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A Preliminary Study on the Selectivity of Linear Polynuclear Aromatic Hydrocarbons in SFC using Phenyl-type Stationary Phases

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Abstract

The retention behaviour of a homologous series of polyaromatic hydrocarbons was evaluated on two phenyl-type stationary phases in reversed phase supercritical fluid chromatography. These phases were the Synergi Polar RP phase and the Cosmosil 5PBB phase, both of which are polar end-capped and incorporate an ether in a propyl chain that tethers the phenyl ring to the silica surface. The Cosmosil 5PBB phase also has five bromine atoms on the phenyl ring. The retention capacity of the Cosmosil column was substantially greater than the Synergi column. However, selectivity on the Cosmosil column was effectively independent of the acetonitrile modifier composition in the CO_2 mobile phase, whereas, selectivity on the Synergi column was greatly affected by the acetonitrile modifier in the CO_2 mobile phase

The results from this study showed that selectivity and retention studies in HPLC cannot be used to predict selectivity and retention behaviour in SFC.

Introduction

The optimisation variables in SFC are far more substantial than in HPLC. In HPLC the separation mode is dictated by the type of stationary phase, while the mobile phase (solvent type, composition, pH etc.) determines the extent of retention and provides scope for selectivity. In contrast, in SFC there are potentially as many options in stationary phases as there are in HPLC, but, the mobile phase also provides for a greater degree of separation control. In SFC CO_2 is the bulk component of the mobile phase, usually a waste by-product that is recycled providing a greener option than HPLC, especially normal phase where the bulk solvents are often heptane or similar. The CO_2 is modified with an organic component, in reversed phase this is usually methanol or acetonitrile, the addition of which serves the same purpose as in RP-HPLC, and that is to increase the solvent strength and provide a level of selective interaction with the solute species of the stationary phase in order to enhance the selectivity provided by the stationary phase. The choice between methanol or acetonitrile as the organic component therefore often comes down to the nature of the solutes being separated, for example, are they proton donors/acceptors, do they have π-bonding capabilities etc. Beyond this level of selection, and the strength of the solvent based on composition, SFC provides for an additional solvent variable; largely the density. The density of the SFC mobile phase can be regulated by the temperature and the back pressure, factors that can greatly change solute solubility in the mobile phase. In HPLC, the density of the mobile phase is largely unaffected by the temperature and back pressure.

As a consequence of being able to greatly alter the properties of the mobile phase, the SFC solvent should play a more substantial role in the separation process than in HPLC. That being the case, optimisation strategies in SFC should not be reliant on prior applications in HPLC, since the nature of the solvent is potentially substantially different. In recent studies, for example, we showed how in the separation of linear polynuclear aromatic hydrocarbons the behaviour observed in RP-HPLC very different from the behaviour was observed in SFC, using the exact same columns in both systems. Should the stationary phase be the dominate aspect of these separation processes, one might assume that the solute behaviours could be similar in HPLC and in SFC, or at least provide some level of predictability. This was not the case, and hence it is clear that the abundant knowledge gained in HPLC cannot be automatically assumed to the starting point for optimisation strategies in SFC. As SFC continues to gain popularity, driven by the demands of a cleaner separations industry, research must be focused on the design of SFC-specific stationary phases and separation protocols.

In this paper we explore further the retention behaviour of linear polynuclear aromatic hydrocarbons on two phenyl-type stationary phases. This study extends on prior work that was undertaken using a methanol modifier.

Stationary Phase Background.

The two phenyl-type stationary phases under study in this work are the Synergi-polar RP phase and the Cosmosil 5PBB phase, the key physiochemical properties of which are given in **Table** 1. The Synergi polar-RP column is prepared on Luna silica. The surface area is 475 m^2/g, particle diameter is 4 μm, the carbon load is 11%, and the ligand density is 2.02 $\mu mol/m^2$. The Cosmosil 5PBB is a modified Cosmosil, 5 μm silica phase; the surface area of which is 300 m^2/g, and the carbon load is 8% with a ligand density of 2.71 $\mu mol/m^2$. The basic structures of these two stationary phases are shown in **Figure** 1 [ref]. Both stationary phases are polar end-capped with a proprietary end-capping agent, and both are ether-linked phenyl phases that are tethered to the silica surface via a propyl alkyl chain. The ether linkages between these phases differs slightly; the Synergi polar RP phase has the oxygen ether link bonded between the propyl alkyl chain and the phenyl ring (phenoxy-ether), whereas the ether functional group on the 5PBB phase is between the propyl alkyl chain and a methyl group (methoxy-ether), which is subsequently tethered to the phenyl ring. Aside from these differences, the 5PBB phase has five bromine atoms bonded to the phenyl ring.

