for the HC-Tol, HGN and HC-C₈ (data not shown) columns, while the adsorption plots (Eq. (2), Figs. 4A and 5A) are much more linear. The data points for both types of plots were fit to a linear regression, and the correlation coefficient (R^2) values for the adsorption

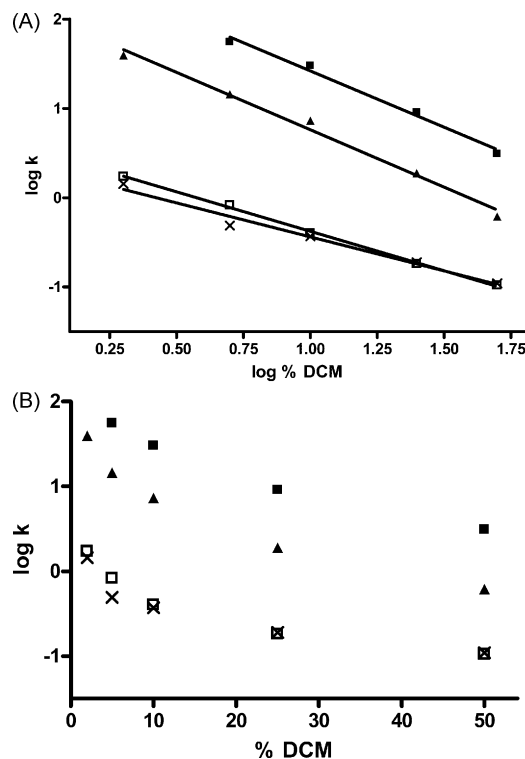


Fig. 4. (A) Plot of $\log k$ vs. \log (% DCM) on the HC-Tol column (adsorption). The lines are a linear fit to the data. (B) Plot of $\log k$ vs. % DCM on the HC-Tol column (partition). The size of the data points includes the error in the data. ▲ indole, □ anthracene, ■ acetophenone, × dibenzothiophene.

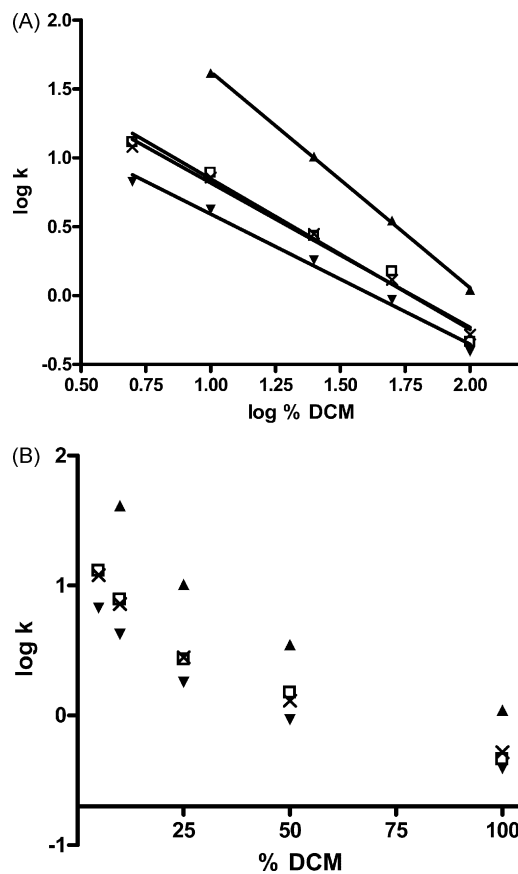


Fig. 5. (A) $\log k$ vs. \log (% DCM) for HGN column (adsorption). (B) $\log k$ vs. % DCM for HGN column (partition). The size of the data points includes error. ▲ indole, □ anthracene, ▽ dibenzofuran, × dibenzothiophene.

Table 2

Correlation coefficients for all standards on the HC-Tol and HGN columns. The R^2 values are for a linear fit to a plot of either $\log k$ vs. % DCM (partition, Eq. (1)) or $\log k$ vs. \log (% DCM) (adsorption, Eq. (2)).

Compound	R^2			
	HC-Tol		HGN	
	Adsorption	Partition	Adsorption	Partition
Pyrene	0.991	0.922	0.983	0.927
Indole	0.992	0.954	0.907	0.956
Carbazole	0.994	0.951	0.995	0.985
Benzene	0.922	0.715	0.872	0.693
Anthracene	0.997	0.884	0.982	0.924
Acetophenone	0.989	0.962	0.991	0.900
Me-benzoate	0.985	0.961	0.990	0.911
Anisole	0.993	0.945	0.987	0.915
Benzofuran	0.985	0.958	0.979	0.938
Diphenyl ether	0.997	0.938	0.982	0.931
Dibenzofuran	0.979	0.957	0.990	0.916
Dibenzothiophene	0.985	0.961	0.991	0.911
Ph-sulfide	0.996	0.930	0.980	0.938
Naphthalanethiol	0.995	0.946	0.997	0.979
Thianaphthalene	0.96	0.922	0.985	0.926

plots are all closer to one than the partition plots (Table 2). The correlation coefficient is used to assess quality of fit; therefore, the adsorption plots are more linear than the partition plots. Based on this data and Eqs. (1) and (2), we assume that the retention mechanism of the hypercrosslinked phases in QNP is that of adsorption.

