Determination of physico-chemical constants in sorption of polycyclic aromatic compounds to soil organic matter

-investigated by use of soil organic matter HPLC columns

Ph.D. Thesis

by

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Preface

The current thesis presents results obtained during my Ph.D.-project carried out at Risø National Laboratory. I have been enrolled as a Ph.D.-student at Roskilde University. My Ph.D.-project has been financed by The Danish Environmental Research Programme as a part of the Center for Biological Processes in Contaminated Soil and Sediment.

Many people has supported me in various ways during my Ph.D.-projeckt:

At Risø I thank Anders Feilberg for his support with laboratory equipments and being an inspiring and good colleague and Helge Egsgaard for interesting dialogues and for showing me remarkable and interesting chromatographic results. Walther Bastbjerg is acknowledged for introducing me to Size Exclusion Chromatography, and for his help in this area.

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I am grateful to laboratory apprentices, employees and students in the former Biogeochemistry Programme at Risø, and to the Plant Nutrition Programme for having more or less adopted me after I returned from my leave. Also thanks to the department administration staff for help and support and to the library staff at Risø for always being helpful.

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Finally, and most of all, I wish to thank my family, Anette and Mattias, my parents and my brother for their love and support when I needed it most.

Kristoffer Jonassen, Risø, December 2003.

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Dansk resumé

Optimering af HPLC kolonner modificeret med kemisk bundet humussyre er blevet udført med det formål at opnå det højest mulige indhold af humussyre og lavest mulige indhold af bindermateriale. Optimeringsprocessen har medført ændringer i såvel valg af kolonnemateriale (silikat) som fremstillingsmetoden under hvilken humussyren er bundet kemisk til silikatoverfladen.
Der er opnået forbedringer med hensyn til begge kriterier. I forhold til tidligere arbejder er mængden af humussyre næsten tredobbelt og samtidigt er mængden af bindermaterialet reduceret med 96 %.

En ny metode til bestemmelse af fordelingskoefficienten mellem organisk kulstof og vand (K_{oc}) ved hjælp af HPLC kolonne modificeret med humussyre er blevet udviklet og valideret.

Bindingen af 69 polycykliske aromatiske forbindelser (PAC) til humussyre er blevet målt ved hjælp af ovennævnte HPLC kolonne indeholdende en kommerciel tilgængelig humussyre fra Aldrich. Efterfølgende er fordelingskoefficienten log K_{oc} blevet beregnet. Generelt øges bindingen af PAC til humussyre med størrelsen (antallet af ringe) af PAC-molekylerne. Tilstedeværelsen af heteroatomer (f.eks. nitrogen) i ringstrukturen influerer også på bindingen, idet polære azaarener (N-PAC) udviser lavere sorption (op til 0,8 log enheder) end de analoge ikke polære PAH. Der ses tydelig substituenteffekt med øget sorption ved hydrofobe substituenter og faldende sorption ved hydrofile substituenter.
For de usubstituerede PAH og N-PAC er sorptionen også målt på en kolonne indeholdende organisk materiale isoleret ved demineralisering af jord fra pløjelaget (Ap horisonten) fra en mark ved Ulsted (Nordjylland). Bindingen af PAC (især PAH) til denne type organisk materiale er højere end til Aldrich humussyre, hvilket primært skyldes højere molekylvægt i Ulsted materialet end Aldrich humussyren.

Der er god overensstemmelse mellem log K_{oc}-værdier bestemt ved hjælp af HPLC-metoden og tilgængelige litteraturværdier, hvilket understøtter anvendelsen af den her benyttede metode. For en lang række af de undersøgte stoffer er der ikke tidligere publiceret data for bindingen til organisk materiale.

HPLC-metoden gør det muligt relativt enkelt at måle miljøpåvirkninger af sorptionen. Ved at måle tilbageholdelsen af PAC på HPLC-kolonnen ved forskellige temperaturer er ændringerne i enthalpi (ΔH) og entropi (ΔS) som følge at bindingsprocessen bestemt for 5 PAHer, 8 N-PACer, dibenzofuran, dibenzothiophen og en række substituerede anthracener. Kun meget få værdier for enkelte af de udvalgte stoffer er tidligere publiceret. For alle stofferne er både ΔH og ΔS negative, hvorfor bindingsprocessen til humussyre er en spontan exoterm proces der medfører en højere orden i systemet.
Fordelingskoefficienter beregnet udfra de fundne ΔH- og ΔS-værdier stemmer godt overens med de direkte målte værdier. Log K_{oc} for de to- og tre-ringede forbindelser samt pyren er beregnet ved temperaturer ned til frysepunktet, et fald i temperaturen med 30 °C medfører stigninger i K_{oc} med op til én log-enhed.

Ændringer af pH i systemet fra pH 7,8 til 2,5 medfører en nærmest lineær forøget tilbageholdelse af de to- og tre-ringede PAHer med op til 3 gange så stor tilbageholdelse ved pH 2,5 som ved pH 7,8. For quinolin og isoquinolin ses den maksimale tilbageholdes mellem pH 3,8 og 4,5, altså lidt under deres pK_a-værdier men omkring pK_a-værdien for humussyren. Den maksimale målte tilbageholdelse var næsten 6 gange så høj som ved pH 7,8. Ved pH 2,5 var tilbageholdelsen omtrent det dobbelte af tilbageholdelsen ved pH 7,8. Variation af ionstyrken i systemet viser at tilbageholdelsen af PAC stiger med faldende ionstyrke. Dog ses den laveste tilbageholdelse ved anvendelse af en upufferet mobilfase.

Resultaterne viser, foruden anvendeligheden af HPLC-metoden med kemisk bundet humussyre, at bindingsprocessen involverer flere molekylære mekanismer. Disse mekanismer er både ikke specifikke vekselvirkninger som hydrofob adsorption og fordeling[†], samt mere specifikke vekselvirkninger, f.eks. brintbinding, ionbinding, dipol-dipol og van der Waalske kræfter.

[†] Oversættelse af det engelske "partitioning".

List of Abbreviations

Abbreviations common used in this thesis, listed in alphabetical order.

BET:	Specific surface area (Brunauer, Emmett and Teller)
C_{sol}:	Aqueous solubility
c_{OC}:	Concentration of a compound sorbed into Soil Organic Matter normalised to Organic Carbon
c_{SOM}:	Concentration of a compound sorbed into Soil Organic Matter
c_{w}:	Concentration of a solute in the water phase
DOM:	Dissolved Organic Matter
f_{OC}:	Fraction of Organic Carbon
f_{SOM}:	Fraction of Soil Organic Matter
FTIR:	Fourier Transformation Infrared Spectroscopy
GC:	Gas Chromatography
GC/MS:	Gas Chromatography with Mass Spectrometry detector
HA:	Humic acid
HPLC:	High Performance Liquid Chromatography
HS:	Humic Substances
ID:	Inside Diameter of a column
k':	Column coefficient on the HPLC column ($k' = (t_r - t_0)/t_0$)
k'_{blank}:	Column coefficient on the blank column using water a mobile phase
k'_{SOM}:	Column coefficient on soil organic matter column using water a mobile phase
k'_{w}:	Column coefficient using water a mobile phase
K_{oc}:	Partitioning coefficient between soil and water
K_{oc}:	Partitioning coefficient between organic carbon and water
K_{ow}:	Partitioning coefficient between 1-octanol and water
ln:	The natural logarithm function with the base e (2.7182818…)
log:	Logarithm function with the base 10
MeOH:	Methanol
MS:	Mass Spectrometry
N-PAC:	Azaarenes, N-heterocyclic PAC
NMR:	Nuclear Magnetic Resonance
NOM:	Natural Organic Matter
O-PAC:	Oxarenes, O-heterocyclic PAC
OC:	Organic Carbon
$\mathrm{OC}_{\mathrm{carrier}}$:	Organic Carbon origin from binding material (carrier carbon)
$\mathrm{OC}_{\mathrm{SOM}}$:	Organic Carbon origin from Soil Organic Matter, e.g. humic acid.
OECD:	Organisation for Economic Co-operation and Development
OM:	Organic Matter

PAC:	Polycyclic Aromatic Compounds
PAH:	Polycyclic Aromatic Hydrocarbons
PAMW:	Peak Average Molecular Weight
R:	Linear regression correlation coefficient
R:	The "gas constant" (8.31451 J mol^{-1} K^{-1})
RSC:	Refractory Soil Carbon
rsd:	Relative standard deviation
S-PAC:	Thiaarenes, S-heterocyclic PAC
sd:	Standard deviation
SEC:	Size Exclusion Chromatography
SOM:	Soil Organic Matter
T:	Absolute temperature (in K)
t_R:	Retention time for a compound through a column
t_0:	Migration time through a column
UV:	Ultraviolet spectroscopy
V:	Volume
V_M:	Volume of the mobile phase in the column
V_S:	Volume of the stationary phase in the column
V_{SOC}:	Volume of organic carbon origin from soil organic matter in the stationary phase in the column
V_{Tot}:	Total column volume
X-N-PAC:	Substituted N-PAC
X-PAH:	Substituted PAH
ΔG:	The free Gibbs energy
ΔG_{sorp}:	The free Gibbs energy of the sorption process
ΔH:	Enthalpy changes
ΔH_{sorp}:	Enthalpy changes for the sorption process
ΔS:	Entropy changes
ΔS_{sorp}:	Entropy changes for the sorption process
π_{Ar}:	Lipophilic aromatic substituent constant. Describes the differences in K_{ow} between substituted benzene and benzene
ρ:	Density
%(V/V):	Fraction by Volume (in %)
%wt.:	Fraction by weight (in %)

1. Introduction

This Ph.D.-thesis describes and discusses experimental studies on sorption of polycyclic aromatic compounds (PAC) to humic substances (HS) using high-performance liquid chromatography (HPLC) columns modified with HS. Emphasis has been on optimising the HS-modified column materials both regarding the way to immobilise HS by cross-linking and by choice of silica base.

The advantage of using this HPLC method is obvious compared to common sorption experiments, involving an experimental part, a sample outtake and treatment part, and finally an analytical part. The method used in this thesis only involves the first and the last part as the experimental and analytical parts are combined.

OECD already accepts the use of HPLC in estimating the partitioning coefficient K_{oc} (OECD, 2001). In that method the stationary phase consists of silica and cyanopropyl, and log K_{oc} is calculated from the relative retention compared to the retention from compounds with known K_{oc} values. In this thesis log K_{oc}-values are calculated directly from the retention to humic acid in the HPLC column.

Polycyclic aromatic compounds are wide spread environmental polluters of both anthropogenic and natural sources. Their presence has been shown in air (Santodonato and Howard, 1981; Nielsen et al., 1986), groundwater (Pereira et al., 1987) and in both marine and freshwater environments (Blumer et al., 1977; Kozin et al., 1997). Important anthropogenic sources include incomplete combustion of fossil fuel, coke and asphalt production, oil refining, aluminium production and other industrial activities (Edwards, 1983; Seinfeld and Pandis, 1997; Kochany and Maguire, 1994; Pereira et al., 1988). Volcanic eruptions, forest fire and burning of other vegetation are among the natural sources. Contamination of soil with PAC is a result of both sources. Either by point sources (e.g. spill and leaking from storage facilities and pipelines, gas manufacturing, coal tar distillation and wood preservation treatment of railway sleepers with creosote) or diffuse sources from e.g. power plants and diesel engines (Nielsen et al., 1986). In Denmark approximately 300 coal tar and asphalt production sites are known (Jacqueline et al., 2002). Minor sources are metabolism of pesticides (Kuhn & Suflita, 1989) and pharmaceuticals containing aromatic parts (Bronwyn et al. 2000).

Polycyclic Aromatic Compounds are a group of compounds all consisting of two or more five and six membered rings in a fused aromatic system. Classifications of PAC are done after the homogeneity of the atoms in the fused ring system and exocyclic substitution. A list of the compounds used in this work including structure, CAS# and alternative names is given in Appendix 1.
The most abundant group of PAC is the non-polar polycyclic aromatic hydrocarbons (PAH). When released to the soil, PAH are strongly bound into the soil organic matter (SOM), reducing the spreading to ground water. Less abundant is the slightly polar groups of PAC e.g. endocyclic N-substituted azaarenes (N-PAC) and PAH with polar substitutes and the non-polar O-substituted oxyarenes (O-PAC) and S-substituted thioarenes (S-PAC). The polar compounds are less hydrophobic than PAH, and are therefore less susceptible to partitioning into SOM and consistently more mobiles in soil.

Several PAH are considered to be carcinogenic (IARC, 1983). Also N-PAC are carcinogenic having potencies similar to those of PAH (Jacob, 1996), although their effects and their metabolism could be different (IARC, 1983; LaVoie et al., 1993). Acute (narcotic) toxic effects of PAH and N-PAC increases with increasing number of rings in the molecules. In general PAH shows a higher toxicity than the analogue N-PAC, but PAH are also more lipophilic than N-PAC (Bleeker et al., 2002). Oxygenated metabolites seem to have lower acute toxic effect than their original compounds, but probably higher genotoxic effect (Bleeker et al. 1999; Müeller et al. 1996).

The sorption of organic chemicals to soil organic matter (SOM) as e.g. humic acid includes several specific (e.g. hydrogen bonding, ionic bonding, charge transfer, ligand exchange, dipole-dipole, van der Waals) and non-specific (e.g. hydrophobic adsorption and partitioning) sorption mechanisms (Senesi, 1993). Several models describing the sorption in soil are used in the literature, e.g. the linear model, Langmuir and Freundlich (Weber and Miller, 1988; Schwarzenbach et al., 1993). In the linear model the isotherm is linear at all concentrations and the partitioning coefficient K_d describes the ratio between the concentration of a compound sorbed to the soil matrix and the soil water concentration. For non-ionic organic compounds like PAC, SOM like humic acid is the dominating sorbent. Thus when the soil consists of a significant amount of SOM, K_d can be given in terms of the fraction of organic carbon (OC) in the soil (f_{oc}) and the partitioning coefficient between SOM normalised to OC and water: $K_d = f_{oc} \times K_{oc}$.

The sorption is affected by the conditions under which the processes are taking place. Typical environmental parameters affecting the sorption are temperature, pH and electrolyte concentration in the pore water. The HPLC method used in this work is extremely suitable in those kinds of investigations, since each parameter is easily controlled, either by changes in the mobile phase or column temperature and a large number of compounds can be examined in relatively short time.

Knowledge of physico-chemical properties is thus crucial in risk assessment modelling transport to ground water and ecotoxicological impacts in soils, see Figure 1.

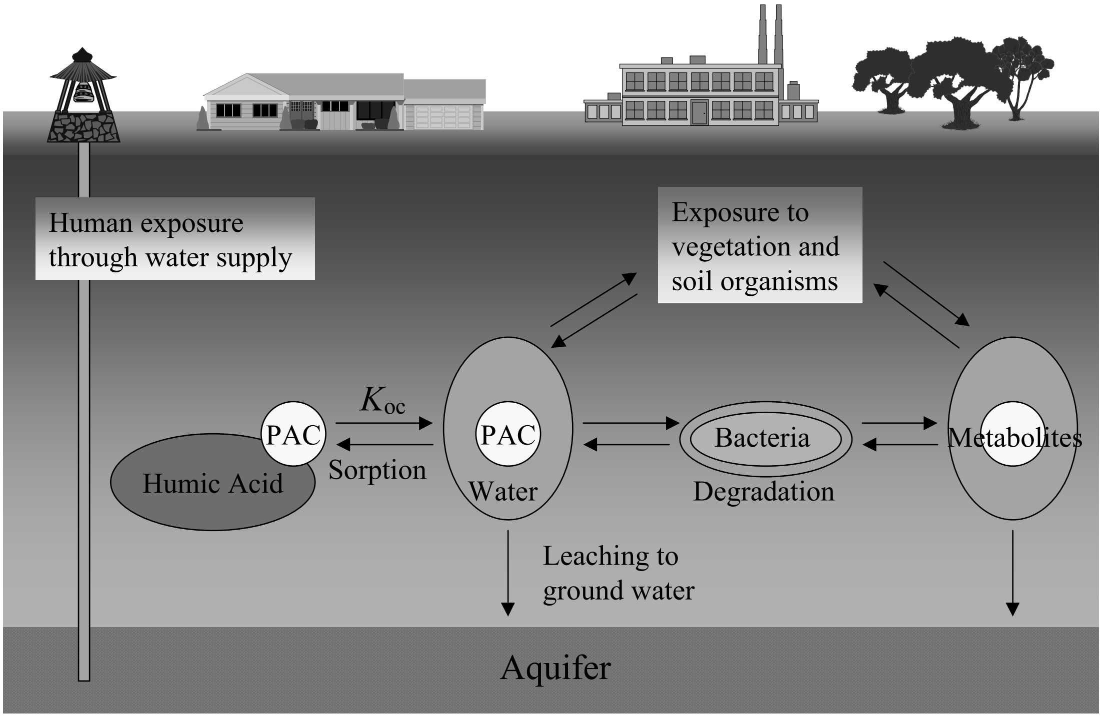


Figure 1.1. Schematically distribution of PAC in the soil environment.

The main purposes of the study are as follows.

- To optimise humic substance HPLC column materials for direct determination of partitioning coefficients between organic carbon (OC) and water (K_{oc}), describing sorption of PAC in soil. This step includes both preparation of humic acid coated columns and analysis of these column materials with respect to extent of coating, authenticity and durability.
- To develop and evaluate a model for experimental use of the HPLC column.
- To provide experimental values for K_{oc} for a variety of PAC having different physico-chemical properties.
- To provide experimental values for enthalpy (ΔH) and entropy (ΔS) changes during the sorption of different PAC.
- To describe the sorption process with special emphasis on the effect of environmental parameters as temperature, pH, and ionic strength.

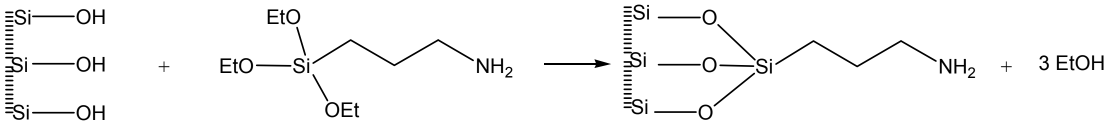
After this introduction and a presentation of the experimental work, the results and a discussion of the optimisation process of the HPLC column materials follows in Chapter 3. Results of the chromatographic work are presented in Chapter 4 and a validation of the HPLC method is carried out in Chapter 5. Discussion of chromatographic results with focus on sorption of PAC (thus determination of log K_{oc}) and how it is affected by environmental changes follows in Chapter 6-8. Determination of the thermodynamic constants (ΔH and ΔS) is discussed in Chapter 7 and the final conclusions and perspectives are given in Chapter 9. Finally a list of all PAC used here, characterisation of the humic substances used in this work, derivation of important equations, and two original papers are enclosed in Appendices.

2. Experimental

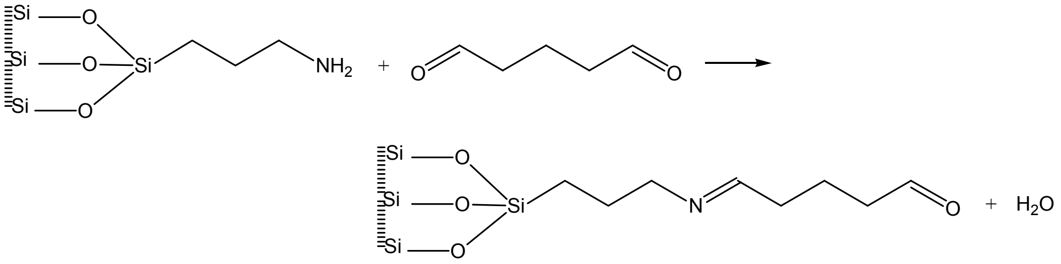
This chapter describes the experimental work forming the basis for the result presented and discussed in the following chapters.
It consists of two central parts. 1) First the optimisation process of column materials is described, followed by a short description of the methods used in characterisation of humic substances and column materials. 2) The use of HPLC column modified with humic substances, both regarding the measurements of sorption of PAC and calculations of K_{oc}, and the investigations of influence of environmental factors (temperature, pH and electrolyte concentration) on the sorption, including calculation of ΔH_{sorp} and ΔS_{sorp}.