The difference in the 'ether-linkage' between these two phases would be expected to influence substantially the spatial orientation of these ligands in relation to the surface of the silica. Ab-initio calculations on energy minimized structures for the unit structure for idealised stationary phases were undertaken using Gaussian© 03W (closed shell restricted Hartree-Fock method and a 6-31G basis set) [68] in order to assess the molecular orientation of each of these ligands. These calculations were based on gas phase conformations, and neglect both solvent and near neighbour interactions; however, the information gives preliminary insight into the solute–surface interactions of these stationary phases [ref]. The geometric data from the energy minimized structures were measured using Cambridge Soft Chem 3D Ultra. The outcomes from these calculations are given in **Table** 2 [ref]. The data in **Table** 2 details the length of the silica-peripheral carbon, silica-peripheral hydrogen, the width of the phenyl ring and the sweep area of the ligand, which is the region bound within the confines of the ligand if in fact it where able to rotate around the bonding site.

The molecular models of these two stationary phases, illustrating the molecular orientation are shown in **Figure** 2. Because the phenyl rings are tethered to the stationary phase by a propyl alkyl chain the orientation of the phenyl ring is likely to be approximately perpendicular to the silica surface [ref]. However, a key difference between these two phases is that the phenoxy-ether link in the Synergi polar-RP column rotates the phenyl ring approximately 90° with respect to the silica surface, but within a plane ~ 90° to that found for the Cosmosil 5PBB chain, which contains a methoxy-ether link. The shape differences of these two phases are therefore substantial; the Cosmosil phase could be considered to act as a 'slot-like' surface potentially serving to guide the PAHs into the body of the stationary phase, whereas since the Synergi phase has a larger width to depth ratio this phase may act more bowl-like rather than slot-like [ref]. The molecular dimensions of these phenyl phases, such as, the measurements of the length of the silica-peripheral carbon, silica-peripheral hydrogen, the width of the phenyl ring and the sweep area (the region bound within the confines of the ligand if in fact it where able to rotate around the bonding site) are given in **Table** 2. Although these measurements in all likelihood do not reflect the true molecular behaviour of these stationary phases in solution state environments, it is clear that the potential sweep area of the Synergi phase is greatly different from that of the Cosmosil phase, and this is supported by the lower ligand density on the Synergi phase compared to the Cosmosil phase. The orientation of the phenyl ring for each of these phases when in close proximity to the PAH solute is likely to be substantially different, and this may influence not only the type of π-π binding that would be apparent, but also the molecular selectivity of the bonding process.

Experimental

Chemicals

Supercritical CO_2 was obtained using food grade CO_2 purchased from Coregas, Yennora, Vic., Australia. HPLC grade ACN was used as an organic mobile phase modifier and HPLC grade tetrahydrofluran (THF) was used for the dissolution of the polynuclear aromatic hydrocarbons (PAH). Both ACN and THF were purchased from Honeywell Burdick & Jackson (Taren Point, NSW, Australia). Polynuclear aromatic hydrocarbon standards were purchased from Sigma-Aldrich (Castle Hill, NSW, Australia).

Separations

All chromatographic separations were performed on a Agilent 1260 Infinity Analytical SFC System, utilizing a Fusion A5 (G4301A) SFC system, 1260 degasser (G1322A), HPLC-SFC binary pump (G4302A), SFC autosampler (G4303A), column compartment (G1316C), DAD UV-detector (G1313C, set at 304 nm), and Agilent Chem Station software on an Intel Core 2 Duo 3.16 GHz processor (Mulgrave, Victoria, Australia). The chromatography columns used in this study were a Synergi polar-RP (4 µm P_d, 150 × 4.6 mm, 80 Å) and a Cosmosil 5PPB (5 µm P_d, 150 × 4.6 mm, 120 Å) purchased from Phenomenex (Lane Cove West, NSW, Australia).