In previous work by Trammell and co-workers, application of the linear solvent strength model (Eq. (1)) demonstrated that the HC-C₈ column behaves under a partition mechanism in RPLC [22]. The HC columns are therefore able to act under either adsorption or partition mechanisms, depending on the use of RP or NP conditions. Similar behavior has also been observed on the HGN-type hypercrosslinked phases [11,12].

The major retention mechanism on the HGN stationary phases has been reported to be an interaction of Pi electrons (Pi–Pi) between the analyte and the stationary phase [12,15,18,26]. To confirm this for our work, we looked at the retention of PAH standards on the HGN phase. If a Pi–Pi mechanism is occurring, we would expect retention to increase with the number of aromatic rings in the compound [18]. Indeed, at 10% DCM, the k values for benzene, anthracene and pyrene are 0.7, 7.8 and 12.3, respectively. This is an excellent indicator that the Pi–Pi interactions are a major contributor to retention on the HGN column. Pi–Pi interactions are dominant in non-polar solvents (hexane in this case), and are weakened by the presence of a more polar solvent (DCM) [12,18]. The retention for all compounds on the HGN column decreases with the addition of more DCM in the mobile phase, consistent with expectations for Pi–Pi interactions.

Other polymeric hypercrosslinked polystyrene stationary phases (MN-200) have been described as acting under a Pi–Pi mechanism [15]. On the MN-200 stationary phase, there are also oxygen groups on the surface that can cause additional retention of heteroatom-containing compounds [32]. The HGN phase is synthesized in a similar manner to MN-200. Thus, it is possible that similar oxygen functionalities exist on the HGN phase, causing retention beyond what we would expect from just Pi–Pi interactions. However, studies have concluded that no such groups are present on HGN [11,33].

If the HC columns were acting under the same Pi–Pi mechanism, we would see similar increasing retention for the PAHs as they increase in size. However, as shown previously, the PAHs all experience very weak retention on the HC columns, with very little dependence on ring size. This indicates that the HC columns experience an adsorptive mechanism that is not Pi–Pi.

Polymeric hypercrosslinked polystyrene can be used for size exclusion chromatography [15,34–36], but we do not believe this to be occurring with the HC phases. On the HC phases, at all solvent strengths examined, there is an increase in retention with the size of the solute for PAHs (i.e. benzene to anthracene to pyrene). On both HC columns, we see much stronger retention of carbazole than of benzene (i.e. k of 16.4 vs. 0.3 at 5% DCM in hexane on HC-Tol). Carbazole is two rings larger than benzene (Table 1). If an exclusion mechanism were occurring, we would expect to see less retention of a larger solute, which is opposite to what is observed.

3.2. Comparison of HC-Tol and HC-C₈

The retention of compounds is generally stronger on the HC-C₈ phase when compared to the HC-Tol phase. The difference between the structures of the HC-Tol and the HC-C₈ phases is the moiety used in the final derivitization step. The HC-C₈ phase is derivitized with octylbenzene, while the Tol phase with toluene. Essentially, the C₈ phase has an eight carbon chain attached to the surface at various positions, while the Tol phase has only one carbon, as illustrated in Fig. 1 [21]. The motivation for the creation of the HC phases was to make phases that are stable under acidic and high temperature conditions for use as the second dimension in 2D-LC [19,21,25,37]. The HC-Tol phase is one generation beyond the HC-C₈, and was made because toluene facilitates an easier reaction; it is more reactive than octylbenzene, and can be used as the solvent in the derivitization step [25].

The HC-C₈ phase is structurally very similar to the HC-Tol phase, but the synthesis steps as well as the underlying silica used are different. The surface area of the HC-C₈ phase is also greater than the HC-Tol phase (250 m²/g vs. 180 m²/g) [19,22,25]. These two factors may account for the differences in retention observed between these two columns. Studies are currently underway to determine if this is the case.

In comparing the two phases, it is useful to revisit Fig. 2, which is at a solvent strength of 25% DCM in hexane. Due to their strong retention on HC-C₈, the pyridines were not plotted. The pyrroles (▲) are isolated from the rest of the compounds on both columns. The similarity in retention between the Tol and C₈ phases is indicated by a correlation coefficient for the k values of 0.87. The sulphur (▽), ether and furan (○) and PAH (■) compounds experience very weak retention on both HC phases ($k < 0.5$).