2.1 Optimisation of SOM content on HPLC column silica material.

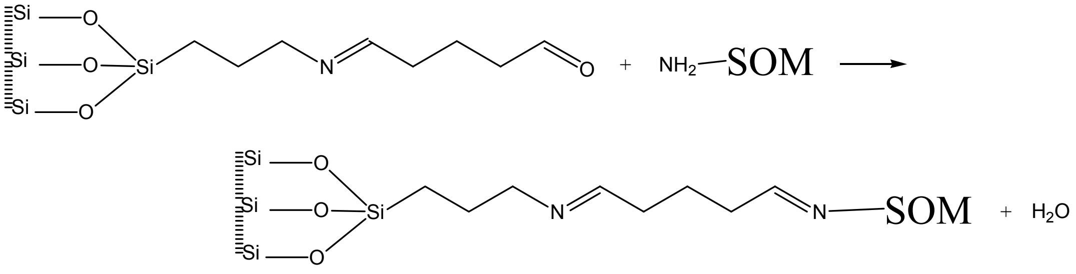
The column material used in previous works presented in Nielsen et al. (1997) based on a method described by Szabó and Bullmann (1994) was adopted for further development. This column material was prepared in four steps as described in the following using Nucleosil 50-10 (Macherey-Nagel, Düren, Germany) as silica base. The numbers in the name refer to the pore diameter (50 Å) and the particle size (10 μm). Earlier works suggested that there was a tenfold excess of organic carbon from binding material compared with the humic contribution (Kollist-Siigur et al. 2001).
Firstly silanol groups in the silica gel were alkylated with 3-aminopropyl triethoxysilane:



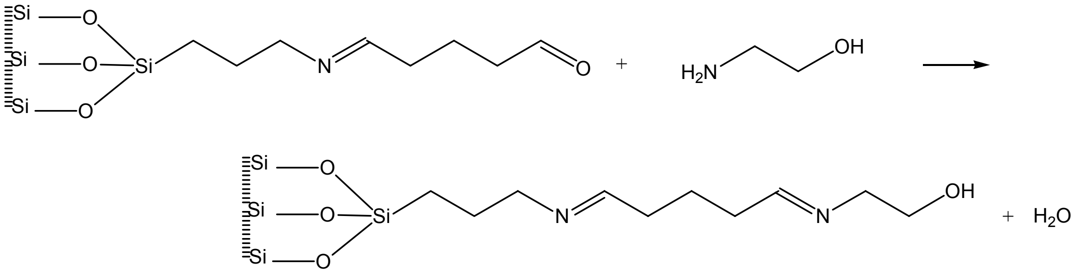
Secondly, a 10-fold excess of glutardialdehyde implies that only one of the carbonyl groups should be transformed to an imine:



In the third step humic acid was immobilised to the silica gel by imine formation between the aldehyde group and amino groups in the SOM molecules:



Where only amino groups were expected to be attached. Residual aldehyde groups were inactivated by imine formation with 2-aminoethanol in the fourth step:



Changes have been done firstly in choice of pore diameter in the silica gel used as basic material, secondly in increasing the amount of humic acid used in the synthesis. Finally both in the way to attach the humic acid to the silica gel and by changing the size of the particles.

2.1.1 Increase of pore diameter

The pore diameter in the silica gel was increased from 50 Å to 500 and 4000 Å respectively (Nucleosil 500-10 and Nucleosil 4000-10, from Macherey-Nagel).

The synthesis was the same for both silica gels and done in the same way as Nielsen et al. (1997).

A suspension of five grams of silica gel was stirred under nitrogen for four hours with 1.25 ml 3-aminopropyl triethoxysilane (pro analysi, >98%, Merck-Schuchardt, Hohenbrunn, Germany) dissolved in 25 ml toluene (pro analysi, Merck, Darmstadt, Germany). The white product was filtrated on glass filter (pore size 10-16 µm), washed twice with 25 ml toluene, 25 ml methanol (99.9% from LabScan, Dublin), 25 ml low organic water (Millipore), 25 ml methanol, and dried over night at 50°C.

The purified aminopropyl silica gel was added to 115ml 5 % aqueous solution of glutardialdehyde (dissolved from a 25 % aqueous solution, pro analysi, Merck-Schuchardt, Hohenbrunn, Germany) and stirred for four hours under nitrogen. Then filtrated on glass filter and washed several times with water (totally 175 ml) and dried over night at 50°C.

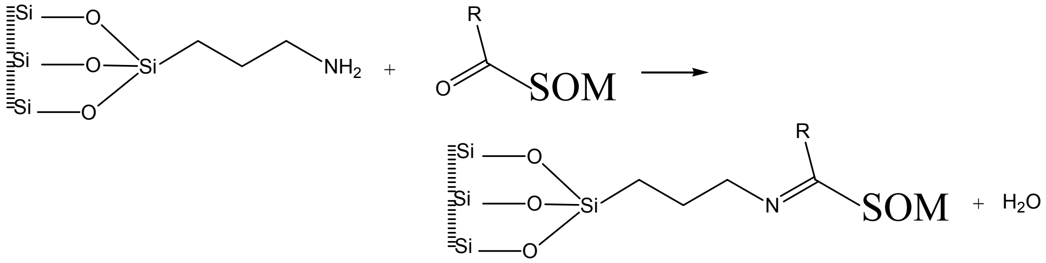
The pink product was added to a suspension of 500 mg humic acid (sodium salt, tech., Aldrich, Steinheim, Germany) in 50 ml water and stirred at room temperature under nitrogen for seven hours. The suspension was split up in to eight centrifuge tubes, the product spun down and separated from the supernatant; washed four times with 5 ml phosphate buffer (0.5 M, pH 7.5) and with water until the supernatant was colourless. The product was pressed as dry as possible and suspended in 50 ml of an aqueous solution of 0.1 M 2-aminoethanol (pro analysi, >99%, Merck, Darmstadt, Germany) buffered to 7.5 by phosphoric acid (85 %wt in water, Aldrich, Steinheim, Germany) to deactivate residual aldehyde groups attached to the silica gel. The suspension was stirred under nitrogen for four hours, filtrated on glass filter and washed five times with water before drying over night at 50°C.

2.1.2 Increasing amounts of humic material

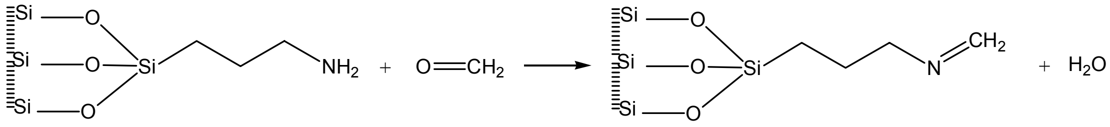
A procedure similarly to above using Nucleosil 500-10 as silica gel was used, the only change was to add five times the amount of humic acid as mentioned, e.g. 2.5 g, in the third step.

2.1.3 Simplifying the procedure

In this procedure the second step using glutaraldehyde was left out so humic acid was immobilised by imine formation between carbonyl groups in the humic acid and amino groups in aminopropyl silica gel.



As a result of this change, residual basic amino groups were deactivated by formaldehyde.

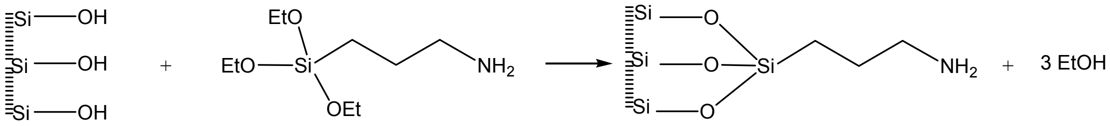


Two different kinds of silica gel were used in this work, the spherical Nucleosil 500-10 and Nucleprep 300-20 (Macherey-Nagel, Düren, Germany) with particle size between 15 and 25 µm and pore diameter 300 Å.

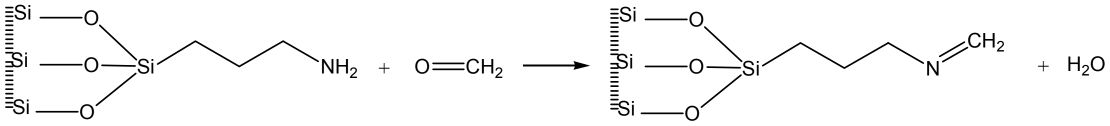
In the second step purified aminopropyl silica gel was added to a suspension of 500 mg humic acid in 50 ml water and stirred at room temperature under nitrogen for seven hours. The suspension was split into eight centrifuge tubes, the product was spun down and separated from the supernatant, washed four times with 5 ml phosphate buffer (0.5 M, pH 7.5) and with water (Millipore) until the supernatant was colourless. The product was pressed as dry as possible and suspended in 50 ml of an aqueous solution of 0.1 M formaldehyde (pro analysi, Merck, Darmstadt, Germany) adjusted to pH 8.0 by sodium hydroxide (pro analysi, Merck, Darmstadt, Germany). The suspension was stirred under nitrogen for four hours, filtrated on glass filter and washed five times with water before drying over night at 50°C.

2.1.4 Blank column

Material for a blank column was made in two steps using Nucleprep 300-20 as silica gel. Firstly silanol groups in the silica gel were alkylated with 3-aminopropyl triethoxysilane:



Secondly the amino groups were deactivated by formaldehyde.



Both steps were carried out as previously described.

2.1.5 Packing of HPLC columns

Column materials were packed in 12.5cm × 4.6mm (ID) stainless steel columns using a pneumatic HPLC pump from Knauer (Berlin, Germany).

Approximately 1.5 g of the column material was suspended, using ultra sound, in 12 ml of a 3:7 mixture of methanol (99.9%, LabScan, Dublin, Irland) and 2-propanol (pro analysi, Merck, Darmstad, Germany). The column and pre-column were filled with the same mixture and a reservoir was mounted on top of the pre-column. The suspension was filled in to the reservoir and methanol was added before closing the system to avoid air pockets in the finished column. Methanol (120 ml) was pumped through the system at a pressure at approximately 35 MPa. Pressure was stabilised by waiting five minutes before dismounting the system finally the column was closed.

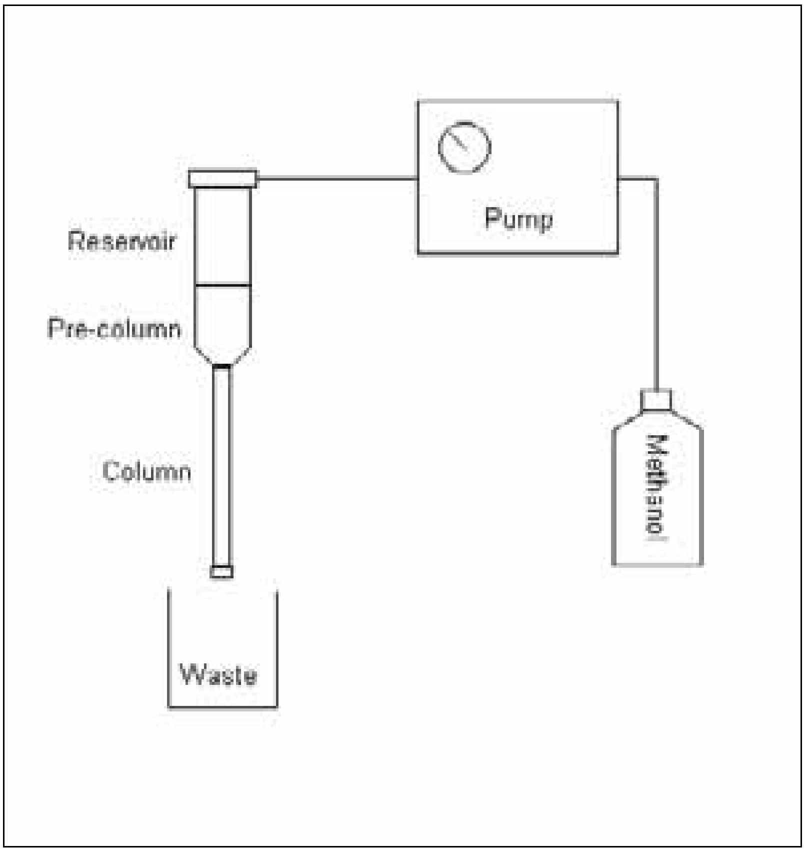


Figure 2.1. Schematic overview of the packing of HPLC columns. A slurry of column material in a mixture of methanol and 2-propanol is placed in the reservoir and pumped into the column at 35 MPa (350 bar) using methanol.

2.2 Characterisation of soil organic matter and column materials

Two kinds of soil organic matter were used. The commercially available Aldrich humic acid (HA), which has been the main SOM in this work, and Ulsted refractory soil carbon (RSC) isolated by demineralisation of a soil from Ap horizon from a field in northern Jutland, Denmark. Column materials containing those two SOM and blank column materials have been characterised.

2.2.1 Organic carbon and sulphur

The amount of carbon and sulphur in the humic substances and column materials were measured by combustion. The measurements were carried out using a Leco CS 200 instrument (St. Joseph, MI, USA) by combustion of column materials in pure O_2 and subsequent infrared analysis of the amount of CO, CO_2 and SO_2 (relative standard deviation of 0.5% for carbon and 1.5% for sulphur).

Organic carbon on column materials

The main criteria in evaluating column material were to measure the content of organic carbon of humic origin in the column materials. A determination of the sulphur contents in both the Aldrich humic acid and the column materials gave the opportunity to calculate the origin of organic carbon from binding material and humic acid respectively, as humic acid is the only source of sulphur. By use of sulphur as internal standard, organic carbon from Aldrich HA (Soil Organic Material) on the column material (OC_{SOM}) was calculated as

$$OC_{SOM} = \frac{OC_{HA}}{S_{HA}} \cdot S_{SOM}$$

OC_{HA} and S_{HA} are organic carbon and sulphur in Aldrich humic acid and S_{SOM} sulphur in the column material. Organic carbon from binding material is calculated as the difference between total organic carbon measured and OC_{SOM}.

For Ulsted RSC column material OC_{SOM} was calculated as the difference between total OC and OC in a sample in the previous step in the synthesis, thus the aminopropyl silica gel.

Additional measurements of total organic carbon have been made by measuring residual humic acid from synthesis of some selected column materials, by means of spectroscopy. Ultraviolet spectroscopic measurements were carried out on a Phillips PU 8800 UV/VIS spectrophotometer (Philips, Eindhoven, Netherlands) by measuring the absorption at 360nm of residual humic acid in an aqueous solution with known volume. Standard aqueous solutions between 0.5 and 5mg l^{-1} of the humic acid were used to obtain a linear regression line.

2.2.2 Fourier Transform Infrared Spectroscopy, FT-IR

Spectra of the soil organic matters and column materials were measured on a Perkin Elmer FT 2000 IR spectrometer using the KBr pressed pellet technique. Different amounts of the two SOM (0.71 mg of the Na-salt of Aldrich humic acid and 0.30 mg Ulsted refractory soil carbon) were separately mixed carefully with 300 mg dried KBr and pellet was pressed at 8 tons under evacuation of air. For column materials 1 mg were used. Samples from the final selected column material using Aldrich HA (before and after use), column material with Ulsted RSC and the blank column materials were measured. Pellets with Aldrich HA and column materials were dried over night at 110°C to avoid interference from water. Each pellet was scanned either 20 times (for SOM) or 360 (column materials) using a pure KBr pellet as reference.

2.2.3 Size exclusion chromatography, SEC

A modification of the method described by Ziechmann et al. (2000) was used applying a Shimadzu LC-10 HPLC system (Shimadzu, Kyoto, Japan) with an Optilab 5902 Interference Refractometer from Teactor (Höganäs, Sweden) as detector, cell length: 1.0 cm.

The size exclusion was done with a column temperature of 40°C using two polymer water based gel-columns in series, a TSK G 3000 PW followed by a G 2500 PW, both 30 cm x 7.5 mm (ID); from TosoHaas Corporation (Stuttgart, Germany). A mixture of 1:9(V/V) of methanol (99.9%, LabScan, Dublin, Ireland) and 0.05 M NaCl (pro analysi, Merck, Darmstad, Germany) in water (Millipore) was used as mobile phase at a flow rate of 1.0 ml min^{-1}. The SOM was dissolved in the mobile phase at concentrations between 0.5 and 2.5 mg OC ml^{-1} using acetone as internal standard (2 µl ml^{-1}), injection volume: 50 µl.

Surplus SOM from the column material synthesis was also injected in similar concentrations but dissolved in the phosphate buffer (pH 7.5) used in purification step in the synthesis of HPLC column materials.

Calibration was performed with dextran standards (Pharmacosmos, Viby Sj., Denmark) having Peak Average Molecular Weights (PAMW) of 1080, 4440, 9890, 21400, 43500, 66700 and 123600 Dalton, all dissolved in mobile phase at concentrations of approximately 1 mg ml^{-1}.

2.2.4 Determination of reactive primary amino-groups in Aldrich humic acid.

A 2.0mM crotonaldehyde (technical quality, Fluka, Buchs, Switzerland) solution was prepared by dissolving 0.2 mmol (16.5 µl) crotonaldehyde in 1ml methanol (99.9 %, LabScan, Dublin, Ireland) and adding low organic water (Millipore) up to a total volume of 100 ml. A suspension of 500 mg humic acid (Aldrich, Steinheim, Germany) in 50 ml of the crotonaldehyde solution was stirred under nitrogen at room temperature for 7.5 hours.

Two aliquots of the suspension, each of a few ml, were centrifuged for 15 minutes at 500 × g, and the supernatant filtrated through a 45μm PTFE filter before analysing.

The concentration of crotonaldehyde in the solution before and after the reaction was determined on HPLC (Shimadzu LC 10, Kyoto, Japan), using a C-18 HPLC column (25.0 cm and × 4.6 mm (ID) column packed with Nucleosil 100-5 C_{18} (Macherey-Nagel, Düren, Germany) at 35 MPa). A mixture of equal amounts of purified water (Millipore) and methanol (HPLC-grade, LabScan, Dublin, Ireland) was used as mobile phase. Crotonaldehyde was measured at 214 nm using a photodiodearray detector and standard aqueous solutions between 0.2 and 2 mmole l^{-1} were used to obtain a linear regression line.

2.2.5 Pyrolysis GC/MS of Ulsted refractory soil carbon

Small aliquots of a few hundred micrograms of the Ulsted RSC have been pyrolysed at both 694 and 804 °C on a Pytol A 85 Foil Pulse Pyrolyzer (Pyrol AB, Lund, Sweden). The pyrolysis products were separated on a Varian 3400 gas chromatograph (Varian, Walnut Creek, CA, USA), using a Varian WCOT fused silica 25m x 0.32mm (id) column with DB5 coating, and He as carrier gas. Injector temperature: 250°C, column temperature program: 60°C for five minutes, raised to 290°C at a rate of 15°C/min, and held at 290°C for 10 minutes; transfer line temperature was set to 280°C.

The detection was carried out using a Varian Saturn II GC/MS mass spectrometer, excluding masses with m/z below 45 and above 550. Electron ionisation was used as scan mode and the manifold temperature was 200 °C.

The total yield of pyrolysed sample was not determined.

2.3 Sorption of PAC to Soil organic matter

2.3.1 Compounds

All test compounds were dissolved in methanol (Lichrosolv 99.8%, Merck, Darmstad, Germany or 99.9%, LabScan, Dublin, Ireland) in typical concentrations of 0.04 g l^{-1} and UV-spectra were verified before use. Lists of all compounds including structural formula, nomenclature alternative to the one used in this work and source of the compound are found in Appendix 1. Most compounds were obtained from commercial sources. Preparation of other compounds is described in Nielsen et al. (1997) (bromopyrenes) and Kollist-Siigur et al. (2001). 9-anthracenecarboxamide, -carboxylic acid methyl ester, 9-formyl-, 9-methoxy-, 9-nitro- and 9-cyanoanthracene were provided by Ole Hammerich and Tove Thomsen, University of Copenhagen.

5H-phenanthro[4,5-bcd]pyran-5-one was prepared from phenanthrene-4-carboxylic acid after Lee-Ruff & Kruk (1990) and Togo et al. (1995) by photolysis: Phenanthrene-4-carboxylic acid (Sigma-Aldrich, Millwaukee, WI, USA), Iodide (unknown source) and Lead tetraacetate (ICN Biomedicals Inc., Aurora, OH, USA) were dissolved in 50 ml 1,2-dichloroethane (pro. analysis, Merck-Schuchardt, Hohenbrunn, Germany) in a ratio of 1:2:2 (0.05 mmole phenanthrene-4-carboxylic acid, 0.1 mmole I_2, and 0.1 mmole $Pb(Ac)_4$), split into ten pyrex preparation tubes with screw cap for photolysis. The tubes were, placed in a rotor, irradiated by a mercury-vapor lamp (Ace-Hanovia high-pressure quartz mercury-vapor lamp 7825-34, 450W, 135 V, Ace Glass Inc., Vineland, NY, USA) in water at room temperature for 30 minutes. The lots were mixed in a separatory funnel, washed three times (1:1 V/V) with 0.01 M $NaHCO_3$ in low organic carbon water (Millipore) and five times (1:1 V/V) with low organic carbon water (Millipore) to remove surplus phenanthrene-4-carboxylic acid together with iodide, lead, and acetate. Identified according to literature (Pitts et al. (1982) and Glover et al. (1981)) by UV, MS, FT-IR and melting point.

2.3.2 Mobile phases

Mixtures of methanol (99.9%, LabScan, Dublin, Ireland) and water (Millipore) with different methanol volume fractions were used as mobile phase. Mobile phases were buffered to pH 7.0 with KH_2PO_4 (pro analysi, Merck, Darmstadt, Germany) and NaOH (pro analysi, Merck, Darmstadt, Germany), having phosphate concentration of 35mM. (For mobile phases with 0.75 and 0.85(V/V) fractions of methanol, the phosphate concentration was 20 mM, to avoid salt precipitation. A test has shown that this has negligible effects on the sorption as long as the total phosphate concentration > 10mM.)

Mobile phases were changed daily and degassed using ultrasound (Bransonic Ultrasonic Cleaner 2210, Bransonic Ultrasonic Corporation, Dansbury, CT, USA) for minimum 10 minutes (90 W, 47 kHz) after mixing.

2.3.3 Instrumentation

A Shimadzu LC-10 HPLC system (Shimadzu, Kyoto, Japan) with photodiode array detection, thermostatic controlled column oven and auto injection was used.
In all experiments (except temperature variation and size exclusion chromatography) the column temperature was held at 25°C. Typical injection volume was between 20 and 50 µl given a load of polycyclic aromatic compounds between 0.2 and 2 µg per injection. Compounds were controlled and identified by means of UV-spectroscopy.