The PAH stock standards were dissolved in THF and made up in concentrations of 10 mg/mL; injectable samples were then prepared by dilution with THF to 1 mg/mL. Each column was tested using five different mobile phase compositions of CO_2 and ACN at a flow rate of 3 mL/min, with a column temperature set at 35 °C and backpressure regulated at 110 bar. Each sample was injected onto the column using a 5 µL injection loop with an overfill factor of 3 and duplicates were performed for each injection.

Retention factors were determined using void volumes calculated by the inflection point of the solvent front resulting from the minor disturbance generated by the injection plug.

Results and Discussion

In this work we assessed the retentivity and global selectivity of the set of homologue PAHs on both stationary phases. In order therefore to quantify their retention behaviour the relationship between the retention factor, k, and the solvent composition, Φ, was evaluated. The model that we used for this assessment was based on the Linear Solvent Strength (LSS) theory [8,9], according to the following relationship:

$$\log k = \log k_0 - S\Phi \qquad (1)$$

where k_0 is the retention factor of the solute in the weak solvent (i.e., water in reversed phase and CO_2 for SFC), and S is the rate of change in log k with Φ. Plots of log k versus Φ are important as they provide a visual depiction of how selectivity changes as the solvent composition changes and the S parameter provides a means to quantify the expected degree of separation – or global selectivity, and then allow the determination of the optimum solvent composition required to bring about the desired level of separation. In HPLC the relationship between log k and Φ is generally linear when the range of retention factors considered is limited to between 1 to 10, beyond which a quadratic relationship is often observed. At present little is known whether or not this relationship maintains linearity over a wider range in SFC, although our initial studies suggest that in SFC there is a greater adherence to linearity.

The first observation with regards to retention behaviour of these stationary phases was that the degree of retention was far more substantial on the Cosmosil phase, than on the Synergi phase. This was consistent with the findings when methanol was used as the modifier in SFC and when both columns were used in HPLC, i.e., the Cosmosil stationary phase retained longer the PAHs. This outcome is likely a result of very strong cohesion forces associated with the nature of the ligand itself, either related to the ring orientation or to the bromination of the ring structure, since, the physiochemical properties of the phases would suggest that retention should be greater on the Synergi phase, moreso than the Cosmosil phase. That is, the Synergi phase had a higher surface area and higher carbon load, yet less retention of the PAHs.

From these initial observations it might be appropriate to hypothesise that using acetonitrile as the mobile phase additive rather than methanol, a reduction in the degree of retention would be expected due to the increase in solvent strength of the acetonitrile, and that acetonitrile would potentially serve as a π-bonding competitor to the PAHs, hence displacing the PAHs from the surface of the stationary phase. The retention studies using the Cosmosil phase certainly supported this hypothesis, where a change in SFC composition from 40% MeOH to 40% ACN resulted in a drop of retention time for Pentacene from 35.2 min to more than half this value - 15.4 min. However, this hypothesis was not supported for the retention behaviour on the Synergi phase, where a significant increase in retention was observed when acetonitrile was employed rather than methanol. For example, the retention time of pentacene almost doubled (rather than decreased) from 17.5 min to 33.0 min when the solvent was 1% MeOH compared to 1% ACN. These examples represent snap shots on the retention behaviour of the PAHs.

To evaluate the retention behaviour of the PAHs more extensively, retention was tested using a range of solvent compositions similar to the method used in our previous study [4]. On the Synergi phase the composition of ACN in the mobile phase was varied between 1 to 5%, consistent with the range employed using methanol as the modifier. Since overall, the retentivity was greater on the Cosmosil phase, the ACN range varied between 20% to an upper limit of 40%. The lower limit of ACN on the Cosmosil phase differs slightly from our prior work, since the acetonitrile was a stronger solvent and we sought to maintain retention factors within a similar band across both solvents.