A contributing factor towards retention on the HC phases may be silanols available on the surface that are not shielded by the hypercrosslinked polystyrene. Silanols can interact strongly with many polar compounds. The HC-Tol phase is designed, in part, to have less activated silanols than the HC-C₈ phase [25]. This could contribute to the different retention observed on the two phases. The silanols may also be part of the strong retention of the ketones and esters (×) observed in Fig. 2. However, the HGN phase also strongly retains ketones and esters, so we cannot disregard the selectivity of the hypercrosslinked polystyrene itself.

3.3. Comparison of HC-Tol and HGN phases

As discussed in Sections 3.1.1 and 3.1.2, the retention mechanism and characteristics of the HGN column are different than the HC columns. This can be partly attributed to the Pi–Pi retention mechanism on the HGN column. Aromatic rings in all of the standards causes them to be retained on the HGN phase where they may experience very little retention on the HC-Tol phase.

As in the comparison of the two HC columns, a plot of the k values on HC-Tol vs. k on HGN at 25% DCM reveals a lot about the retention on these two phases (Fig. 3). On this figure, there is a very distinct scatter of points. There is overlap of all the groups on the HGN column, and the standards experience more retention on

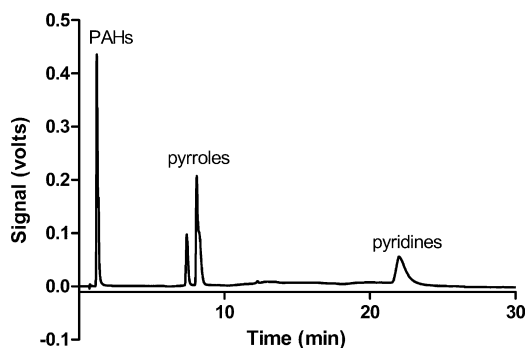


Fig. 6. Chromatogram of nitrogen/PAH mix under a step gradient on the HC-Tol column. Temperature was 35 °C, at a flow rate of 1 mL/min, detection wavelength of 254 nm. PAHs: benzene, pyrene, anthracene. Pyrroles: indole, carbazole, 1H-benzo[g]indole. Pyridines: quinoline, phenanthridine, acridine. Step gradient was 20 min long, with 12 min of equilibration time. Initial solvent condition was 5% DCM in hexane, increasing by 20% DCM every 4 min. Equilibration time was 12 min between each gradient separation.

the HGN column. Where the majority of the compounds on HC-Tol have a k of less than one, almost all of the compounds have a k greater than one on HGN. There is almost no correlation in retention between the two columns ($R^2 = 0.05$). This reinforces that the HC-Tol column is not acting under a π - π mechanism as was the HGN [12,15,18,26]. As the main goal of this study was to separate N-containing groups, the inability to elute the pyridine-type compounds, as well as the overlap of groups, points to the conclusion that the HGN packing did not meet our needs. However, the exceptional orthogonality exhibited by these two phases indicates that they may be used together in a 2D-LC experiment, to perhaps attain even better resolution and group separation of the compounds in petroleum.

3.4. Separation of model compounds on HC-Tol

The HC-Tol phase is ideal for the type of separation we want to achieve because both nitrogen groups as well as aromatic compounds have different retention on the column. To illustrate the abilities of this column, a mix of nine compounds (three PAHs, three pyridines and three pyrroles) was run under a step gradient (Fig. 6). The gradient is necessary because of the strong retention of the pyridine groups relative to the pyrrole and PAH groups, and a step gradient was used because it gave sharper peaks than a linear gradient for later eluting compounds. An equilibration time of 12 min was used; 10 min was insufficient to return the column to equilibrium after the separation, but increasing the time beyond 12 min did not show any additional improvement. The aromatic compounds are essentially unretained, while the two different nitrogen groups show very different selectivity. Within the pyrrole group, two peaks are visible, with a resolution of 1.5. The pyridine group elutes as a single peak, as does the PAH group. However this is not a problem, as the objective with petroleum analysis is to separate groups, not compounds within a group.