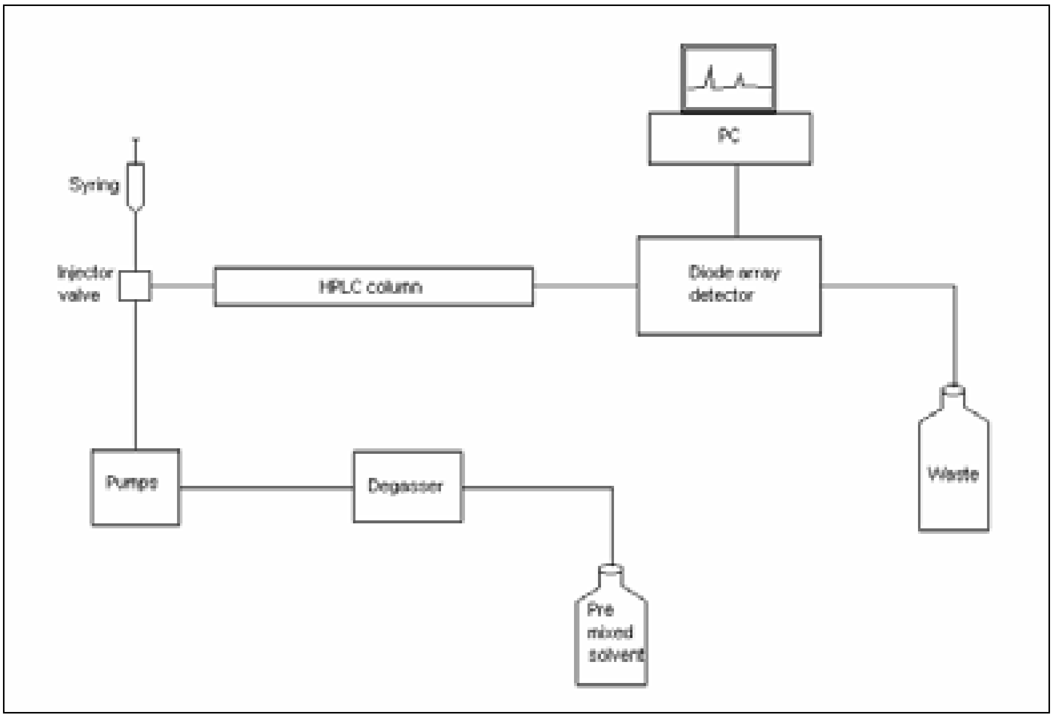


Figure 2.2. Flow diagram of the HPLC set-up used in this work. Premixed solvents were used as mobile phases to avoid trouble with air by low pressure mixing of methanol and the aqueous phosphate buffer. Another advantage was the knowledge of the exact pH value in the mobile phase entering the column.

2.3.4 Determination of column coefficients

SOM column

Suitable mixtures of three or four PAC at approximately 10 mg l^{-1} in methanol were injected and the retention time (t_R) of each compound was measured at least twice at each mobile phase. The mean value of the two measurements was used for further calculations. Relative standard deviations (rds) on the mean values were in general less than 1%, even though values of 5% are observed.

The migration time (t_0), measured as the time it took for water to migrate through the column, was measured minimum ten times for each mobile phase, by injection of water (Millipore) uniformly distributed through out the measuring series. The mean value (rds<1.6%) was used for determination of column coefficients $\left(k' = \frac{t_R - t_0}{t_0} \right)$. Relative standard deviation on k' where calculated from rsd from the mean values of t $\left(rsd_{k'} = \sqrt{(rsd_{t_R})^2 + (rsd_{t_0})^2} \right)$.

Mixtures of methanol and water with a methanol volume content of 0.35 to 0.85 were used as mobile phases.

Blank column

The compounds dissolved in methanol (40 mg l^{-1}), were injected individually on to the blank column to avoid insufficient separation because of short retention times. Injection volumes were regulated so the loaded amount of each compound was similar to what was used on the humic acid column. All measurements were repeated for each mobile phase, mean values of retention times (rsd<4.8%, in general below 1%) were used for calculation of k'. Migration times were measured using water as described for the humic acid column (rsd < 1.6%) and the same calculations of rsd on k' were made.

As mobile phases, mixtures of methanol and water with a methanol volume content of 0.05 to 0.50 were used.

2.3.5 Direct determination of log K_{oc}

The main idea in this work was to use the humic acid HPLC column in a direct determination of log K_{oc} for polycyclic aromatic compounds (PAC). Measuring the retention times for the various compounds through the column at a series of mixtures of water and methanol makes it possible, by linear extrapolation, to estimate capacity coefficients for each compound in a system using a mobile phase without methanol (k'_w) (Snyder et al., 1979; Szabo and Bulman, 1994; Gawlik et al., 1997). Knowing k'_w for both the humic acid column (k'_{SOM}) and the blank column (k'_{blank}), log K_{oc} is calculated by the equation below (Jonassen et al., 2003) See Appendix 3 for derivation of the equation.

$$\log K_{oc} = \log\left(\frac{\left(k'_{SOM} - k'_{blank}\right) \cdot \mathrm{V}_M}{\mathrm{V}_S \cdot \rho \cdot OC_{SOM}}\right) \qquad \text{(Eq. 2.1)}$$

Where $\mathrm{V}_M = t_0 \cdot \text{flow rate}$, is the volume of the mobile phase in the HPLC column, $\mathrm{V}_S = \mathrm{V}_{\mathrm{Tot}} - \mathrm{V}_M$ is the volume of the stationary phase, $\mathrm{V}_{\mathrm{Tot}}$ the total inner volume of the HPLC column and ρ the density of the column material.

The columns used in this work all have length of 12.5 cm and an inner diameter of 4.6 mm, resulting in a total volume of 2.08 cm^3.

Soil organic matter carbon content ($\mathrm{OC}_{\mathrm{SOM}}$) in the Aldrich HA HPLC column was before use measured to 1.91 %wt, but as humic acid was released from the column during use, this value cannot be expected to be accurate at all times. After measurements were finished $\mathrm{OC}_{\mathrm{SOM}}$ was therefore measured again, this time to 1.08 %wt. As an estimate for the "true" value the mean value of the two measurements (1.50 %wt) is used as $\mathrm{OC}_{\mathrm{SOM}}$ in the calculations of log K_{oc}. This give a correction of 0.1 on log K_{oc} compared to the values obtained using 1.91 %wt.

Soil organic matter carbon content in the Ulsted RSC HPLC column was measured to 4.44 %wt.

2.3.6 Determination of column coefficients

The retention of PAC dissolved in methanol was measured on the two columns (humic acid column and blank column) as described above. Migration time (t_0) was also measured both for determination of column coefficients $\left(k' = \frac{t_R - t_0}{t_0}\right)$ and to control the stability of the HPLC system. Relative standard deviation on k' was calculated from rsd from the mean values of t $\left(rsd_{k'} = \sqrt{\left(rsd_{t_R}\right)^2 + \left(rsd_{t_0}\right)^2}\right)$.

2.3.7 Linear determination of k'_{w}

The ideal concept has obviously been to measure the retention on the column using pure, or buffered, water as mobile phase. Because of low solubility in water and high affinities for the column materials, such measurements have been impossible, or highly time demanding. The work was therefore carried out at several different mixtures of water and methanol; and a linear correlation between log k' and the fraction of methanol content in mobile phases was made according to e.g. Szabo et al. (1999). The values of log k'_w using buffered water on both humic acid and blank columns were calculated as the intercept with the ordinate on the plots (Figure 2.3 exemplifying the correlation for some selected PAH). Correlation was carried out in Microsoft Excel by the “Linest” function (which uses the least squares method in calculating the straight line best fitting the data points). Standard deviation on the intercept was calculated during the linear correlation and used for calculations of sd on log K_{oc}.

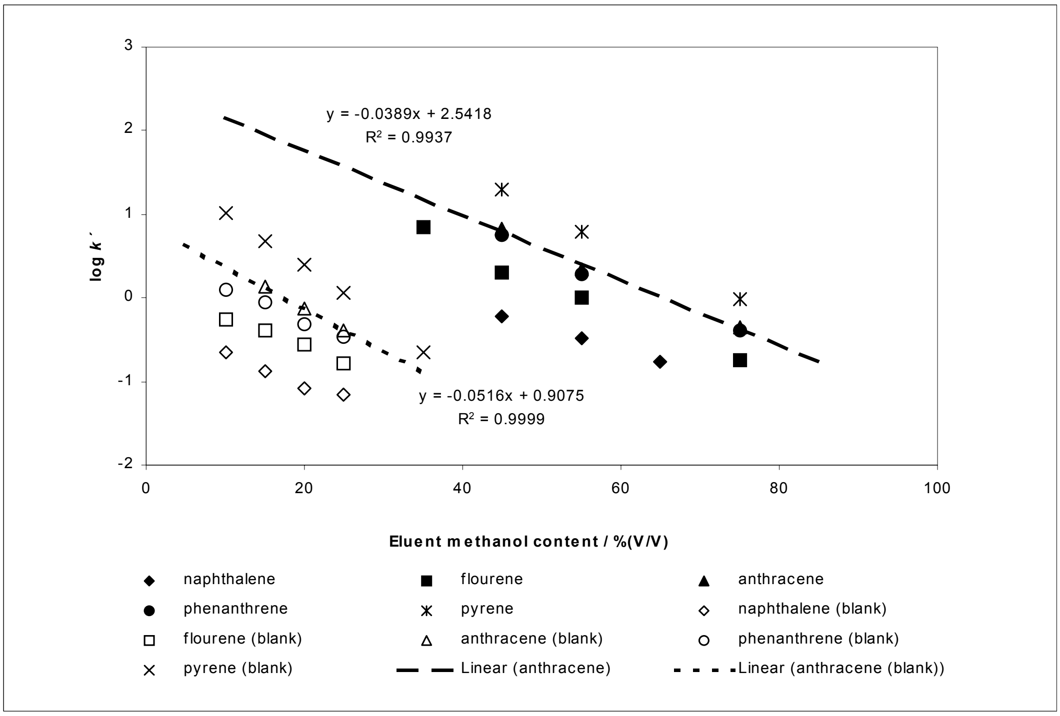


Figure 2.3. Linear determination of log k'_{w} on both the Aldrich humic acid column (closed marks) and the blank column (open marks) exemplified by five PAH. The linear regression lines for anthracene on the two columns are included.

2.3.8 Calculation of standard deviation on log K_{oc}

Standard deviations on the direct determined values of log K_{oc} was calculated from sd on log k'_{w} achieved from the linear extrapolations on both humic acid and blank columns $\left(sd_{\log K_{oc}} = \log\sqrt{\left(sd_{k'_{SOM}}\right)^2 + \left(sd_{k'_{blank}}\right)^2}\right)$.

The standard deviation on the other terms in Eq. 2.1 is neglected as either being small compared to $sd_{\log k'}$ (V_M, V_S and ρ) or impossible to quantify (the used mean value of OC_{SOM} for the Aldrich HA column).

2.3.9 Indirect determination of K_{oc}

For the four and five ring PAC (except pyrene), 9,10-dibromoanthracene and the mono- and di-bromopyrenes it was impossible to use the direct method to determine K_{oc}. This was probably caused by insufficient solubility of the compounds in the mobile phase resulting in precipitation when lowering the methanol content. The partitioning coefficients were therefore calculated by an empirical correlation found between log K_{oc} from all bi-, tri- and tetracyclic compounds without oxygen-containing substitutes determined by directed determination, and log k' measured on the humic acid column using a mobile phase with a methanol fraction volume of 0.75. The correlation is based on 18 compounds giving the empirical equation log $K_{oc} = 2.2483 \times \log k'_{75} + 5.2002$ with a correlation coefficient (R^2) of 0.79.

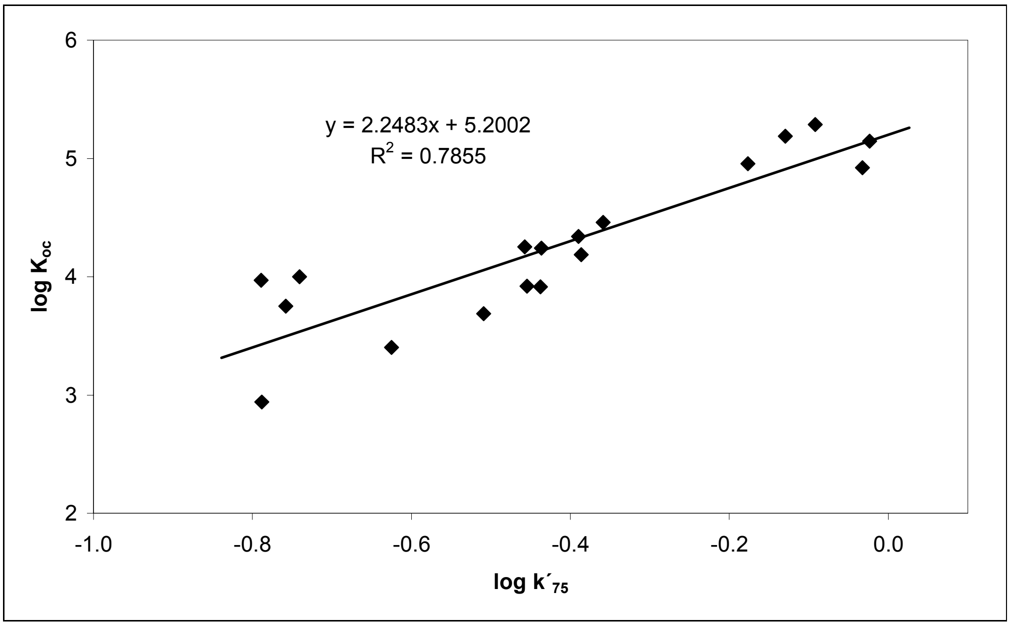


Figure 2.4. Linear correlation between log K_{oc} determined by directed determination and log k'_{75} based on 18 bi-, tri- and tetra-cyclic compounds without oxygen-containing substitutes. The correlation was used in calculations of log K_{oc} for the four and five ringed compounds (except pyrene), 9,10-dibromoanthracene and the bromo- and dibromo-pyrenes.

2.4 Temperature variation and determination of ΔH_{sorp} and ΔS_{sorp}.

The retention of non-substituted compounds and a series of substituted anthracenes were measured at different temperatures using several different mobile phases (see Table 2.1) holding pH and phosphate concentration constant at 7.0 and 35 mM respectively. Injection volume was 20 µl and the flow rate 1.0 ml min^{-1}. Temperature was decreased in steps of either 5 (or 3)°C and retention times measured twice for each compound (injected individually) at four temperatures.

Table 2.1. Temperature ranges and mobile phases under which the sorption of PAC has been investigated. In all mobile phases pH was 7.0 and the phosphate concentration 35 mM.

	Mobile phase methanol fraction (V/V)			
Compounds	**0.25**	**0.45**	**0.65**	**0.85**
PAH				
Naphthalene	20-35°C	20-35°C	17-30°C	
Fluorene	20-35°C	20-35°C	17-30°C	
Anthracene		20-35°C	17-30°C/ 20-35°C	
Phenanthrene		20-35°C	17-30°C	
Pyrene		20-35°C	17-30°C	20-35°C
N-PAC				
Quinoline	20-35°C	20-35°C	17-30°C/ 20-35°C	
Isoquinoline	20-35°C	20-35°C	17-30°C	
4-azaflourene	20-35°C	20-35°C	17-30°C	
Acridine	25-35°C	20-35°C	17-30°C	
5,6-benzoquinoline	25-35°C	20-35°C	17-30°C	
7,8-benzoquinoline	25-35°C	20-35°C	17-30°C	
Phenantridine	25-35°C	20-35°C	17-30°C	
Carbazole	20-35°C	20-35°C	17-30°C	
O,S-PAC				
Dibenzofuran	20-35°C	20-35°C		
Dibenzothiophene	25-35°C	20-35°C		
X-anthracenes				
9-acetylanthracene			20-35°C	
9-anthracene carboxamide			20-35°C	
9-anthracene carboxylic acid methyl ester			20-35°C	
9-bromoanthracene			20-35°C	
9-chloroanthracene			20-35°C	
9-cyanoanthracene			20-35°C	
9-formylanthracene			20-35°C	
9-metoxyanthracene			20-35°C	
9-methylanthracene			20-35°C	
9-nitroanthracene			20-35°C	
9,10-dibromanthracene			20-35°C	
Anthraquinone			20-35°C	

Retention times (t_R) were measured twice for each compound at each temperature, and column capacities (k') calculated for each measurement using the time for the solvent peak as dead time (t_0). Mean value of the k'-values at each temperature (rsd between 0.05% and 4.79%, with values typically below 1.5%) was used for further calculations.

The natural logarithms of k' for each series were plotted against the reverse absolute temperature (exemplified in Figure 2.5 for the small PAH) all showing a linear relationship. This it not surprising as the sorption process is a thermodynamic process of which the equilibrium constant is inversely linear related to the temperature according to van’t Hoff (Atkins, 1994).

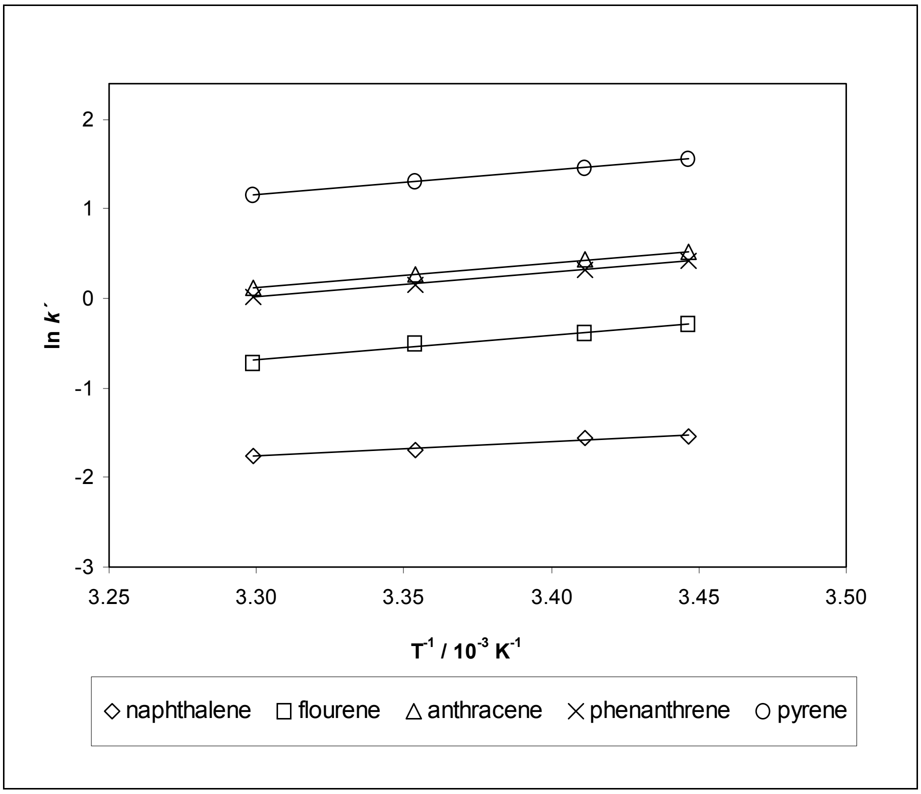


Figure 2.5. The relationship between ln k' and the reverse temperature illustrated for five PAH. Mobile phase with 65 %(V/V). Similar relationships (not shown) were achieved from all the combinations of compounds and mobile phases shown in Table 2.1

From Appendix 4 it is seen, that it is necessary to take the ratio (φ) between the volumes of the solid phase (V_S) and the dead volume (V_M) into account calculating ΔH_{sorp} and ΔS_{sorp} from the shown relationship. Using this knowledge, an expression similar to what is seen in the literature, e.g Stalcup et al. (1988) and Tran et al. (2001), is achieved (Eq. 2.2).

$$\ln k' = -\frac{\Delta H_{sorp}}{R \cdot T} + \frac{\Delta S_{sorp}}{R} + \ln\left(\frac{\mathrm{V}_{SOC} \cdot \rho}{\mathrm{V}_M}\right) \qquad \text{(Eq. 2.2)}$$

Because PAC primarily sorbs to organic material, volume of the stationary phase is reduced to the volume of organic carbon origin from SOM on the stationary phase ($\mathrm{V}_{SOC} = \mathrm{V}_S \cdot OC_{SOM}$). So the factor $\varphi_{SOM} = \frac{\mathrm{V}_{SOC} \cdot \rho}{\mathrm{V}_M}$ is used, as it is only the sorption to organic material, not the total column that is to be quantified.

Enthalpy changes for the sorption process (ΔH_{sorp}) are calculated as minus the slope (thus the first derivative of Eq. 2.2 with respect to T^{-1}) in (Figure 2.5) times the gas constant (R):

$$\frac{d \ln k'}{d\left(T^{-1}\right)} = -\frac{\Delta H_{sorp}}{R} \Rightarrow \Delta H_{sorp} = -\frac{d \ln k'}{d\left(T^{-1}\right)} \cdot R \qquad \text{(Eq. 2.3)}$$

The entropy changes (ΔS_{sorp}) are calculated from the intercept with the ordinate.

$$\Delta S_{sorp} = R \cdot \left(\ln k'_{\mathrm{int}} - \ln \varphi_{SOM}\right) \qquad \text{(Eq. 2.4)}$$

2.5 The influence of other environmental factors on the sorption of PAC

2.5.1 Variation of pH

To study the effect of pH on the sorption of bi- and tri-cyclic PAH as well as the bicyclic azaarenes, an experiment using mobile phases with different pH values was set up. Seven series were made decreasing pH from 7.8 to 2.5, the other parameters: methanol fraction (0.35(V/V)), phosphate concentration (35mM, KH_2PO_4/H_3PO_4), column temperature (25 °C) and flow rate (2.0 ml min^{-1}) were constant. Retention times were measured twice for each compound (quinoline, isoquinoline, naphthalene, flourene, anthracene and phenanthrene) at each pH value. All compounds were injected individually. The solvent peak from each injection was measured as t_0.