Plots of log *k* versus *Φ*, expressed as volume fraction of ACN in supercritical CO_2, are shown in **Figure** 3 for both the Synergi and Cosmosil stationary phases. In all cases these plots were linear over the solvent composition ranges tested even across retention factor ranges that exceeded 30 units. Visual evaluation of these curves indicates that selectivity between respective PAH members in this homologue series would increase at a far more substantial rate on the Synergi column, than on the Cosmosil column. This was verified by evaluating the values of *S* derived from these graphs illustrated in **Figure** 3, which are given in **Table** 3. Plots of S versus the number of aromatic rings (N) are shown in **Figure** 4, curves (a) and (b) for the Synergi polar-RP and Cosmosil 5PBB columns, respectively. These curves are distinctly different for each phase. The relationship between *S* and *N* on the Cosmosil phase was linear, with a very shallow gradient (0.3), but on the Synergi phase the relationship was barely linear, tending more towards being exponential. This was similar to the case when methanol was used as the modifier, but here, using acetonitrile the outcome was more extreme. Hence, gaining separation on the Synergi phase required a simple manipulation of the mobile phase modifier, whereas on the Cosmosil phase, selectivity was almost fixed, irrespective of the modifier concentration. Furthermore, on the Cosmosil phase, the separation outcome using acetonitrile or methanol as the modifier was almost the same, hence selectivity options would be very limited beyond the separation that first eventuates, irrespective of the mobile phase conditions. For example, the plots in **Figure** 5 show the relationship between *S* values obtained when methanol was used as the modifier compared to when acetonitrile was used (the data neglects the retention of benzene, since its elution on the Synergi phase was almost on the void, and strictly speaking it is not a PAH) – **Figure** 5a is the data for the Synergi phase, **Figure** 5b is the Cosmosil phase. On the Cosmosil phase, the slope of the relationship was essentially unit, signifying that aside from differences in the degree of retention, the selectivity change was an absolute direct, and unit change proportion for the molecular descriptor – number of aromatic rings. Whereas on the Synergi column the relationship between the methanol and acetonitrile systems was also linear, but the slope was almost equal to 2, indicating that per unit content of acetonitrile the rate of change in global selectivity as a function of the molecular descriptor was twice that of the methanol system. Hence selectivity on the Synergi column was a function of both solvent composition and the type or the organic modifier employed. This data therefore shows that the retention process on the Cosmosil phase is very much dominated by the physio-chemical properties of the stationary phase, the organic modifier playing a very passive role in the retention process, whereas, on the Synergi-RP phase, the role of the mobile phase is far more significant to the retention process. In particular, the magnitudes of the *S* values on the Synergi phase were very large, indicating that this phase potentially offers a very great scope for controlling separation of these types of compounds. The results from these studies yielded very conflicting outcomes for stationary phases that have as their basis, very similar attributes. That is they are both π-bonding selective stationary phases, and their collective behaviours in HPLC are very different to that observed in SFC

Conclusion

This preliminary investigation into the behaviour of PAHs on phenyl-type stationary phases in SFC has highlighted several important factors: First and foremost, retention and selectivity data obtained using HPLC cannot be used as an indicator for retention behaviour in SFC, secondly, strongly retentive phases in SFC environments may provide for limited selectivity optimisation, conversely, weakly retentive phases in SFC environments may provide for very high levels of selectivity optimisation, and thirdly, subtle changes in the nature of the stationary phase may lead to very substantial differences in the manner by which that phase acts as a retention surface; in particular, whether the role of the stationary phase serves as the dominating factor in the retention process, or otherwise.

The studies we have conducted here have reported preliminary findings. The outcomes were unexpected and it is clear that we do not fully understand the nature of these retention processes. We suspect that a greater understanding of mobile phase solvation with the stationary phase surface is warranted and future studies will be directed towards this.

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Figure Captions.

Figure 1. Structures of the (a) Synergi Polar RP stationary phase, and (b) the Cosmosil 5PBB stationary phase.