The HC-Tol phase also exhibits unique selectivity for oxygen-containing compounds (Fig. 7). A separation of eight oxygen compounds was achieved using a similar step gradient. There is better resolution between compounds, and it would appear that the HC-Tol phase has a different selectivity for oxygen group-types as well. Although this was not the target of this study, this may prove useful to studies in the future. Fig. 7 also reveals more about the retention mechanism on the HC-Tol column. The HGN column acts under a π - π mechanism [12,15,18,26], with compounds with more aromatic rings experiencing more retention. On the HC-Tol column, anisole and phenyl ether elute in an order opposite to what

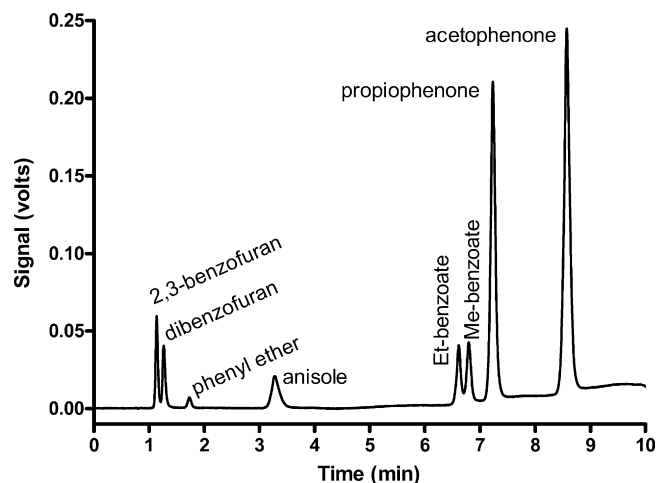


Fig. 7. Chromatogram showing the separation of oxygen-containing model compounds on the HC-Tol column. Conditions: 35 °C, 1 mL/min, detection wavelength of 254 nm. A step gradient was used, beginning at 5% DCM in hexane, increasing 20% DCM every 2 min up to 100% DCM. Equilibration time was 12 min between each gradient separation.

would be expected based on a π - π mechanism. Anisole, which has only one ring, is retained stronger than phenyl ether, which has two rings. This would suggest that the retention is based on the polar group, as the phenyl ether has more steric hindrance around the oxygen than the anisole. A similar elution order is observed with ethyl and methyl benzoate, with methyl benzoate experiencing stronger retention.

Efficiencies were calculated for the HC-Tol phase for anthracene, carbazole and methyl benzoate. As the compounds become more retained, the efficiencies of the standards decrease, but the peak shapes remain the same (Table 3a). These efficiencies are lower than the 3560–4210 observed in RP mode [25].

All of the standards were run at a high concentration (up to 10 mg/mL) during the experiments, and a suite of four compounds were run at one-third the elevated concentration to see if the concentration had any effect on retention and efficiency. The retention of the weakly retained anthracene, dibenzofuran and dibenzothiophene were the same within error at both concentrations. However, retention of the strongly retained indole decreased with an increase in concentration (Table 3b). Qualitatively, the peak shapes are the same between the different concentrations, despite the shift in retention time. This suggests that overload is not a problem here,

Table 3

(a) Efficiencies and retention on the HC-Tol column with varying solvent strengths. (b) Efficiencies and retention on the HC-Tol column, from retention data collected at a solvent strength of 2% DCM in hexane. k values are an average of three runs.

Conditions	Compound					
	Anthracene		Carbazole		Me-benzoate	
	N	k	N	k	N	k
50% DCM	2500	0.11	2000	0.51	2400	1.2
25% DCM	2500	0.18	1100	1.7	1600	3.2
10% DCM	1100	0.40	730	7.2	870	10.7
5% DCM	1800	0.83	770	16.4	600	17.6
2% DCM	840	1.7				
	1.0 mg/mL			0.3 mg/mL		
	N	k	N	k	N	k
Anthracene	840	1.7	1300	1.8		
Indole	420	39.4	800	45.3		
Dibenzothiophene	1800	1.4	2000	1.5		
Dibenzofuran	2200	1.5	2400	1.5		

and the signal-to-noise is too poor to allow for a quantitative comparison of asymmetry.

Previous work completed in our group had shown that an aminopropyl bonded silica NP column was capable of group-type separation of N compounds in petroleum [38]. However, when these experiments were repeated on a new column, it was found that the chemistry of the previous column had somehow been altered by harsh solvent and temperature conditions, and that retention was due to residual silanols. The hypercrosslinked columns in this work have shown to be far more useful than an aminopropyl column, serving to separate the N groups from each other, as well as from PAHs that may interfere with future analysis.

4. Conclusions

The HC-Tol stationary phase provides unique and useful selectivity for N-containing groups in petroleum. Using a step gradient, all pyrrole and pyridine group-types can be separated in under 25 min, with PAHs, sulphur compounds and the majority of oxygen compounds weakly retained and well separated from the N compounds. The HC-Tol phase also provides an excellent separation of oxygen standards.

The HC-C₈ and HGN packings show similar retention characteristics to HC-Tol, but both retain pyridine compounds with such strength that they are unable to provide a group-based separation. This strong retention could be a concern when using real petroleum samples, as irreversible adsorption could occur in the column. The HGN column also retains PAH compounds, which may overlap with the N fractions we wish to analyze. Both of these types of columns have been used in reversed phase and normal phase mode, which speaks to their potential for a very wide variety of applications and solvent conditions.

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