2.5.2 Electrolyte concentration

The effect of electrolyte concentration was investigated for a series of bi-, and tri-cyclic PAH and N-PAC by measuring the retention times changing the total phosphate concentration in the mobile phase. All compounds were injected individually twice and t_0 was defined as the time for the solvent peak for each injection. Besides the phosphate concentration (KH_2PO_4), pH and the methanol fraction of the mobile phase were the only other parameters that were changed. Column temperature was 25 °C and flow rates 2.0 ml min^{-1}.

At pH 6.0 the retention of naphthalene, flourene, anthracene, phenanthrene, quinoline, isoquinoline, acridine, 5,6-benzquinoline, 7,8-benzoquinoline and phenantridine were measured changing phosphate concentration from no electrolyte to 35 mM having a methanol fraction of 0.35(V/V).

At pH 8.0 the total phosphate concentration was changed between 1 and 35mM (35%(V/V) methanol) measuring retention times adding pyrene to the series of compounds above. Retention of the bi-cyclic compounds naphthalene, quinoline and isoquinoline were also measured changing phosphate concentration from 5 to 35 mM having 10%(V/V) methanol in the mobile phase.

3. Optimising the SOM content on HPLC column silica material

One of the aims of this project was to improve the quality of HPLC column material, consisting of silica gel with chemically immobilised soil organic matter (SOM). As measure for the improvement two criteria was chosen: 1) Increase loading of organic carbon in order to have a higher content of soil organic matter on the silica, and 2) To improve the analytical accuracy by decreasing the background contribution coming from the carrier carbon, witch contributed with approximately 95 % of the organic carbon (Kollist-Siigur et al., 2001). More specific description of the processes in immobilising SOM to the silica is given in the previous chapter, “Experimental”.

3.1 Results and Discussion

3.1.1 Humic acid HPLC column, developments and improvements

Basis for this work was the work represented by Nielsen et al. (1997) using silica gel, a Nucleosil Si 50-10 (with a pore diameter at 50 Å and a particle size of 10 μm) from Macherey-Nagel. The changes in the column materials, was evaluated by both the amount of organic carbon from humic acid (SOM) and binding material (carrier carbon) and the ratio between these two values (organic carbon ratio = $OC_{SOM}/OC_{carrier}$).

All values mentioned in the following discussion (including particle size, pore diameter and specific surface area, BET) are given in Table 3.1 to facilitate comparison between the column materials.

Table 3.1. Comparing of the column materials fabricated and/or reported to in this work.

Column material	**Particle size / µm**	**Pore diameter / Å**	**BET / $m^2 g^{-1}$**	**Total OC / %wt.**	**SOM OC / %wt.**	**Carrier OC / %wt.**	**OC ratio, $OC_{SOM} / OC_{carrier}$**
With Aldrich humic acid							
Nucleosil 50-10, amino procedure*	10	50	450	13.2	0.7	12.5	0.056
Nucleosil 500-10, amino procedure	10	500	35	2.82	1.05	1.77	0.59
Nucleosil 4000-10, amino procedure	10	4000	10	1.28	0.58	0.69	0.84
Nucleosil 500-10, amino procedure, extra HA	10	500	35	3.35	1.32	2.03	0.65
Nucleosil 500-10, carbonyl procedure	10	500	35	2.68	2.04	0.64	3.17
Nucleoprep 300-20, carbonyl procedure	15-25	300	100	2.42	1.91	0.51	3.73
With Ulsted refractory soil carbon							
Nucleoprep 300-20, carbonyl procedure	15-25	300	100	5.07	4.44	0.63	7.05
Blank							
Nucleosil 500-10, amino procedure, blank	10	500	35	2.52		2.52	
Nucleosil 500-10, carbonyl procedure, blank	10	500	35	0.63		0.63	
Nucleoprep 300-20, carbonyl procedure, blank	15-25	300	100	0.54		0.54	

* From Kollist-Siigur et al. (2001)

Changes have been done 1) firstly in choice of pore diameter of the silica gel used as carrier material. The reason for this was to improve the possibility for humic acid to enter the pores in the silica, hereby increase the loading of humic acid on the column material. The specific surface area (BET) was decreased from 450 m^2 g^{-1} in Nucleosil Si 50-10 to 35 and 10 m^2 g^{-1} in Nucleosil Si 500-10 and Nucleosil Si 4000-10, causing a lower load of carrier carbon and hereby increasing the accuracy of the sorption of PAC on the modified HPLC-column. The choice of pore diameter has a double effect on the ratio between organic carbon originating from humic acid and from binding materials. a) The binding of humic acid is dependent of the pore diameter with an optimum at 500 Å. b) The amount of carrier carbon decreases with decreasing pore diameter. The dependence is not linear, as the y-axis is the ratio between carrier OC and the surface area (see Figure 3.1).

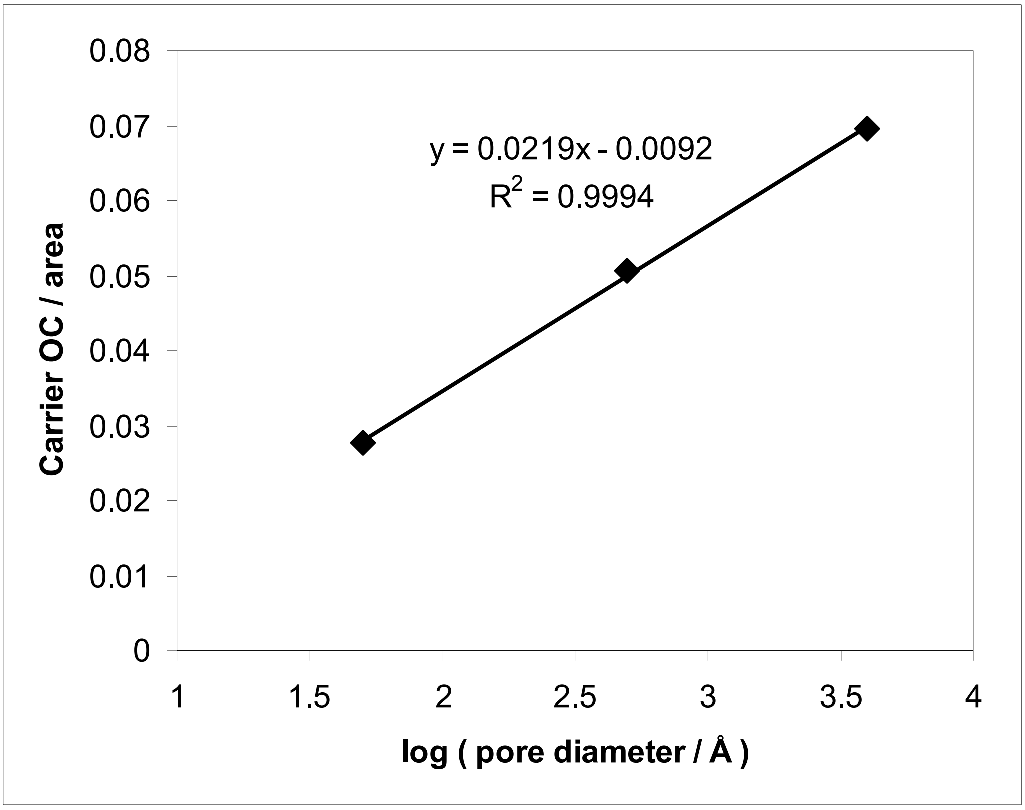


Figure 3.1. The relationship between carrier carbon and the pore diameter (as the carrier OC to specific surface area (BET) ratio vs. log pore diameter) for column materials fabricated by the amino procedure.

2) The second change was to increase the amount of humic acid used in the synthesis, to obtain a higher load of humic acid on the column.
3) Finally, the way to immobilise the humic acid onto the silica gel was changed as the step using glutardialdehyde was left out in the synthesis, hereby immobilising the humic acid through imine bonds directly between its carbonyl groups and amino groups on the activated silica gel. This change was done primarily to decrease the amount of background carbon by decreasing the chain length of the carrier from eight carbons and one nitrogen atom to three carbons and one nitrogen atom.

The first change 1) was to increase the pore diameter using 500 Å and 4000 Å, respectively. Those changes caused an increase in the ratio between organic carbon originating from humic acid and from binding materials by more than ten fold from approximately 0.06 to 0.59 and 0.84 for 500 Å and 4000 Å, respectively. In absolute figures from 0.7 %wt to 1.1 and 0.6 %wt organic carbon originating from humic acid, and on the same time a decrease in the amount of binding material organic carbon from 12.5 %wt to respectively 1.8 and 0.7 %wt. Because of the highest load of humic acid OC, 500 Å was chosen as pore diameter.

2) Use of the 500 Å pore diameter (Nucleosil Si 500-10) and a fivefold increase of humic acid during synthesis only resulted in an minor increase in the humic acid binder material organic carbon ratio from 0.6 to 0.65 (1.3 and 2.0 %wt organic carbon from humic acid and binding material, respectively). Because of a large excess of residual humic acid, the purification step was complicated and time-consuming, and not worth this extra effort compared to the result.

3) The next step was to leave out glutardialdehyde in the synthesis, immobilising humic acid through imine binding between its carbonyl groups and amino groups on the activated silica gel. Residual amino groups were finally deactivated by formaldehyde to avoid, or at least reduce, their basic properties. The new way to fix humic acid resulted in an organic carbon ratio of 3.2 (compared to 0.59) decreasing the amount of binding carbon to the third from 1.8 to 0.6 %wt and doubling the humic acid organic carbon from 1.0 to 2.0 %wt. Unfortunately, the column packed with this material had a low permeability resulting in very high pressure and long retention times. This was probably a result of a high humic load compared with the small particle size. To solve this problem, the particle size was increased to 20 µm (Nucleoprep Si 300-20) and the same procedure resulted in an organic carbon ratio of 3.7, with a content of 1.9 and 0.51 %wt. OC from humic acid and binding material, respectively. By choosing Nucleoprep Si 300-20 not only the size of the particles was changed, the pore diameter (300 Å) and BET (100 $m^2 g^{-1}$) was decreased compared with Nucleosil Si 10-500, but it seems that the load of neither carrier materials nor humic acid was affected.

Fabrication of blank material (only silica gel and binder) on the same silica gel (Nucleoprep Si 300-20) gave an organic carbon content of 0.54 %wt.

As discussed in Kollist-Siigur et al. (2001) it seems that the sorption properties of humic acid was not affected by the way it was immobilised. If this is correct, it can be expected that the immobilisation of at least Aldrich humic acid do not change the spherical structure or the composition of the humic material. This will be discussed further in the following chapters.

3.1.2 Effects of silica, -surface area and pore sizes.

The effects of the kind of silica are investigated closely looking at the amount of carrier carbon on the different types of silica gel. The amount of carrier OC on the five used combinations of silica and manufacturing procedure are shown in Figure 3.2A. It is seen that “Nucleosil 50-10, amino procedure” shows a content that are seven times higher than the second highest value and almost 25 times as much as in the final selected column material, so improvements on reducing carrier OC are definitely obtained.

In Figure 3.2B, the ratio between the measured amounts of carrier OC and the specific surface area are shown. If the possible number of sites for silanisation was the only controlling factor, the ratios were expected to be uniform for materials using the same procedure. Even though the data set is small there are some evidence that the amount of carrier carbon pr. unit of area increases with decreasing specific surface area and/or pore diameter. Comparing the two procedures (with the same kind of silica) the ratio between the amount of carrier carbon is 2.7, the same ratio between the number of cross-linking carbon atoms in the two procedure is also 2.7 (the number of carbon atoms in the links in the two procedures is 8 and 3 respective). This indicates that same number of sites on the silica surface is occupied and that a possible physical binding of glutardialdehyde as mentioned by Koopal et al. (1998) can be neglected here.

According to Skoog and Leary (1992) the typical concentration of chemically active Si-OH groups on the silica surface are 8 μmol m^{-2}, but due to steric effects, the surface coverage by silanisation is limited to 4μmol m^{-2} using organochlorosilane. In this work triethoxysilane is used for silanisation so the surface coverage should maximally be 1.3 μmol m^{-2}. Load of organic carbon in the blank column material was 0.54 %wt. or 5.4 mg g^{-1}. Each carbon chain contains four carbon atoms (three from aminopropyl and one from the end-capture with formaldehyde), thus 48 g C mol^{-1}. As the specific surface area of the silica used is

100 m^2 g^{-1}, the surface coverage in this work is 1.1 μmol m^{-2}, more or less the maximally possible.

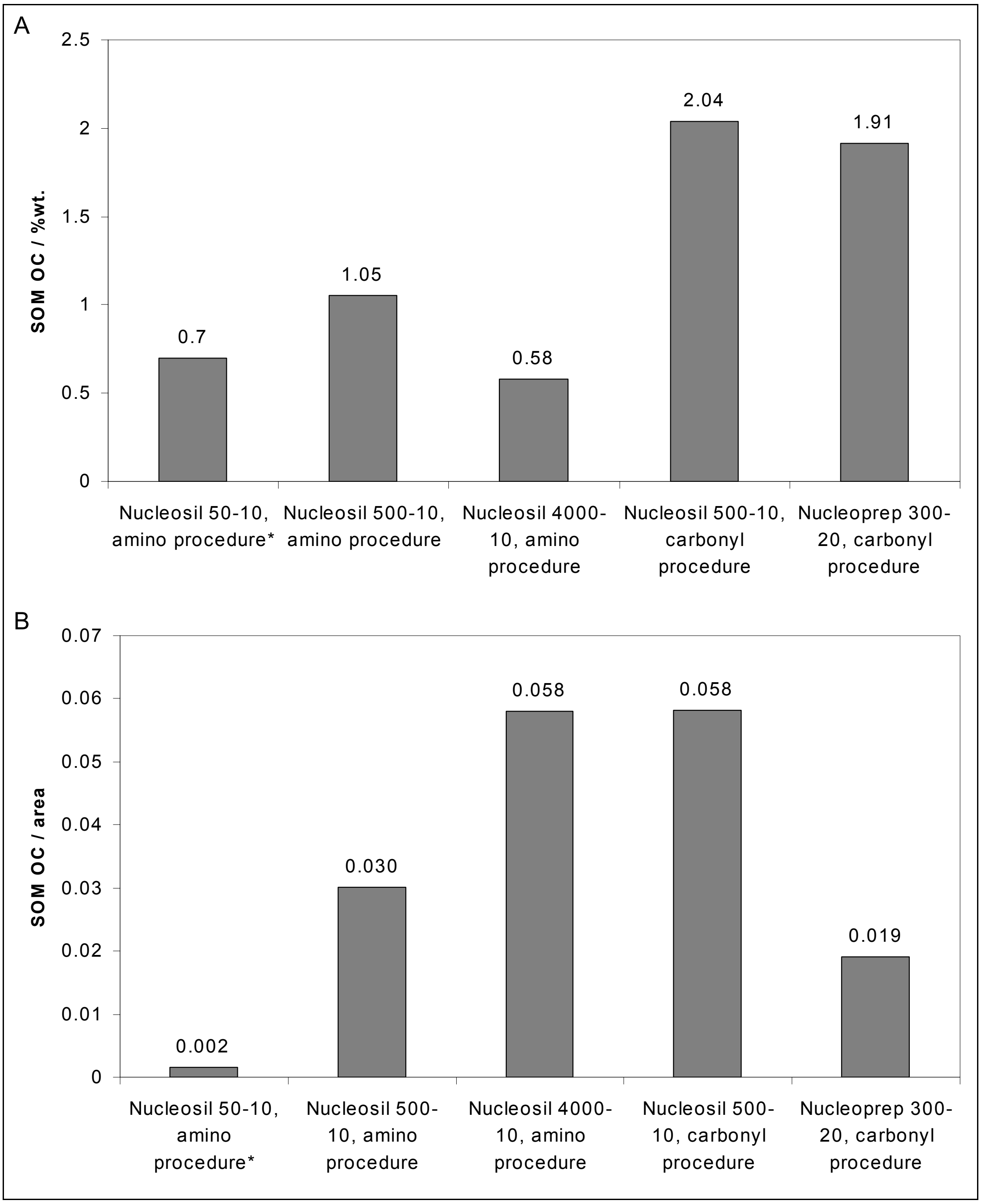


Figure 3.2. Effect of silica and manufacturing procedure on the amount of carrier OC on the column materials. A. Amount of carrier carbon in %wt. calculated as mentioned in the text. B. The ratio between the amount of carrier carbon and specific surface area (BET) in m^2 g^{-1}, there are no unit shown on the ordinate.

*Data from Kollist-Siigur et al. (2001).

3.1.3 Organic carbon on column materials

The content of organic carbon in column materials is already mentioned in the paragraph above (and Table 3.1). Figure 3.3 shows the distribution of organic carbon from SOM and binding material for the different column materials using Aldrich HA as well as Ulsted RSC.

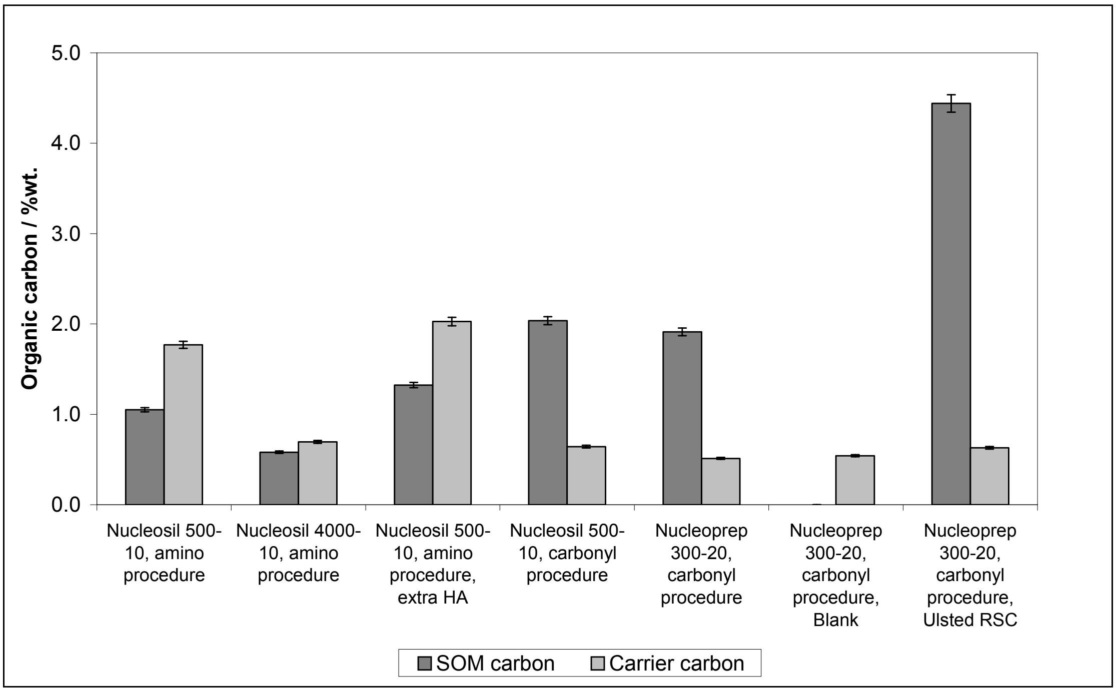


Figure 3.3. The distribution of organic carbon on the column materials. Dark grey indicates OC from soil organic matter (where no sources are mentioned Aldrich HA is used), light grey from carrier carbon. The estimated distribution in the material using Nucleosil 50-10 and humic acid was 0.7 and 12.5 %wt. respectively (Kollist-Siigur et al., 2001). Error bars indicate relative standard derivations (2.18 % and 2.24 % relatively for the two series) calculated from standard derivations on the measurements (0.5 % on carbon and 1.5 % on sulphur).

Material from the selected column material was also measured after use now containing 1.085 %wt. organic carbon from Aldrich humic acid, resulting in a loss of 42.6 % of the humic acid during use. The high load of organic carbon in the column material with Ulsted RSC compared with Aldrich HA column material using the same procedure is partly explained by the higher content of carbon in Ulsted RSC (60 %wt.) than Aldrich HA (42.5 %wt.). The higher load of carrier carbon is naturally also an explanation.

There are some discussion going on about whether the SOM is physical or chemically bound to the silica. Koopal et al. (1998) found a load of 42 mg HA (g $SiO_2)^{-1}$ using a method similar to the one designated “carbonyl procedure” in this work. The work of Koopal et al. (1998) did not come to our knowledge until after the “carbonyl procedure” was developed. The methods used in the two works are almost identical as they both extend the work of Szabo and co-workers (e.g Szabo et al., 1992 and Szabo and Bulman, 1994). The main difference is in the way to end caps the residual amino groups. In this work the end capping was carried out in an aqueous solution using formaldehyde, while Koopal and co-workers used 5% acidic anhydride in DMF (N,N-dimethyl-formamide) and also taking extra efforts to avoid or minimise the physical bounding by curing the product at 120 °C for five hours.

The load of humic acid on the column used in this work is 45 mg HA (g $SiO_2)^{-1}$ before and 25.5 mg HA (g $SiO_2)^{-1}$ after use. It is unknown whether this loss corresponds to humic acid being physically bound or just a result of breaking down of the column material as the column has been used in the pH range from 2.5 to 8.1 and at pressures up to 150 bar.