Figure 2. Energy minimised molecular models of the (a) Synergi Polar RP stationary phase and (b) the Cosmosil 5PBB stationary phase. The phenyl rings are the symbols at the top right of each model.

Fgure 3. Plot of Log k versus the solvent composition (ϕ) for the linear PAHs on the Synergi Polar RP and the Cosmosil 5PBB stationary phases.

Figure 4. Plot of S versus the number of aromatic rings (N) in the PAHs on (a) the Synergi Polar RP and (b) the Cosmosil 5PBB stationary phases.

Figure 5. Plots of S(Acetonitrile) versus S(Methanol) on:

(a) The Synergi Polar RP stationary phase
(b) The Cosmosil 5PBB stationary phase

Table 1. Physio-chemical properties for the Synergi-polar RP and the Cosmosil 5PBB stationary phases, as reported by the manufacturer.

Physio-chemical properties						
Column	**Pore Size (Å)**	**Surface area (m^2/g)**	**Particle size (μm)**	**Carbon load (%)**	**Ligand density ($\mu mol/m^2$)**	**End-capping**
Synergi-polar RP	80	475	4	11	2.02	Polar
Cosmosil 5PBB	120	300	5	8	2.71	Polar

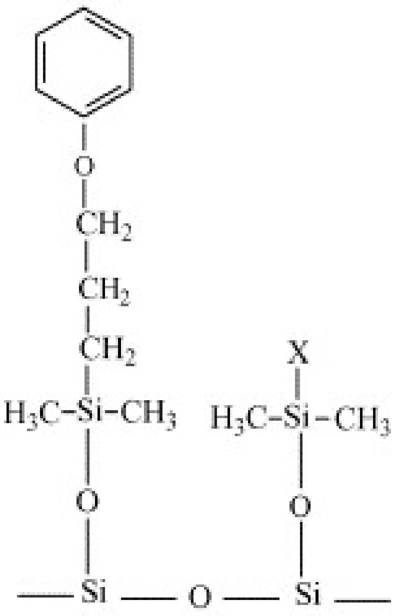
Table 2 Important molecular parameters determined from energy minimised molecular models for the Synergi polar RP and the Cosmosil 5PBB stationary phases.

Molecular dimensions of the Synergi Polar RP and Cosmosil 5PBB stationary phases (measured relative to the silicon of the dimethylsiloxane group)				
Stationary Phase	Length Si-C (Å)	Length Si-H (Å)	Width (Å)	Sweep Area (Å^2)
Synergi	6.604	10.419	4.261	245
Cosmosil	7.599	11.406	5.717	39

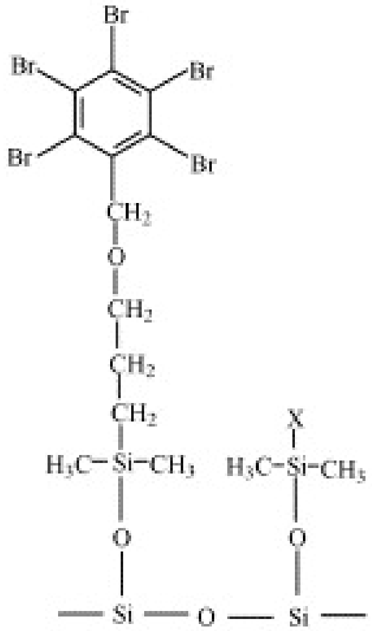
Table 3. Values of S derived from plots of log *k* versus ϕ for the linear PAHs on the Synergi Polar RP and Cosmosil 5PBB Stationary phases.

Compound	**Column** **Synergi Polar RP**	**Cosmosil 5PBB**
Benzene		0.47
Naphthalene	0.95	0.77
Anthracene	4.24	1.09
2,3-Benzanthracene	9.17	1.33
Pentacene	11.75	1.68

Figure 1

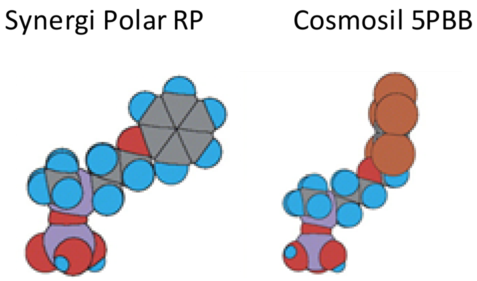


Synergi polar-RP



Cosmosil 5PBB

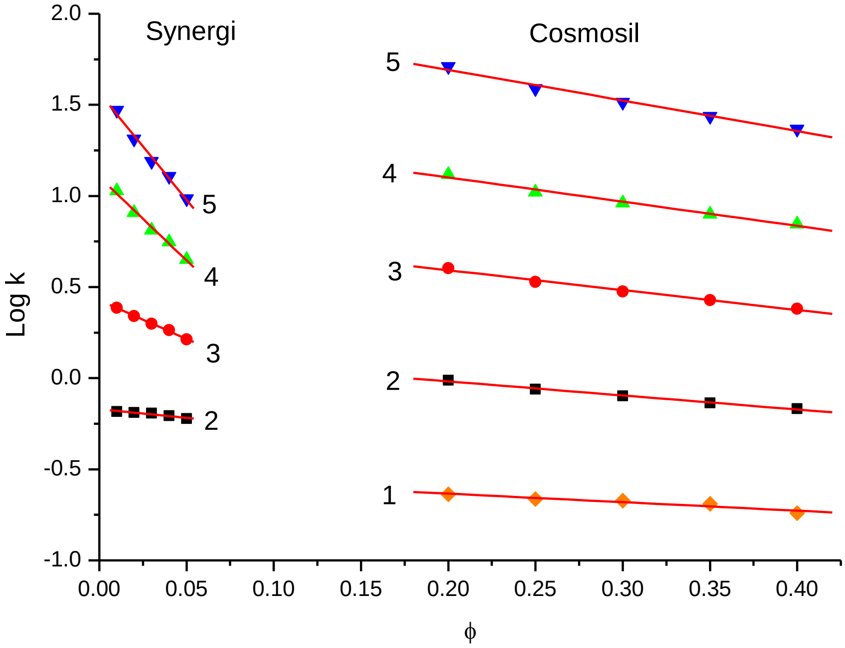
Figure 2



Synergi Polar RP

Cosmosil 5PBB

Figure 3


Synergi
Cosmosil
Log k
ϕ
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
0.00
0.05
0.10
0.15
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0.30
0.35
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5
4
3
2
5
4
3
2
1

Figure 4.