Another aspect is how similar the amount of humic acid is as the silica used in this work and by Koopal at al. (1998) are different. They use non-porous silica with a specific surface area at 52 $m^2\ g^{-1}$, and we used porous silica with a specific surface area of 100 $m^2\ g^{-1}$. Looking further on the similarities both Koopal at al.(1998) and Kollist-Siigur et al. (2001) found a load of 16 mg HA (g $SiO_2)^{-1}$ using a method similar to the amino procedure, again using silica with different specific surface area (52 and 450 $m^2\ g^{-1}$ respectively). An interesting question enters comparing these similar figures: Is this just a coincidence or is the main factor determining the load of humic the way of binding humic acid (either by amino- or carbonyl groups in humic acid).

3.1.4 Determination of column material density

The density was measured in three replicates to 2.26 g cm^{-3} by use of pyknometers. There was no systematic difference between pure silica gel and the Aldrich humic acid column materials.

According to Macherey-Nagel the bulk density for the porous Nucleoprep 300-20 particles is 0.45, as the particles are made of silica a material density similar to quartz (2.65 g cm^{-3} (Rocks & Minerals)) is to be expected. In general there is no doubt that the particle density is higher than unity as they settle in water.

Humic acid from Aldrich is incompletely decomposed organic material with a very dark colour and a relatively high content of aromatic carbon. Compared with graphite (2.23 g cm^{-3}), it seems reasonable that the density must be in the same range, furthermore this can explain why the densities of the silica gel and the humic acid column material are very similar.

3.1.5 FTIR of column materials

Spectra of the four examined column materials are compared in Figure 3.4. The functional groups from the blank column material dominate all spectra. The main reasons for this apparent similarity may be that Si-O-Si stretch from approximately 1150 cm^{-1} to 1000 cm^{-1} totally dominates the spectra. In addition the Aldrich humic acid and Ulsted refractory soil carbon only accounts for respectively 4.5 and 7.4 %wt of total mass, so only small differences compared to the blank material was to be expected.

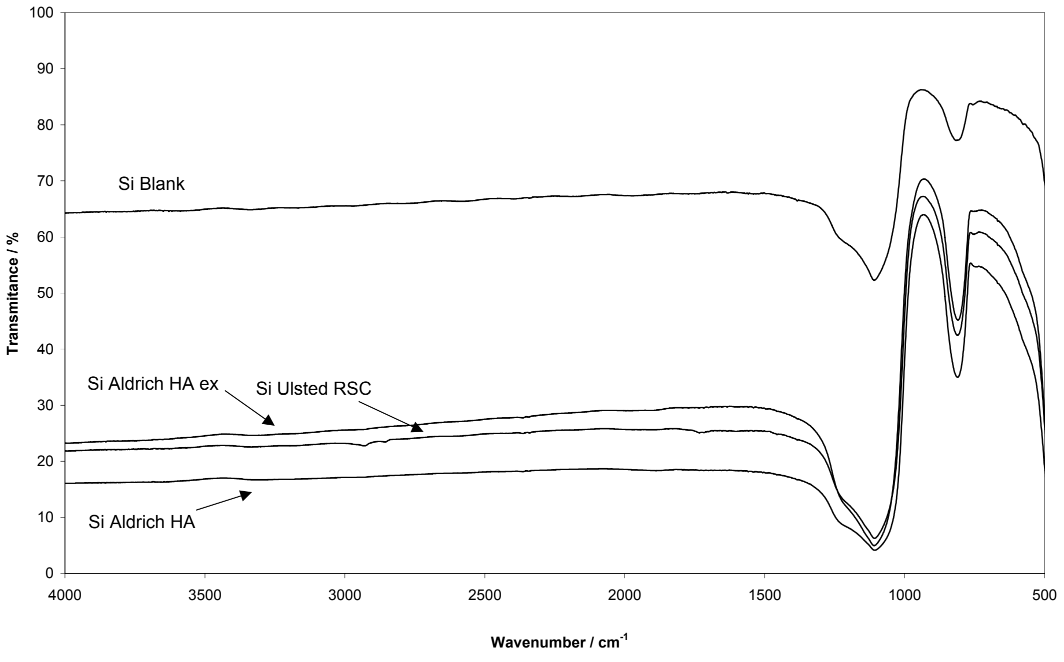


Figure 3.4. Infrared spectra of the three column materials based on Nucleoprep Si 300-20; Si Blank, Si Aldrich HA before and after use (Si Aldrich HA ex), and Si Ulsted. Si-O-Si asymmetric stretch and Si-OH bending dominate the range below 1300 cm-1.

When magnifying the spectre with Ulsted RSC column material (not shown), there is a recognisable pattern from the pure Ulsted RSC in the area around 2900 cm^{-1} (C-H stretch from aliphatic CH_2 and CH_3), 1750 cm^{-1} (C=O, carbonyl) and 1650 cm^{-1} (C=C stretch). Those characteristics are totally missing in the Blank column material spectrum.

Looking at the same analysis of Aldrich HA (not shown), the similarities are not so striking, but evidence for aliphatic C-H stretch is seen. An explanation for this difference between the two SOM column materials might be the higher load of SOM on the Ulsted RSC column (se Figure 3.3). Even though the differences between Blank- and Aldrich HA column materials are small, the presence of Aldrich HA is undoubted.

Finally the column material with Aldrich HA shows no distinct difference between before and after use. Thus, although some of the humic acid is lost from the column during use, the basic characteristic is retained.

3.2 Summary

Distinct improvements were achieved during the optimisation of the column material. By changes in both the way of preparation and choice of silica gel, column materials with a high load of humic acid and a low amount of carrier carbon was obtained. Those improvements lead to fewer artefacts and thus more reliable results in determination of sorption of PAC to the humic acid.

The usefulness of the improved column materials is discussed in the following chapters with special emphasis on comparisons of results of PAC sorption measured by other methods and how pH changes affect the column materials and the sorption.

The question whether the SOM is physical or chemically bound is not fully answered. It seems that at least the cross-linking molecules (carrier carbon) are chemically bound to the silica particles. Even though humic acid is lost during use of the column, IR data indicates that the basic characters of the column remain.

4. Chromatographic Results

Data from use of the HPLC column is presented in this chapter. At first the partitioning coefficients K_{oc} for a variety of PAC. For most of the compounds a direct determination of log K_{oc} based on correlations between the composition of the mobile phase and the column coefficient is made. For the largest compounds a linear correlation is found between the results using one mobile phase and log K_{oc}. Secondly, based on the measurements with temperature variation, enthalpy- and entropy-changes are calculated and presented here. Finally the results from the influence of pH and electrolyte concentration on the sorption are shown. Discussions of the results are given in the following chapters.

4.1 Sorption of PAC to Soil organic matter

4.1.1 Direct determination of log K_{oc}

Values for the direct determined partitioning coefficients for PAC to the two kinds of SOM are shown in Table 4.1 together with the octanol-water-partitioning coefficient log K_{ow} and the aqueous solubility. Use of the method is validated in Chapter 5. The sorption data will be discussed in the Chapter 6 regarding molecular properties as size, shape and lipophilicity.

4.1.2 Indirect determination of K_{oc}

For a series of large compounds it was impossible to use the direct method to determine K_{oc}, probably caused by insufficient solubility of the compounds in the mobile phase. The partitioning coefficients are therefore calculated by an empirical correlation found between log K_{oc} and log k' for one mobile phase (75 %(V/V) MeOH, see Chapter 2, Experimental).

A validation of the indirect determination is made in the next chapter, and the data are enclosed in the discussion in Chapter 6.

Table 4.1. The log K_{oc} values for Aldrich HA and Ulsted RSC determined by the direct method. The values are calculated by Eq. 2.1. For determination of standard deviations, see the text.

Compound	**log K_{oc} Aldrich HA**	**log K_{oc} Ulsted RSC**	**log K_{ow}**	**C_{sol} mg l^{-1}**	**C_{sol} mol l^{-1}**
PAH					
Naphthalene	2.98 ± 0.17	3.96 ± 0.20	3.47[a]	31[f]	$2.42\cdot10^{-4}$
Fluorene	4.00 ± 0.21	4.61 ± 0.23	4.32[a]	1.8[f]	$1.08\cdot10^{-5}$
Anthracene	4.46 ± 0.27	5.19 ± 0.24	4.53[a]	0.06[f]	$3.71\cdot10^{-7}$
Phenanthrene	4.34 ± 0.28	5.16 ± 0.25	4.48[a]	1.3[f]	$7.30\cdot10^{-6}$
Pyrene	5.14 ± 0.27	5.27 ± 0.21	4.84[a]	0.16[f]	$7.92\cdot10^{-7}$
1,2-benzanthracene		5.94 ± 0.19	5.54[a]	0.11[f]	$4.82\cdot10^{-8}$
N-PAC					
Quinoline	2.69 ± 0.20	2.99 ± 0.21	2.23[a]	6330[f]	$4.91\cdot10^{-2}$
Isoquinoline	2.86 ± 0.21	3.02 ± 0.21	2.26[a]	5430[f]	$4.20\cdot10^{-2}$
4-azaflourene	3.40 ± 0.23	3.82 ± 0.24	2.96[a]	14.6[g]	$8.74\cdot10^{-5}$
Acridine	4.24 ± 0.22	4.31 ± 0.21	3.27[a]	46.6[f]	$2.60\cdot10^{-4}$
5,6-benzoquinoline	4.18 ± 0.22	4.20 ± 0.24	3.51[a]	78.7[f]	$4.40\cdot10^{-4}$
7,8-benzoquinoline	3.92 ± 0.21	4.18 ± 0.24	3.37[a]	5.08[h]	$2.84\cdot10^{-5}$
Phenantridine	3.92 ± 0.23	4.19 ± 0.25	3.44[a]	300[b]	$1.68\cdot10^{-3}$
1,2-benzacridine		5.08 ± 0.25	4.48[a]	0.346[b]	$1.51\cdot10^{-6}$
Carbazole	3.69 ± 0.22	4.30 ± 0.22	3.22[a]	1.2[f]	$7.19\cdot10^{-6}$
O,S-PAC					
Dibenzofuran	3.97 ± 0.19		4.09[a]	6.6[f]	$3.93\cdot10^{-5}$
Dibenzothiophene	4.25 ± 0.21		4.41[a]	1.0[f]	$5.43\cdot10^{-6}$
X-PAH					
1-hydroxy-2-naphthoic acid	3.74 ± 0.22		3.42[b]	229[g]	$1.22\cdot10^{-3}$
Fluorenone	3.84 ± 0.24		3.58[c]	25.3[b]	$1.41\cdot10^{-4}$
9-acetylanthracene	4.05 ± 0.25		3.60[d]	0.972[g]	$4.42\cdot10^{-6}$
9-anthracene carboxamide	3.78 ± 0.18		2.23[d]	6.04[g]	$2.73\cdot10^{-5}$
9-anthracene carboxylic acid	2.93 ± 0.18		3.85[c]	85[h]	$3.83\cdot10^{-4}$
9-anthracene carboxylic acid methyl ester	4.37 ± 0.23		3.83[d]	0.588[g]	$2.49\cdot10^{-6}$
9-bromoanthracene	5.19 ± 0.28		5.37[d]	0.0567[g]	$2.21\cdot10^{-7}$
9-chloroanthracene	5.29 ± 0.27		5.27[d]	0.160[g]	$7.55\cdot10^{-7}$
9-cyanoanthracene	4.92 ± 0.28		3.98[d]	0.409[b]	$2.01\cdot10^{-6}$
9-formylanthracene	3.84 ± 0.20		3.77[d]	1.07[g]	$5.20\cdot10^{-6}$
9-methoxyanthracene	4.30 ± 0.20		4.36[d]	0.511[g]	$2.46\cdot10^{-6}$
9-methylanthracene	4.95 ± 0.23		4.96[d]	0.261[h]	$1.36\cdot10^{-6}$
9-nitroanthracene	4.93 ± 0.25		3.70[d]	0.724[i]	$2.25\cdot10^{-6}$
Anthraquinone	4.46 ± 0.23		3.28[d]	1.35[g]	$6.49\cdot10^{-6}$
1,8-dihydroxy-anthraquinone	4.76 ± 0.57		3.94[b]	2.43[g]	$1.43\cdot10^{-5}$
Phenanthrene-4-carboxylic acid	3.89 ± 0.30		4.23[e]	0.639[g]	$2.88\cdot10^{-6}$
4-carboxyl-5-phenanthrene carboxaldehyde	3.51 ± 0.28		3.94[e]	0.784[g]	$3.14\cdot10^{-6}$
5H-phenanthro [4,5-bcd]pyran-5-one	4.92 ± 0.36		2.58[e]	16.8[g]	$7.65\cdot10^{-5}$
Benzanthrone	5.12 ± 0.65		4.73[e]	0.24[b]	$1.04\cdot10^{-6}$
1-hydroxypyrene	4.82 ± 0.71		4.45[e]	1.34[g]	$7.50\cdot10^{-6}$
1-acetylpyrene	4.88 ± 0.71		4.18[d]	0.227[g]	$9.29\cdot10^{-7}$
2-acetylpyrene	5.26 ± 0.67		4.29[d]	0.227[g]	$9.29\cdot10^{-7}$
4-acetylpyrene	5.05 ± 0.73		4.16[d]	0.227[g]	$9.29\cdot10^{-7}$
1-formylpyrene	3.29 ± 0.27		4.23[d]	0.251[g]	$1.09\cdot10^{-6}$

Table 4.1. Continued

Compound	**log K_{oc} Aldrich HA**	**log K_{oc} Ulsted RSC**	**log K_{ow}**	**C_{sol} mg l^{-1}**	**C_{sol} mol l^{-1}**
1-pyrene carboxylic acid	4.59 ± 0.27		4.81[e]	0.149[g]	$6.06 \cdot 10^{-7}$
1-pyrene carboxylic acid methyl ester	5.49± 0.18		4.73[d]	0.136[g]	$5.27 \cdot 10^{-7}$
2-pyrene carboxylic acid methyl ester	5.97 ± 0.39		4.85[d]	0.136[g]	$5.24 \cdot 10^{-7}$
4-pyrene carboxylic acid methyl ester	5.32± 0.27		4.73[d]	0.136[g]	$5.24 \cdot 10^{-7}$
1,6-pyrene-dione	3.78 ± 0.21		3.60[e]	1.92[g]	$8.28 \cdot 10^{-6}$
4,5-pyrene-dione	4.54 ± 0.23		4.15[e]	0.662[g]	$2.86 \cdot 10^{-6}$
X-N-PAC					
2-hydroxyquinoline	2.65 ± 0.20		1.56[d]	1050[h]	$7.24 \cdot 10^{-3}$
6-methylquinoline	2.94 ± 0.21		2.57[c]	631[b]	$4.41 \cdot 10^{-3}$
2,6-dimethylquinoline	3.75 ± 0.21		3.24[e]	121[g]	$7.71 \cdot 10^{-4}$
Quinoline-N-oxide	2.11 ± 0.19		1.13[d]	3920b[g]	$2.70 \cdot 10^{-1}$
N-methyl quinolinium iodide	3.48 ± 0.27		-0.29[d]	9420[i]	$9.72 \cdot 10^{-1}$
Acridone	3.90 ± 0.24		2.84[e]	13.5[g]	$6.94 \cdot 10^{-5}$
6[5H]-phenanthridon	4.35 ± 0.19		1.30[e]	279[g]	$1.43 \cdot 10^{-3}$

[a] Helweg et al. (1997a)

[b] Meyland et al., (1996)

[c] Hansch et al., (1995)

[d] Helweg et al. (1997b)

[e] Calculated based on the molecular structure using the “Kowwin” software from the EPI Suite v3.11, US EPA

[f] Pearlmann et al., (1984)

[g] Calculated based on the molecular structure using the “Wskowwin” software from the EPI Suite v3.11, US EPA

[h] Dannenfelser and Yalkowsky (1991):

[i] Calculated from log K_{ow} by using the “Wskowwin” software from the EPI Suite v3.11, US EPA

Table 4.2. Partitioning coefficients on Aldrich HA for the largest compounds used in this work. Log K_{oc} values are estimated by an empirical correlation between log K_{oc} and log k'_{75}. Log K_{ow} and solubility are from the literature.

Compounds	**Log K_{oc}**[*]	**Log K_{ow}**	**C_{sol} mg l^{-1}**	**C_{sol} mol l^{-1}**
PAH				
1,2-benzanthracene	5.96	5.54[a]	0.011[c]	$4.82\cdot10^{-8}$[c]
benzo(a)pyrene	6.85	6.02[a]	0.004[c]	$1.59\cdot10^{-8}$[c]
1,2,3,4-dibenzanthracene	7.36	6.40[a]	0.0016[d]	$5.76\cdot10^{-9}$[d]
1,2,5,6-dibenzanthracene	7.22	6.54[a]	0.00056[c]	$2.01\cdot10^{-9}$[c]
1,2,7,8-dibenzanthracene	7.30	6.54[a]	0.013[c]	$4.68\cdot10^{-8}$[c]
N-PAC				
1,2-benzacridine	5.70	4.48[a]	0.346[e]	$1.51\cdot10^{-6}$[e]
10-azabenz(a)pyrene	6.62	5.69[a]	0.0333[e]	$1.32\cdot10^{-7}$[e]
1,2,3,4-dibenzacridine	6.97	5.85[a]	0.0184[e]	$6.59\cdot10^{-8}$[e]
1,2,5,6-dibenzacridine	7.05	5.73[a]	0.16[c]	$5.73\cdot10^{-7}$[c]
1,2,7,8-dibenzacridine	7.13	5.63[a]	0.018[e]	$6.45\cdot10^{-8}$[e]
3,4,5,6-dibenzacridine	6.87	6.27[a]	0.00389[e]	$1.39\cdot10^{-8}$[e]
X-PAH				
9,10-dibromoanthracene	5.86	6.29[b]	0.00343[f]	$1.02\cdot10^{-8}$[f]
1-bromopyrene	5.86	5.81[b]	0.00130[f]	$4.64\cdot10^{-9}$[f]
2-bromopyrene	5.89	5.80[b]	0.00130[f]	$4.64\cdot10^{-9}$[f]
4-bromopyrene	5.92	5.84[b]	0.00130[f]	$4.64\cdot10^{-9}$[f]
1,6-dibromopyrene	6.79	6.69[b]	0.000773[f]	$2.15\cdot10^{-9}$[f]
1,8-dibromopyrene	6.81	6.73[b]	0.000773[f]	$2.15\cdot10^{-9}$[f]

[*] Estimated by the empirical correlation Log $K_{oc} = 2.2483 \times \log k'_{75} + 5.2002$, based on 18 compounds ($R^2 = 0.7855$).
[a] Helweg et al. (1997a)
[b] Helweg et al. (1997b)
[c] Pearlmann et al., (1984)
[d] Dannenfelser and Yalkowsky (1991):
[e] Meyland et al., (1996)
[f] Calculated based on the molecular structure using the "Wskowwin" software from the EPI Suite v3.11, US EPA

4.2 Temperature variation and determination of ΔH and ΔS

4.2.1 Enthalpy changes, ΔH_{sorp}

The retention of a series of PAC has been measured at different temperatures as described in Experimental. Enthalpy changes for the sorption process (ΔH_{sorp}) are calculated by Equation 4.1 (same as Eq. 2.3), thus from the slope in the van't Hoff plot (Fig. 2.5):

$$\frac{d\ln k'}{d(1/T)} = -\frac{\Delta H_{sorp}}{R} \Rightarrow \Delta H_{sorp} = -\frac{d\ln k'}{d(1/T)} \cdot R \qquad \text{(Eq. 4.1)}$$

The values are shown in Table 4.3. The most striking observation is the fact that all values are negative, thus the sorption is an energetically favourable process. Further discussion of the data and comparison with some literature values are given in Chapter 7.

Table 4.3. Values of ΔH calculated from Eq. 4.1. Each plot is a result of two measurements at four different temperatures (Table 2.1). Standard deviations come from the standard deviation on the slope of the linear fit (Fig. 2.5).

Compounds	**ΔH_{sorp} / kJ mol^{-1} Mobile phase methanol fraction (V/V)**			
	0.25	**0.45**	**0.65**	**0.85**
PAH				
Naphthalene	-22.3 ± 1.7	-16.3 ± 0.58	-13.0 ± 2.0	
Fluorene	-28.3 ± 1.3	-24.0 ± 0.96	-23.0 ± 2.0	
Anthracene		-29.8 ± 0.74	-22.7 ± 1.1 / -23.0 ± 1.4	
Phenanthrene		-27.2 ± 1.3	-22.7 ± 0.62	
Pyrene		-32.3 ± 0.18	-22.6 ± 0.25	-15.2 ± 0.95
N-PAC				
Quinoline	-26.3 ± 1.9	-16.3 ± 1.3	-23.5 ± 2.8 / -32.3 ± 3.8	
Isoquinoline	-27.9 ± 1.8	-17.2 ± 0.87	-23.4 ± 1.4	
4-azafluorene	-30.0 ± 1.9	-22.0 ± 1.4	-21.3 ± 0.75	
Acridine	-43.8 ± 1.9	-31.1 ± 1.4	-20.9 ± 1.3	
5,6-benzoquinoline	-39.6 ± 4.6	-28.6 ± 1.3	-23.7 ± 1.7	
7,8-benzoquinoline	-32.1 ± 3.2	-25.5 ± 1.3	-23.5 ± 0.29	
Phenantridine	-39.4 ± 0.64	-27.2 ± 1.2	-23.8 ± 1.9	
Carbazole	-31.3 ± 1.3	-23.6 ± 1.4	-21.4 ± 1.2	
O,S-PAC				
Dibenzofuran	-28.8 ± 3.2	-22.4 ± 1.5	-18.2 ± 1.1	
Dibenzothiophene	-36.3 ± 4.1	-24.7 ± 0.93	-20.7 ± 1.5	
X-anthracenes				
9-acetylanthracene			-23.3 ± 1.2	
9-anthracene carboxamide			-19.2 ± 2.8	
9-anthracene carboxylic acid methyl ester			-22.0 ± 2.5	
9-bromoanthracene			-23.9 ± 2.0	
9-chloroanthracene			-22.7 ± 0.36	
9-cyanoanthracene			-24.1 ± 1.1	
9-formylanthracene			-23.6 ± 6.6	
9-metoxyanthracene			-22.1 ± 3.7	
9-methylanthracene			-23.8 ± 0.48	
9-nitroanthracene			-26.1 ± 0.92	
9,10-dibromanthracene			-24.3 ± 2.4	
Anthraquinone			-26.2 ± 0.73	

4.2.2 Enthalpy changes, ΔS_{sorp}

The entropy changes (ΔS_{sorp}) are calculated from the intercept with the ordinate of the van’t Hoff plot (Fig. 2.5) by Equation 4.2:

$$\Delta S_{sorp} = R \cdot \left(\ln k'_{\mathrm{int}} - \ln \varphi_{SOM} \right) \qquad \text{(Eq. 4.2)}$$

And presented in Table 4.4. Again all values are negative meaning that the degree of order in the system is increased during the sorption.