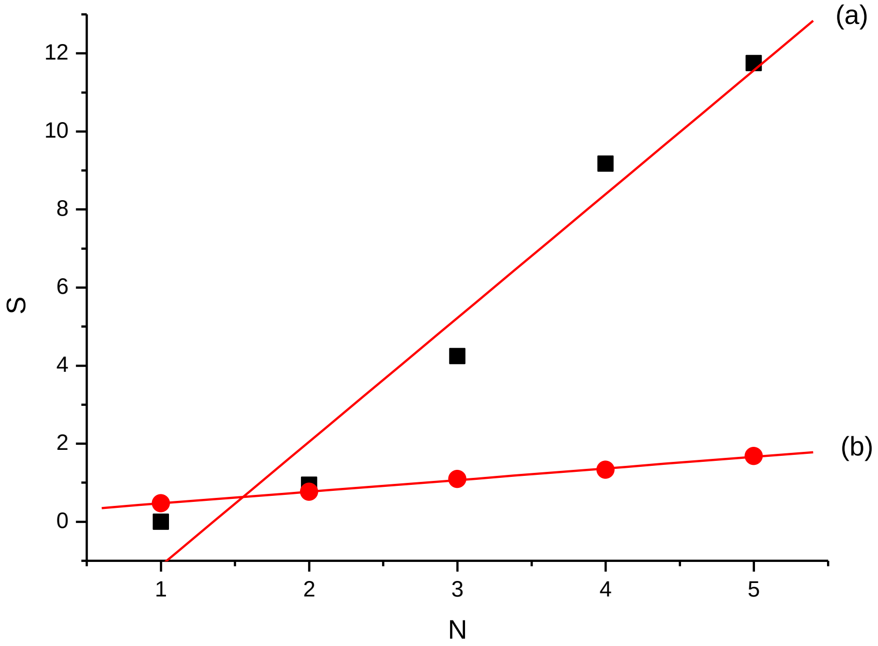


Figure 5a

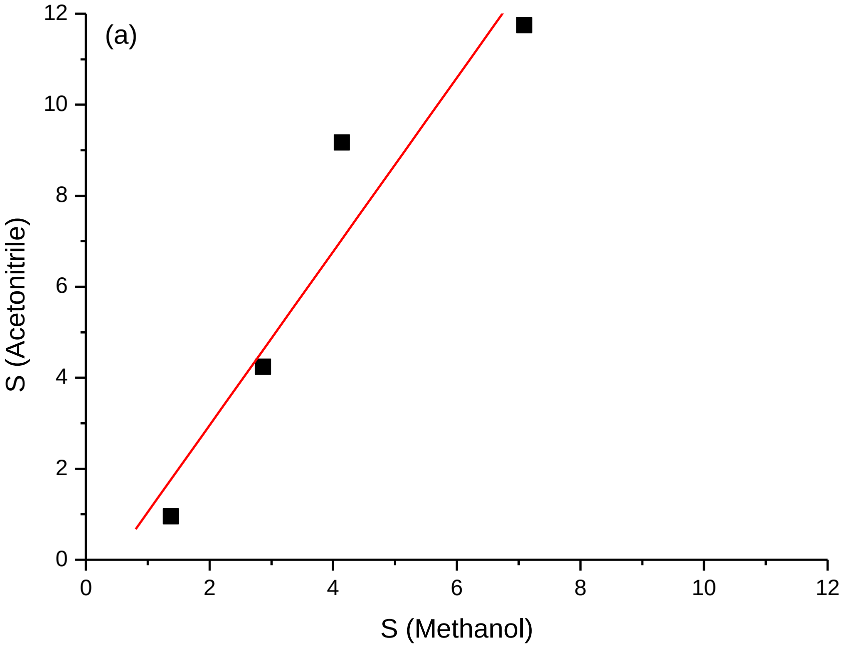
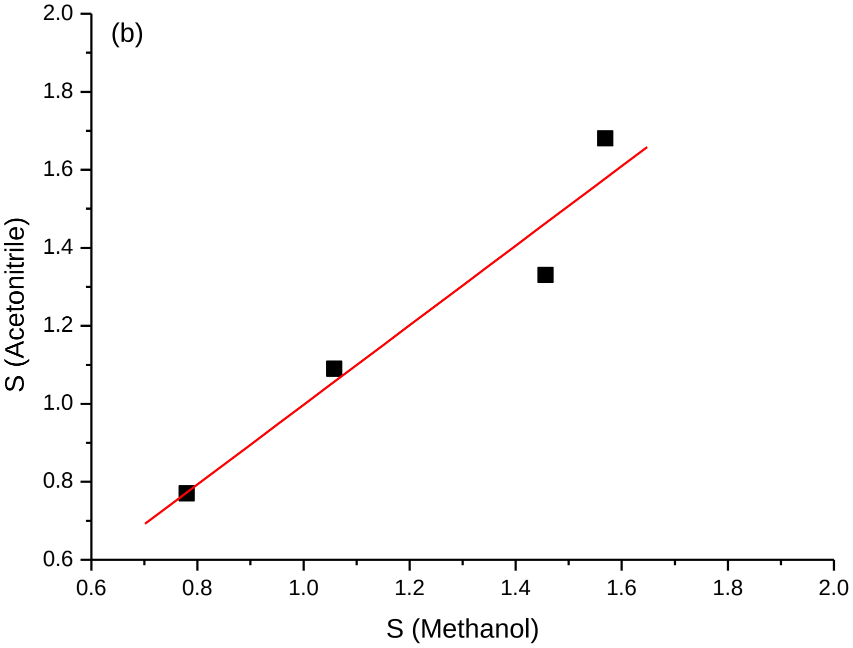


Figure 5b


(b)
S (Acetonitrile)
S (Methanol)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0