Table 4.4. Values of ΔS calculated from Eq. 4.2. Each plot is a result of two measurements at four different temperatures (Table 2.1). Standard deviations come from the standard deviation on the intercept of the linear fit (Fig. 2.5).

Compounds	**ΔS_{sorp} / J mol^{-1} K^{-1} Mobile phase methanol fraction (V/V)**			
	0.25	**0.45**	**0.65**	**0.85**
PAH				
Naphthalene	-27.6 ± 2.5	-22.6 ± 0.77	-22.4 ± 2.7	
Fluorene	-32 ± 2.1	-36.9 ± 1.7	-46.6 ± 3.8	
Anthracene		-47.1 ± 1.4	-39.0 ± 1.9 / -40.0 ± 2.5	
Phenanthrene		-40.8 ± 2.3	-39.7 ± 1.1	
Pyrene		-47.8 ± 0.35	-29.8 ± 0.39	-17.6 ± 1.1
N-PAC				
Quinoline	-45.9 ± 3.5	-26.2 ± 1.9	-60.2 ± 6.0 / -88.5 ± 9.2	
Isoquinoline	-46.4 ± 3.3	-27.4 ± 1.3	-57.7 ± 2.9	
4-azafluorene	-46.0 ± 3.6	-35.7 ± 2.4	-44.2 ± 1.4	
Acridine	-76.9 ± 4.3	-55.8 ± 2.9	-34.5 ± 2.1	
5,6-benzoquinoline	-64.9 ± 9.8	-48.4 ± 2.5	-44.2 ± 3.3	
7,8-benzoquinoline	-43.3 ± 5.7	-40.6 ± 2.5	-45.8 ± 0.55	
Phenantridine	-64.5 ± 1.4	-44.3 ± 2.2	-44.9 ± 3.6	
Carbazole	-46.5 ± 2.4	-37.4 ± 2.4	-40.4 ± 2.1	
O,S-PAC				
Dibenzofuran	-35.5 ± 5.4	-33.1 ± 2.4	-32.2 ± 1.7	
Dibenzothiophene	-53.5 ± 8.1	-35.5 ± 1.6	-36.0 ± 2.5	
X-anthracenes				
9-acetylanthracene			-45.4 ± 2.1	
9-anthracene carboxamide			-33.4 ± 4.5	
9-anthracene carboxylic acid methyl ester			-39.2 ± 4.5	
9-bromoanthracene			-34.5 ± 3.4	
9-chloroanthracene			-32.5 ± 0.57	
9-cyanoanthracene			-38.8 ± 1.9	
9-formylanthracene			-52.8 ± 13.2	
9-metoxyanthracene			-41.3 ± 6.7	
9-methylanthracene			-37.5 ± 0.83	
9-nitroanthracene			-47.3 ± 1.8	
9,10-dibromanthracene			-28.1 ± 3.5	
Anthraquinone			-48.9 ± 1.4	

4.3 Other environmental parameters

4.3.1 Variation of pH

The sorption of six by- and tri-cyclic PAC to the humic acid column was measured decreasing pH from 7.8 to 2.5 holding other parameters constant and shown in Figure 4.1.

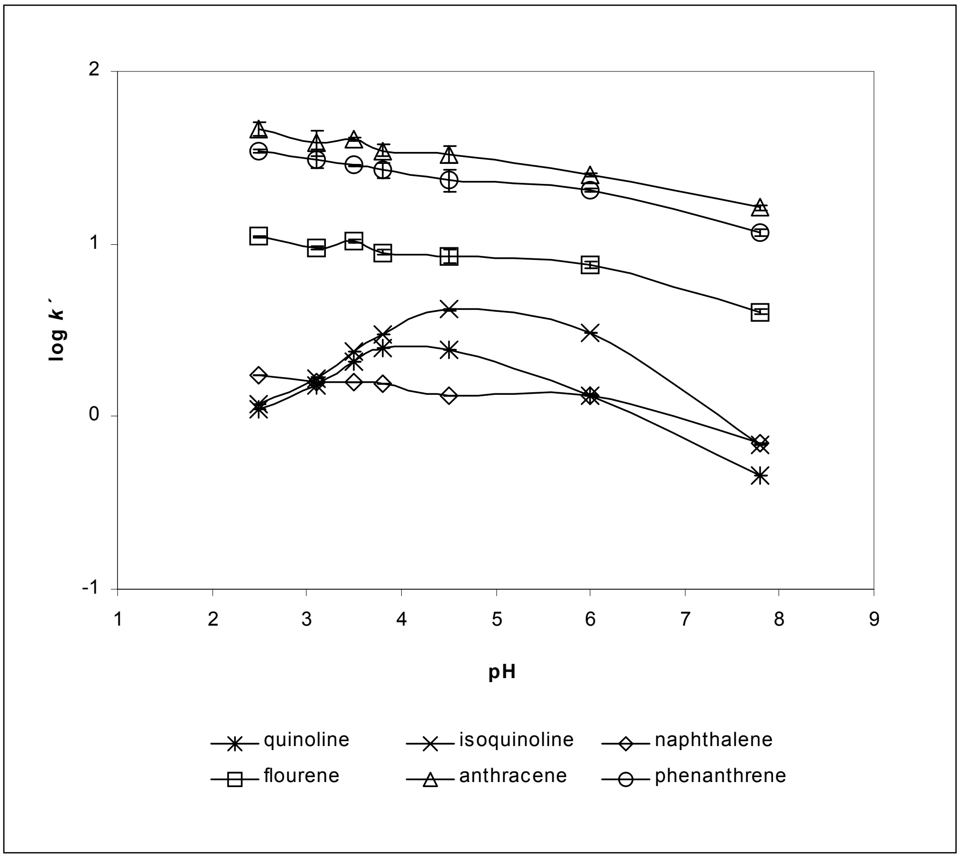


Figure 4.1. Changes of retention as a result of pH changes in the mobile phase. Each point is a result of repeated measurements, error bars indicate standard deviation on the mean value. Other experimental parameters (column temperature, electrolyte concentration, mobile phase methanol content, and flow rate) were kept constant.

Looking at Figure 4.1 the maximum sorption of the N-PAC (quinoline and isoquinoline) are observed at slightly acidic condition around pH 5, while the sorption of PAH increased when pH was decreased. Further discussions of the results are in Chapter 8.

4.3.2 Electrolyte concentration

In order to investigate the influence of electrolyte concentration on sorption of the small N-PAC and PAH, the sorption was measured under the conditions described in Experimental and log *k*' was calculated and presented on the diagrams in Figure 4.2-4.4.

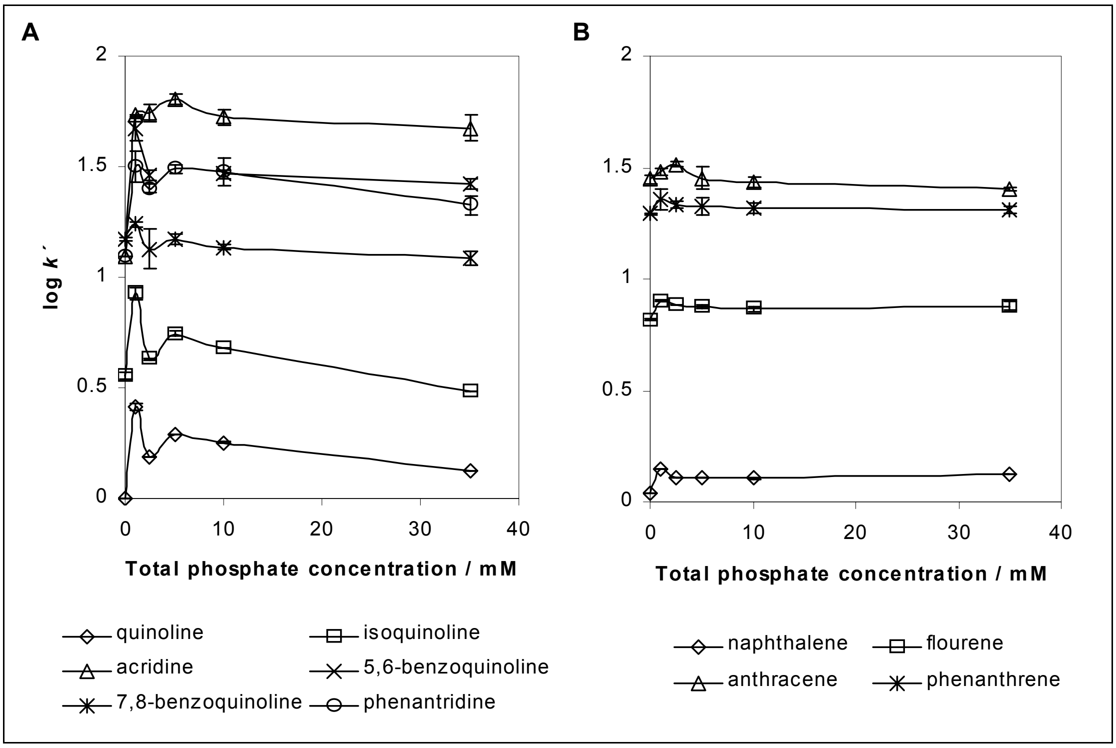


Figure 4.2. Variation of the sorption (measured as log *k´*) of six bi- and tri-cyclic N-PAC (A) and four bi- and tri-cyclic PAH (B) as a function of phosphate concentration. Methanol fraction in the mobile phase: 0.35(V/V), pH: 6.0. Temperature: 25°C. Error bars indicate standard error on mean values of log *k´*.

From Figure 4.2 it is seen that sorption of the N-PAC (Fig. 4.2A) shows an increase in sorption (especially quinoline and isoquinoline), while PAH (Fig. 4.2B) is almost unaffected by the ionic strength in the mobile phase (pH 6.0) when lowering the total phosphate concentration from 35 to 10mM. The highest sorption at pH 6.0 for all the investigated PAC is seen about 1mM phosphate. In the non-buffered mobile phase, the sorption decreases to the lowest point for all compounds.

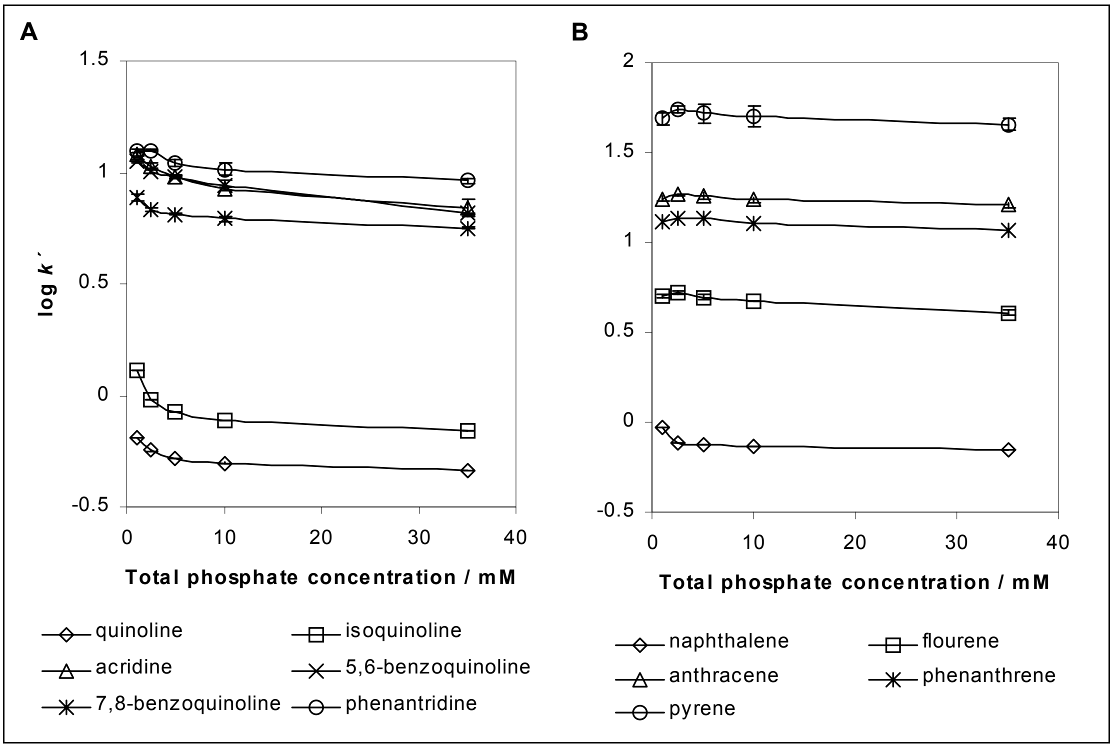


Figure 4.3. Variation of the sorption (measured as log *k´*) of six bi- and tri-cyclic N-PAC (A) and five bi-, tri- and quarto-cyclic PAH (B) as a function of phosphate concentration. Methanol fraction in the mobile phase: 0.35(V/V), pH: 8.0. Temperature: 25°C. Error bars indicate standard error on mean values of log *k´*.

At the higher pH value (8.0) the same pattern is seen for the N-PAC (Fig. 4.3A) and naphthalene, whereas the other PAH (Fig. 4.3B) shows maximum sorption at 2.5 mM phosphate.

For the three bicyclic compounds (quinoline, isoquinoline, and naphthalene) the retention, measured using a mobile phase with only 10 %(V/V) MeOH at pH 8.0 (Fig. 4.4) is higher than at 35 %(V/V) MeOH. Again the same pattern is seen. The sorption is increasing as the phosphate concentration decreases.

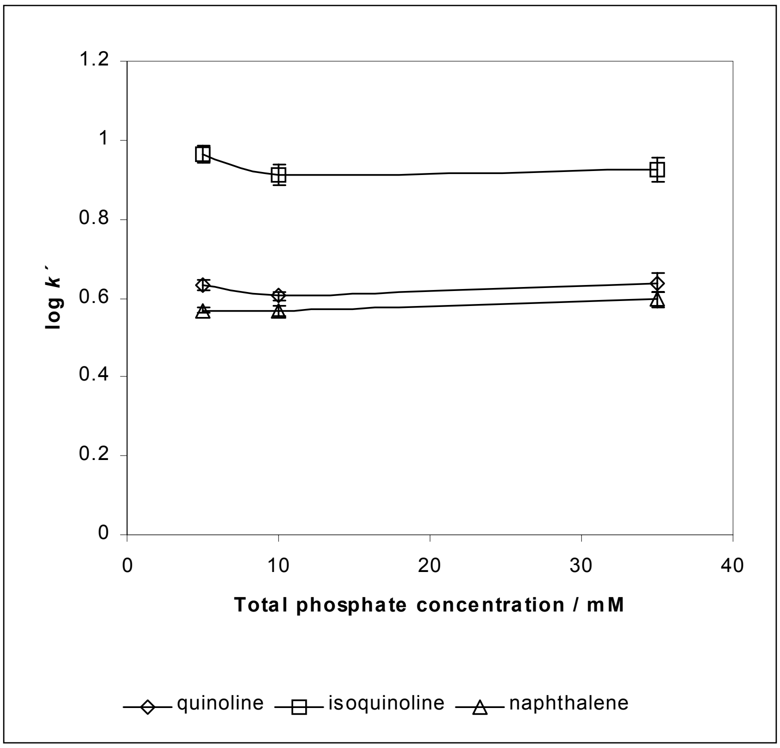


Figure 4.4. Variation of the sorption (measured as log *k*´) of three bicyclic PAC as a function of phosphate concentration. Methanol fraction in the mobile phase: 0.10(V/V), pH: 8.0. Temperature: 25°C. Error bars indicate standard error on mean values of log *k*´.

5. Method validation

The central part of this work is to use the optimised humic substance HPLC columns to determine partitioning coefficients K_{oc} for a variety of polycyclic aromatic compounds. For most of the compounds a successful direct determination of log K_{oc} based on correlations between the composition of the mobile phase and the column coefficient is made (see results in previous chapter). For the largest compounds log K_{oc} was calculated from a linear correlation found between the retention using one mobile phase and log K_{oc} from the direct method for a selected series of compounds. All together partitioning coefficients for 69 PAC are reported.

The methods for determine log K_{oc} is validated and the result of this work is compared with data from the literature obtained by batch experiments. In the end of this chapter is comparisons between HPLC column with Aldrich HA made by different procedures made.

5.1 Validation of the direct method

The main idea in this work was to use the humic acid HPLC column in a direct determination of K_{oc} for polycyclic aromatic compounds (PAC). This was done by measuring the retention on the HPLC column of the individual compounds using a series of different mobile phases (variating the content of MeOH) and calculating of log K_{oc} by equation 2.1.

$$\log K_{oc} = \log\left(\frac{\left(k'_{SOM} - k'_{blank}\right)\cdot \mathrm{V}_M}{\mathrm{V}_S \cdot \rho \cdot OC_{SOM}}\right) \qquad \text{(Eq. 2.1)}$$

Thus the only decisive factor is the linear extrapolation estimating capacity coefficients in a system using a mobile phase without methanol k'_{w}, thus k'_{SOM} and k'_{blank} for the soil organic matter and blank columns respectively. The linearity is tested by a two-tailed t-test on the correlation coefficients R (e.g. Miller and Miller 1993), and for all compounds in Table 4.1 significant linear correlation within a 95 % confidence level is found (for 5H-phenanthro [4,5-bcd]pyran-5-one, benzanthrone, 1-hydroxy, 1-acetyl, 2-acetyl and 4-acetylpyrene within 90 %). Thus the preciseness of the method must be considered to be good. The accuracy of the method will be discussed by comparison with literature values later in this chapter.

The use of the direct method seems to be limited by the aqueous solubility and/or the octanol-water-partitioning coefficient. Based on the data obtained by the Aldrich HA column the method is restricted to compounds having an aqueous solubility above 10^{-7} mol l^{-1} and/or a log K_{ow} value below 5.5. Even though 1,2-benzacridine fulfils both demands (C_{sol}: $1.5 \cdot 10^{-6}$ mol l^{-1} and log K_{ow}: 4.45) like a handful of other compounds, there is at the present no explanation for the missing usefulness of the method on that single compound. For the compounds tested on the Ulsted RSC column no such limitation is observed (lowest tested C_{sol}: $4.8 \cdot 10^{-8}$ mol l^{-1} and highest tested log K_{ow}: 5.54).

If the limitation is caused by the solubility, formation of clusters is a reasonable explanation for the lack of linearity between measurements using different methanol content in the mobile phase. A simple way to avoid clusters could be to decrease the load of PAC onto the column, which thus demands a higher sensitivity on the detection equipment.

5.2 Comparison with log K_{oc} from the literature

To confirm the reliability (thus the accuracy) of the HPLC method used in this work the results are compared with data from literature using different kinds of batch methods (Table 5.1).

In general there is a good agreement between the values in this work and those of others, even thought there is a tendency towards lower sorption especially for the bicyclic compounds. This minor difference perhaps reflects that there in batch experiments is time enough to achieve sorption at sites requiring a long process time in contrast to the HPLC SOM column method. Another possible explanation for the lower sorption seen by the method used here compared to batch methods using Aldrich humic acid could lie in the assumption of linearity between log k'_w and fraction of methanol in the mobile phase. This possibility has been tested on a humic acid HPLC column, showing full linearity of log k' for the bicyclic compounds decreasing the methanol fraction in the mobile phase from 25 %(V/V) to 0 %(V/V), hereby excluding this explanation.

Table 5.1. Log K_{oc} values from this work (Aldrich HA and Ulsted RSC) compared with data from the literature determined by batch experiments.

	log K_{oc}					
Compound	**HPLC method**			**Batch experiments**		
	Aldrich HA	**Ulsted RSC**		**Aldrich HA**	**Other humic substances**	
PAH						
Naphthalene	2.98 ± 0.17	3.96 ± 0.20	3.74 ^{a}	3.32^{b}	3.91	^{e}
Fluorene	4.00 ± 0.21	4.61 ± 0.23	4.68 ^{a}		4.81	^{e}
Anthracene	4.46 ± 0.27	5.19 ± 0.24	4.65 ^{a}	4.64^{c}, 4.87^{b}, 5.0^{d}	4.1 - 5.31	^{c,e}
Phenanthrene	4.34 ± 0.28	5.16 ± 0.25	4.65 ^{a}	4.33^{c}, 4.81^{b}	5.34	^{e}
Pyrene	5.14 ± 0.27	5.27 ± 0.21		5.24^{c}, 5.4^{d}	4.01 - 5.90	^{c,d,e,f,g,h}
1,2-benzanthracene	5.96	5.94 ± 0.19	5.62 ^{a}	5.48^{b}	6.11	^{e}
Benz[a]pyrene	6.85			6.30^{b}	5.3 - 6.12	^{e,g}
1,2,5,6-dibenzanthracene	7.22				6.03	^{e}
N-PAC						
Quinoline	2.69 ± 0.20	2.99 ± 0.21	2.89 ^{a}	3.05^{b}		
Isoquinoline	2.86 ± 0.21	3.02 ± 0.21	3.09 ^{a}			
4-azaflourene	3.40 ± 0.23	3.82 ± 0.24	3.63 ^{a}			
Acridine	4.24 ± 0.22	4.31 ± 0.21	4.00 ^{a}			
5,6-benzoquinoline	4.18 ± 0.22	4.20 ± 0.24	4.07 ^{a}			
7,8-benzoquinoline	3.92 ± 0.21	4.18 ± 0.24	4.13 ^{a}			
Phenantridine	3.92 ± 0.23	4.19 ± 0.25	4.06 ^{a}			
1,2-benzacridine	5.70	5.08 ± 0.25				
Carbazole	3.69 ± 0.22	4.30 ± 0.22	4.74 ^{a}			

[a] From Nielsen et al. (1997). Indirect determination by an empirical correlation between log K_{oc} and log k' measured at one water-methanol mixture as mobile phase on a HPLC columns with chemically immobilised Aldrich humic acid.

[b] From Nielsen et al. (1997). Batch experiments on Aldrich humic acid.

[c] From Laor et al. (2002). In batch experiments using humic substances bound to an inert Sol-Gel, *i.e.* a silane-polymer.

[d] From Perminova et al. (1999). Determined by fluorescence quenching using 26 different humic materials including Aldrich HA.

[e] From Krauss and Wilcke (2001). Achieved by desorption of PAH by methanol-water mixtures.

[f] From Kopinke et al. (2001). Sorption of pyrene to 11 kinds of DOM. Pyrene was extracted by use of SPME (solid-phase microextraction).

[g] From Haitzer et al. (1999). Estimated from bioconcentration factors (BCF) in systems using seven different types of DOM.

[h] From Chefetz et al. (2000). Pyrene sorption to six kinds of SOM.

Figure 5.1 shows a correlation between the results obtained by the Aldrich humic acid HPLC column (using the direct method) and literature values using Aldrich humic acid in batch experiments (values from Table 5.1). The fact that the slope is close to one (1.0132) clearly shows the similarity between the results obtained by use of different methods.

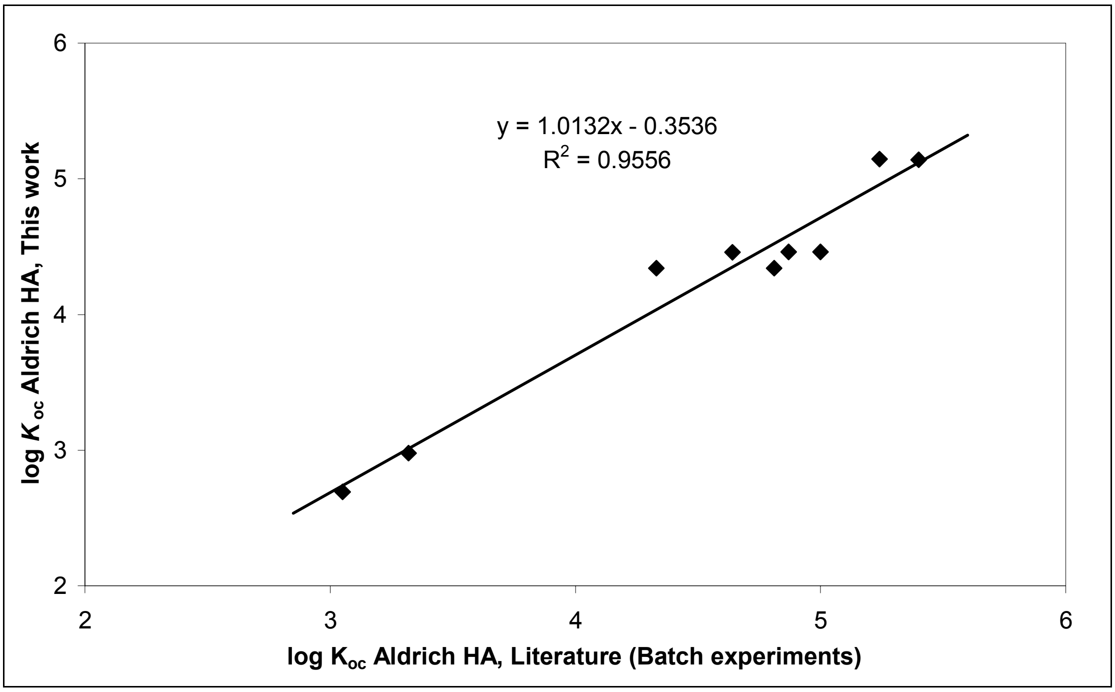


Figure 5.1. Relationship between log K_{oc} values obtained by use of the Aldrich humic acid HPLC column (direct method) and data from the literature using Aldrich humic acid in batch experiments (Table 5.1). Results from quinoline and four PAH take part of the correlation.

Ideally the intercept should be zero, so the negative intercept (-0.3536) indicates general lower log K_{oc}-values obtained by the method used here compared to batch experiments. Analyses of the line (95 % confidence limits, t-value = 2.36, degree of freedom = 7) gives confidence interval of 0.195 on the slope and 0.892 on the intercept, thus indicating no systematic variation between the literature values obtained by batch experiments and the data from the HPLC method used here. The agreement between the values presented in this work and those of others using Aldrich humic acid in batch experiments is a good confirmation of use of the HPLC-method to determine partitioning coefficients. Special attention should be focused on the data from Laor et al. (2002), since those results are achieved using Aldrich humic acid bound to silane-polymers in batch experiments. Although the methods are different, the sorption properties should be identical with the ones in this work. The similarity between the results of Laor et al (2002) and this work indicates that the use of HPLC SOM columns in determination of log K_{oc} is fully acceptable.

5.3 Comparison of the two methods

Caused by insufficient solubility of the largest compounds in the mobile phase lowering the methanol content it was impossible to use the direct method to determine log K_{oc}. The partition coefficient for the four and five ringed PAC (except pyrene), dibromoanthracene and mono- and di-bromopyrenes was therefore calculated by an empirical correlation found between log K_{oc} from all bi-, tri- and tetracyclic compounds without oxygen-containing substitutes determined by directed determination and log k'_{75} (the capacity coefficient measured on the humic acid column using a mobile phase with a methanol fraction volume of 0.75). The correlation is based on 18 compounds having the empirical equation log K_{oc} = 2.2483 × log k'_{75} + 5.2002 with a correlation coefficient (R^2) of 0.79, t-test on R shows a significant linear correlation (95 % confidence level).

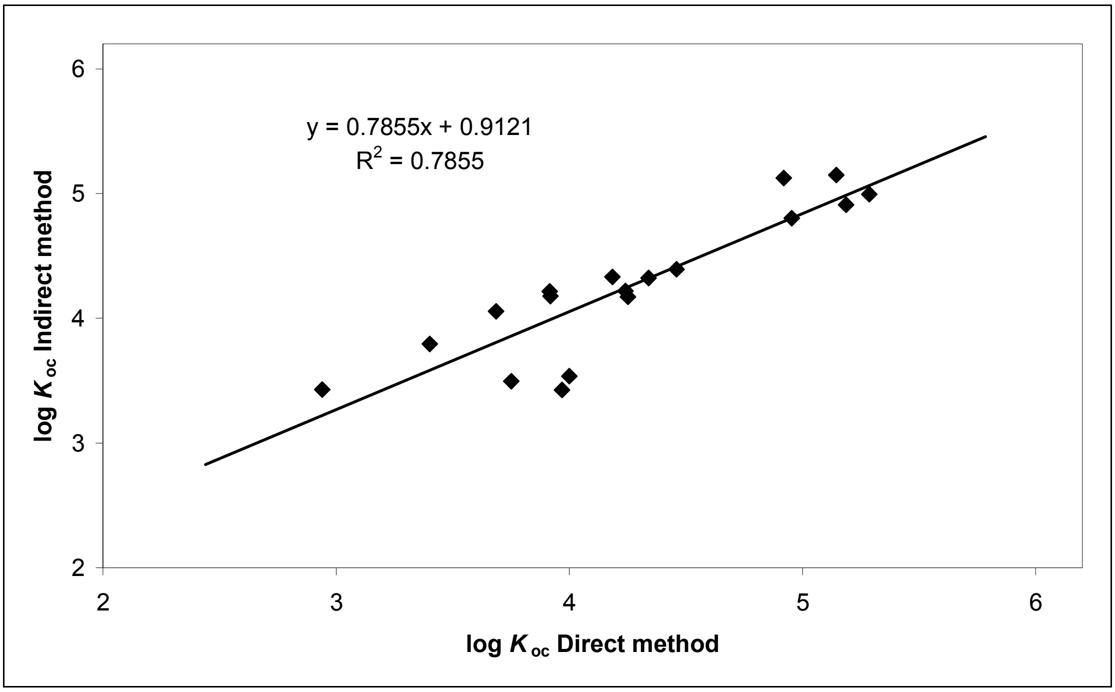


Figure 5.2. Relationships between log K_{oc} values determined by the indirect and direct method for the 18 PAC on which the indirect method is built.

Since the indirect method is built on a linear correlation between log K_{oc} from the direct method and the retention of the compound on the column at one mobile phase (75 % MeOH) it is interesting to evaluate how good this correlation is. An obvious way to do so, is to calculate log K_{oc} by the indirect method for the compounds on which the method is built and compare the data with the results from the direct method (Fig 5.2).

If the two sets of data are identical the slope and the intercept in figure 5.2 should be 1 and zero respectively. Even though a first glance at the equation for the line gives the impression that this is not true, a closer analysis of the data validates the usefulness of the indirect method since the 95 % confidence interval (t-value = 2.12, degree of freedom = 16) gives the equation:

$$\log K_{\mathrm{oc\ indirect}} = 0.7855\ (\pm\ 0.2175) \times \log K_{\mathrm{oc\ direct}} + 0.9121\ (\pm 0.9352).$$

Both demands are just fulfilled and there is thus no systematic evidence for differences between the two sets of data.

Another way to evaluate the indirect method is to look at the compounds not used for the empirical correlation between log K_{oc} (from the direct method) and k'_{75}. Of the 52 compounds for which log K_{oc} was determined by the direct method 18 was used for the indirect method. Of the rest (34 compounds) the retention of 26 X-PAH and the 4 X-N-PAC was measured using the mobile phase with 75 %(V/V) MeOH. Using the same approach as in the previous paragraph a linear correlation was found (Fig 5.3) with the equation (95 % confidence interval t-value = 2.05, degree of freedom = 28):

$$\log K_{\mathrm{oc\ indirect}} = 1.0736\ (\pm\ 0.671) \times \log K_{\mathrm{oc\ direct}} - 0.0184\ (\pm 2.929).$$

As seen the ideal relationship is included in the confidence intervals and a two-tailed t-test on R conforms a significant (95 % confidence level) linear correlation between the two sets of data. Even though, the used of the indirect method on compounds with different substituted groups as here should be reconsidered since the calculated log K_{oc} values are up too three log units to high for e.g. 1,6-pyrene-dione and three log units lower than the measured values for the acids, e.g. phenanthrene-4-carboxylic acid and 1-hydroxy-2-naphtholic acid.

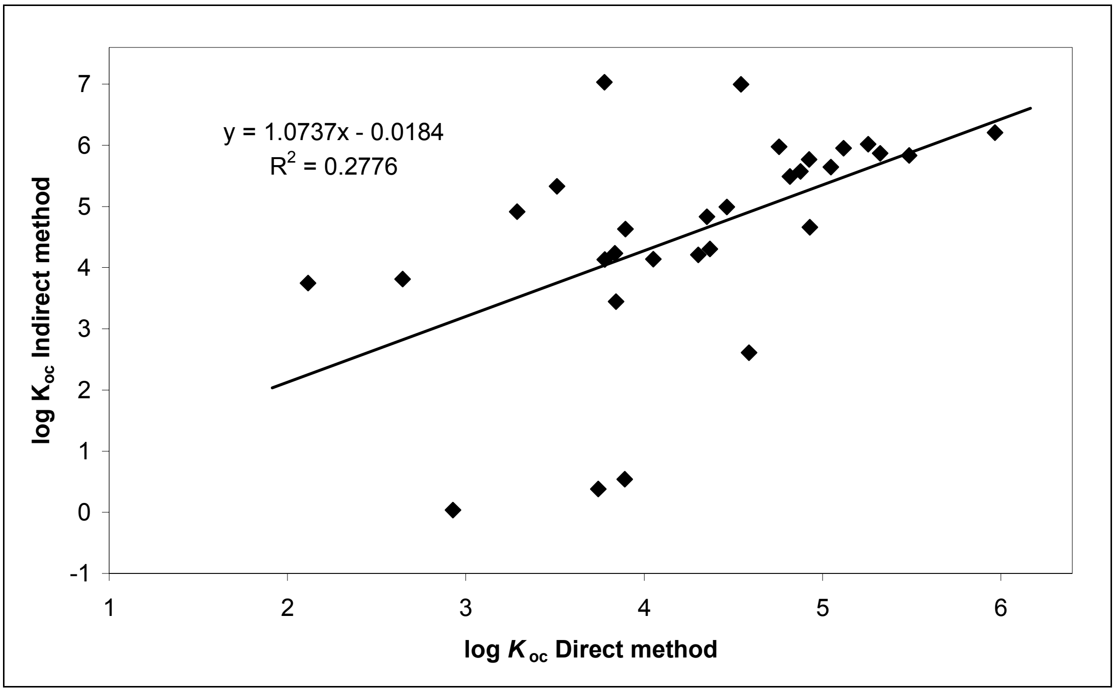


Figure 5.3. Relationship between log K_{oc} values determined by the indirect and direct method for 30 substituted PAC.

The indirect method was built for determination of the large unsubstituted compounds, 9,10-dibromoanthracene and bromopyrenes, and the model compounds were therefore selected based on their molecular structure and an assumption of similar sorption mechanisms. Since the compounds investigated here are all substituted PAC, which were rejected in the creation of the indirect method differences were expected. A separate discussion of the effect of substitution on anthracene and pyrene will follow in the next chapter.

The two examples above clearly demonstrate that the usefulness of a model like the indirect method is restricted, and that one should by aware of those limitations using different models.

5.4 Similarities to, and differences from earlier work

As a part of this discussion of the method it is natural to look at the results achieved by the column material used here and makes comparisons to earlier work, both using Aldrich HA as SOM.

Figure 5.4 shows the relationship between log *k′* (for 44 PAH, N-PAC, O, S-PAC and X-PAC) for the column used in this work (carbonyl) and the one being basis for this work (amino), both set of data are achieved using 65 %(V/V) methanol in the mobile phase.

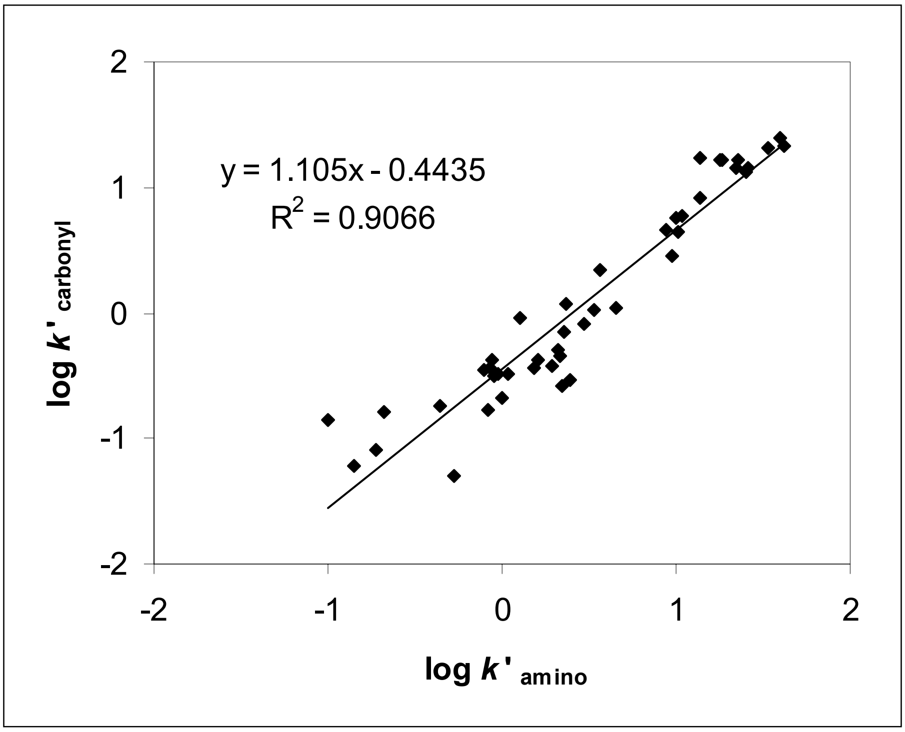


Figure 5.4. The relationship between this work (carbonyl) and the one being basis for this work (amino) illustrated by the retention (log *k′*) for 44 compounds achieved using 65 %(V/V) methanol in the mobile phase.

In the carbonyl procedure used in this work residues of carbohydrates and aromatic quinones in the humic substance are used for linking the column material. In the amino procedure peptide residues in the humic acid take part in the cross-linking. Thus a comparison of the retention on the two types of column materials should make it possible to determine if the attachment procedure causes major changes in the humic acid composition. In general there is a good accordance between the results. However there are significant differences for some compounds especially at low retention.

The retention capacity of a compound depends both on the column surface area, of the silica column material (BET) and its humic acid content, expressed as humic acid organic carbon (OC_{SOM}) $k' = A \times BET \times OC_{SOM}$, A being a constant. The slope of the regression line in Figure 5.4 should be similar to the ratio between log (BET × OC_{SOM}) for the two columns, if the way to attach humic acid has no or little effect on the properties of the humic acid.

$$\frac{\log\left(\mathrm{BET}\cdot\mathrm{OC}_{\mathrm{SOM}}\right)_{\mathrm{Carbonyl}}}{\log\left(\mathrm{BET}\cdot\mathrm{OC}_{\mathrm{SOM}}\right)_{\mathrm{Amino}}}=\frac{\log\left(100\mathrm{m}^2\mathrm{g}^{-1}\cdot 1.9\%\mathrm{wt}\right)}{\log\left(450\mathrm{m}^2\mathrm{g}^{-1}\cdot 0.7\%\mathrm{wt}\right)}=0.94$$

This ratio of 0.94 is reasonable close to the slope in the figure (Fig. 5.4) of 1.10 ± 0.11 (95 % confidence limit) considering there is a negative intercept. Thus in general terms there was fairly good agreement in the properties of the humic acid bonded by the two methods. This probably means that the properties of the bonded humic acid should not be very different from those of the free humic acid, at least for the Aldrich humic acid. This observation is also reported by Laor et al (2002), when comparing data for sorption of PAH to humic acid bound to silane-polymers with sorption to from freely dissolved humic acid.

A way to illustrate if other factors than hydrophobicity contribute to the partitioning is the approach suggested by Seth et al. (1999). In this method log K_{ow} is a reference indicator (axis of abcissa) and the expression log (k'/K_{ow}) is the ordinate, where k' values are achieved using 65 %(V/V) MeOH in the mobile phase. As a reference line for hydrophobicity the regression line for the hydrophobic PAH is incorporated in Figure 5.5.

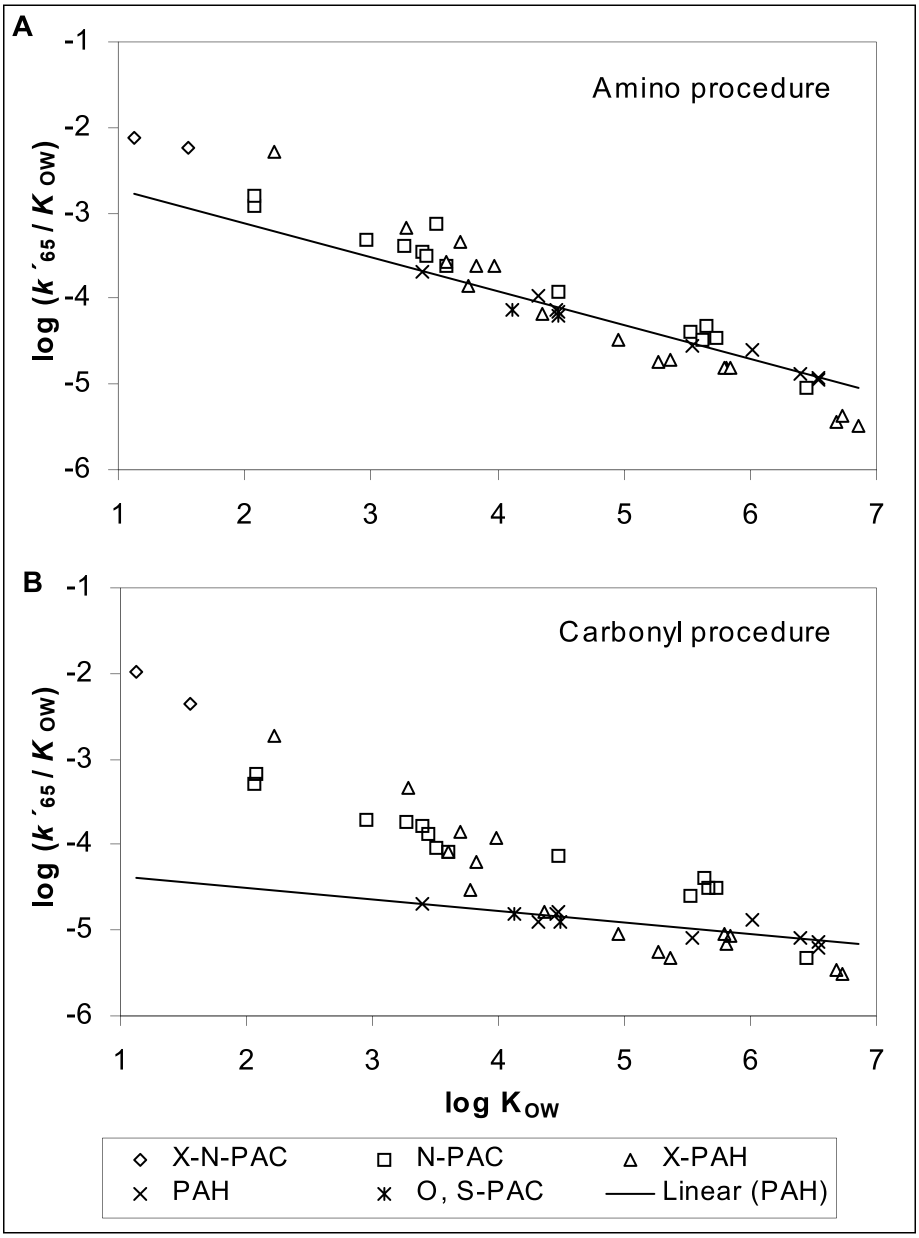


Figure 5.5. Relationships between log K_{ow} and log (k'/K_{ow}) on columns made by the two procedures. The retention of either 43 (amino procedure) or 42 (carbonyl procedure) polycyclic aromatic compounds are measured using mobile phases with 65 %(V/V) methanol. The regression lines are for PAH only. Data for K_{ow} is from Helweg et al. (1997a).

In general the figure indicates a good correlation between the log k' (plotted as log (k'/K_{ow})) and log K_{ow} even though the sorption is relatively stronger for the polar compounds (N-PAC, substituted N-PAC and polar substituted PAH) compared to PAH. The relative increase is particularly strong for those polar PAC having low K_{ow} values, indicating that chemical interactions in addition to the hydrophobicity is contributing to the partitioning process for polar compounds.

The polar compounds are adsorbed relatively stronger on the column synthesised by the carbonyl procedure (Fig. 5.5B) than by the amino procedure (Fig 5.5A) compared to the non-polar compounds. An explanation for this observation could be that nitrogen-containing moieties in the humic acid are attractive sites for polar compounds to enter into specific adsorption mechanisms. As the amount of amino groups are decreased compared to the free humic acid, due to the cross linking in the amino procedure, the differences in sorption between polar and non-polar are reduced.

A similar approach could be claimed about carbonyl groups in the carbonyl procedure. But as the concentration of carbonyl groups (Malcom and MacCarthy, 1986) are several (almost 25) times higher than amino groups (see Appendix 2 "Characterisation of Humic Materials") in Aldrich humic acid, the relative reduction of sites can be neglected in the carbonyl procedure.

When comparing data obtained by columns made by the two procedures, the differences in carrier carbon has to be taken into account. In the column used in this work (carbonyl) about 75 % of the carbon has humic origin, whether the one referred to as the "amino procedure" contains approximately 17 times as much carrier carbon as carbon from humic acid. This large excess of carrier carbon has to affect the retention and might be an additional explanation for the differences between the two columns to adsorb polar compounds.

When using the amino procedure for attachment of humic acid to the HPLC column material, the pH effect on sorption of N-PAC to bound humic acid gave only a small increase of k' at pH above 5 (T. Nielsen, personal communication). This suggests a low content of carboxylate anions in humic acid on the column. Thus the previous column material did not represent the pH properties of the free Aldrich humic acid very well.

In this work the effect was significant in the pH range from 2.5 to 8 (Fig. 4.1), and similar to what was observed for quinoline in batch experiments (Chorover et al., 1999; Nielsen et al., 1997). The variation of electrolyte concentration gave similar result to what was achieved by work using the amino procedure (Kollist-Siigur et al., 2001), so the column made by that procedure appears not to be so bad in this context.

5.5 Summary

It has been possible to use the HPLC SOM columns in a direct determination of the partition coefficient log K_{oc} for a broad variety of PAC. The determination involves a linear extrapolation between mobile phases with different methanol content. This linearity has been proved for 54 different compounds using two different kinds of SOM. The use of the Aldrich HA column is (in this work) restricted to compounds having aqueous solubility above 10^{-7} mol l^{-1} and/or log K_{ow} value below 5.5. Those limitations could be surmounted by use of more sensitive detectors.

For the PAC not fulfilling the demands of solubility, an indirect determination, based on data from the direct determination and measurements using one mobile phase, is used. The usefulness of the indirect method has been demonstrated by comparing results from the two methods.

Results of this work are compared to data from the literature, concluding that the usefulness of the HPLC SOM column method in determining log K_{oc} is good. For the sorption of small PAH and quinoline to Aldrich HA, no systematic differences between this work and the literature was observed.

Similarities in sorption onto column material used in this and earlier works indicate that the properties of humic acid in general are retained after being immobilised. Thus modification improvements are observed, especially in the ability to show the differences in sorption of polar and non-polar compounds. Great improvements in HPLC column materials were observed, as the new fabrication procedure makes it possible to investigate the pH effect on sorption of PAC. Earlier works on HPLC SOM column using the former procedure only showed minor pH effects on sorption of N-PAC. This column gives data of the same quality as obtained in batch experiments (Nielsen et al., 1997).

6. Sorption of PAC to Soil organic matter

The central part of this work was to use the optimised humic substance HPLC columns to determine partitioning coefficients K_{oc} for a variety of polycyclic aromatic compounds. In this chapter the presented partition coefficients (Chapter 4) and effects of molecular properties such as size, shape and lipophilicity on sorption discussed, and so are the effects of substituents. Finally the linear free energy relationships (LFER), between log K_{oc} and log K_{ow} and the aqueous solubility (C_{sol}) respectively are examined.

6.1 Influence of molecule size and ring heteroatoms

Considering the sorption of unsubstituted compounds to Aldrich HA (Table 4.1 and 4.2) several major observations are done. An increasing sorption with the molecule size (number of rings) is seen for both the PAH and their heterocylic nitrogen containing analogues (N-PAC) (Figure 6.1A). Between these two kinds of compounds the PAH show a higher sorption than the basic N-PAC; this is in good agreement with the hydrophobicity of the compounds, e.g. K_{ow} (Helweg et al., 1997a).

The five compounds with fluorene structure show a decrease in sorption in the range (dibenzothiophene, fluorene, dibenzofuran, carbazole, and 4-azafluorene) following the same order as for log K_{ow}. This indicates that non-specific interactions are the major mechanism in sorption and that a specific process in which 4-azafluorene creates hydrogen bonds with SOM is of less importance. Changes of enthalpy and entropy during the sorption (next chapter) also indicate some kinds of specific interactions for dibenzofuran and dibenzothiophene.

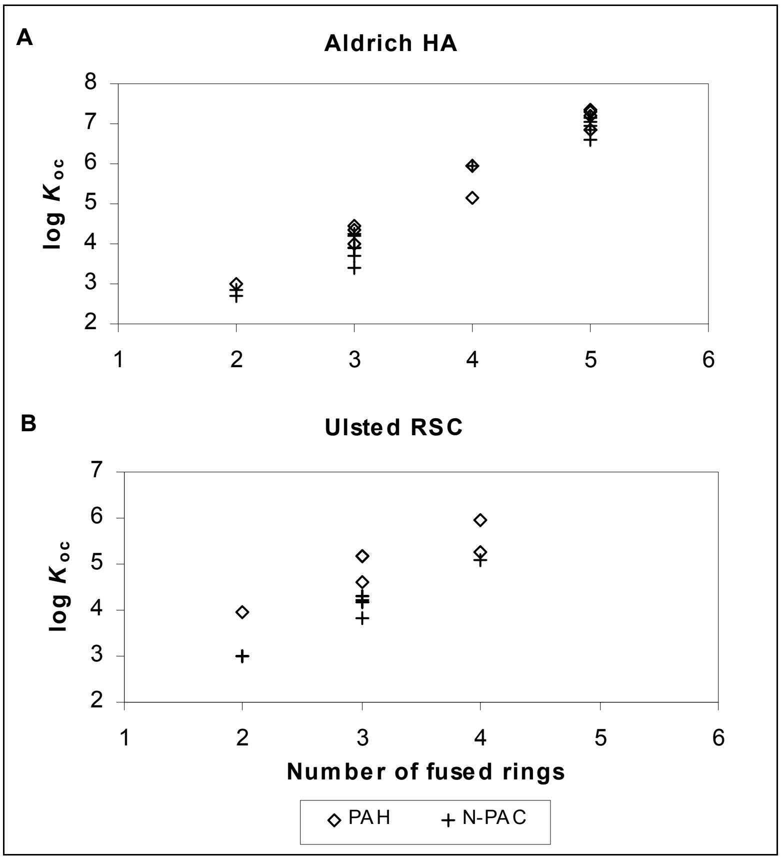


Figure 6.1. Relationship between the sorption (as log K_{oc}) and the number of fused rings in the unsubstituted PAH and N-PAC. Data for log K_{oc} are from Table 4.1 and Table 4.2.

A pattern similar to the results for Aldrich HA is seen for Ulsted RSC (Table 4.1 and Figure 6.1). Most striking is the higher log K_{oc}-value for the PAH, especially for naphthalene where the distribution coefficient is one log unit higher than for Aldrich HA. The main reason for this observation must be that Ulsted RSC consist of larger molecules than Aldrich HA does (see Appendix 2). For the N-PAC log K_{oc}-values are in the same ranges for the two kinds of SOM. The difference in sorption between PAH and N-PAC to the two kinds of SOM, indicates that the formation of H-bonds are more prevalent between N-PAC and Aldrich HA than between N-PAC and Ulsted RSC. This explanation seems quite reasonable, since Ulsted RSC contains more carbon (60 %wt.) than Aldrich HA (42.5 %wt.), and hence less functional groups capable to create hydrogen-bonds with N-PAC.

6.2 Steric effects

Not only molecular size and endocyclic substitution, but also the shape of the molecules seems to influence the sorption, as the linear molecules anthracene and acridine have higher log K_{oc} values than their bent isomers phenanthrene and the three other benzoquinolines. Surprisingly the shielding of the nitrogen atom by benzene rings does not seem to affect the sorption in contrast to what has been observed earlier (Nielsen et al., 1997). One should of course be aware that the difference lies within the standard deviations and that this trend therefore is not systematic.

A shielding effect is on the other hand seen for the dibenzacridines (Table 4.2), for which the sorption increases with decreasing K_{ow}, showing the highest log K_{oc}-value for 1,2,7,8-dibenzacridine (with N sitting in an unshielded position) and the lowest for 3,4,5,6-dibenzacridine (with N sitting shielded by two benzene rings) (see Appendix 1 for structures of the compounds). The fact that the highest sorption is seen where N is unshielded and the opposite order of K_{oc}- and K_{OW}-values of the compounds, indicates that formations of hydrogen bounds between the nitrogen atom in N-PAC and humic acid increases the sorption.

6.3 Substitution effects on anthracene and pyrene

The effect on sorption of substituents to the fused ring system has been investigated. For anthracenes and pyrenes the changes in sorption relative to anthracene and pyrene designated "Δlog K_{oc}" (log $K_{oc\ X\text{-}PAH}$ – log $K_{oc\ PAH}$) has been compared with the lipophilic aromatic substituent constants (π_{Ar}). This constant describes the differences for substituted benzenes in partition coefficient in an octanol-water system (K_{ow}) relative to benzene (Hansch et al., 1973).

In Figure 6.2 Δlog K_{oc} is plotted vs. π_{Ar}. Most of the compounds are lying close around the 1:1 line indicating that the substituent effects are equal whether the system consists of humic acid and water or octanol and water, thus the sorption process solely is a matter of partitioning.

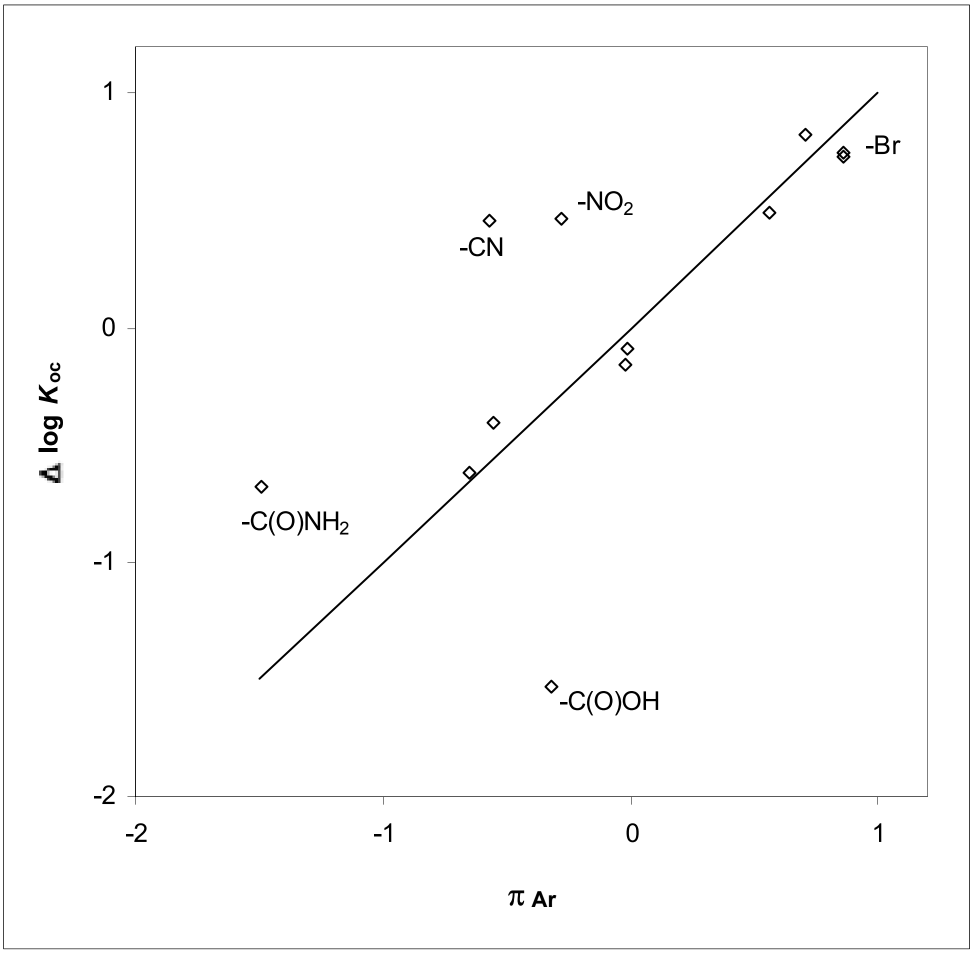


Figure 6.2. The substituent effect on the sorption of 12 mono- and di-substituted anthracenes described as Δlog K_{oc} (log $K_{oc\ \text{X-anthracene}}$ – log $K_{oc\ \text{Anthracene}}$) vs. the lipophilic aromatic substituent constants (π_{Ar})*. Δlog K_{oc} for 9,10-dibromanthracene are split equally on the two bromine atoms. All substituted anthracenes from Table 4.1, except the two anthraquinones, are included. The line shown is a 1:1 line and not a regression line.

*From Hansch et al. (1973).

The three substituents containing nitrogen all lying above (left to) the line disturbs this assumption as they emphasise the importance of more specific sorption mechanisms, e.g. the capability of creating hydrogen binding, dipole-dipole or charge transfer to the humic acid. 9-anthracene carboxylic acid is falling out side, as Δlog K_{oc} is small, most probably because the acid has a pK_a value of 3.65 (Kortum et al., 1961) and is totally dissociated at pH 7. The anion has a lipophilic aromatic substituent constant of -4.36 (Hansch et al., 1973), and is therefore expected to lie on the left side of the line, also indicating that other mechanisms than partitioning takes part in the sorption process. In the next chapter a similar approach of substitution effect is made on changes in entropy in the sorption process (ΔS_{sorp}). The results (Figure 7.3) also there show that the nitrogen containing substituent in some way takes part in specific interactions, probably H binding.

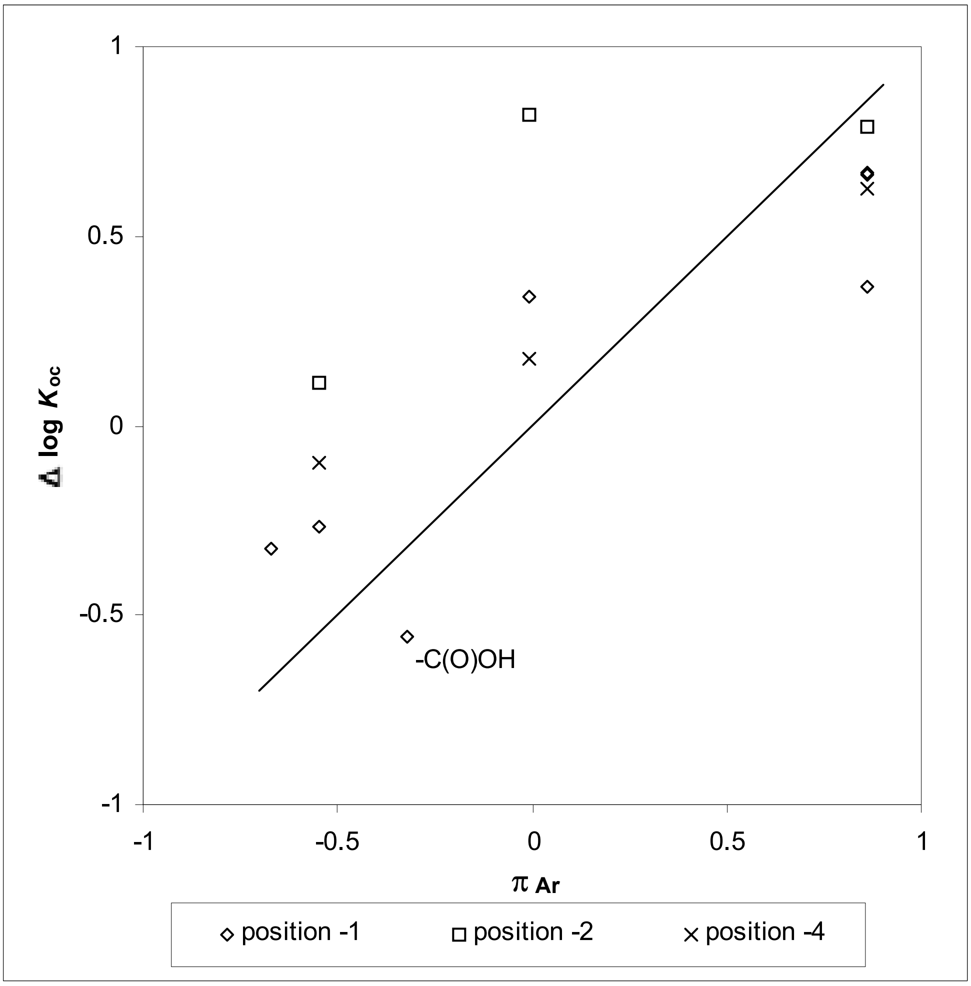


Figure 6.3. The substituent effect on the sorption of 13 mono- and di-substituted pyrenes described as $\Delta\log K_{oc}$ ($\log K_{oc\ x\text{-pyrene}} - \log K_{oc\ pyrene}$) vs. the lipophilic aromatic substituent constants (π_{Ar})*. Position of the substituent is marked by either rhombus (position -1), squares (position -2), or crosses (position -4). $\Delta\log K_{oc}$ for 1,6-dibromopyrene and 1,8-dibromopyrene are split equally on the two bromine atoms, both analogue to position -1. All substituted pyrenes from Table 4.1 and Table 4.2, except 1-formylpyrene and the pyrene-diones, are included. The line shown is a 1:1 line and not a regression line. *From Hansch et al. (1973).

For the substituted pyrenes (Figure 6.3) the same pattern as for anthracene is seen. Again the polar substitute is lying above the 1:1 line and only the lipophilic bromide lies below the line. For 1-pyrene carboxylic acid a similar assumption as for 9-anthracene carboxylic acid can be made.

When looking at the positions of the substituent it seems that position -2 on pyrene gives the most distinct effect (higher $\Delta\log K_{oc}$) on the sorption compared to position –1 and -4.

6.3.1 Effect of keto compounds

For quinone-like structures a decrease in the sorption compared to the corresponding PAH is seen. For fluorene (fluorenone) $\Delta\log K_{oc}$ is -0.16, for anthracene (anthraquinone) there is no effect, and for pyrene (1,6-pyrenedione, 4,5-pyrenedione and 5H-phenanthro [4,5-bcd]pyran-5-one) $\Delta\log K_{oc}$ are -1.36 and -0.60, respectively for two oxygen atoms in the pyrene-diones. For the lactone 5H-phenanthro [4,5-bcd]pyran-5-one the effect ($\Delta\log K_{oc}$ = -0.22) has to be combined with the in-ring substitution effect of oxygen, which is modest (fluorene and dibenzofuran).

Making the same evaluation as Hansch and co-workers (1973) on log K_{ow} for benzene (2.13, Hansch et al., 1995) and 1,4-benzoquinone (0.19, Schultz et al., 1997), the lipophilic constant π_{Ar} are –0.97 pr. oxygen atom, a value none of the compounds investigated here reaches. This indicates once again that specific interactions are involved in sorption to humic acid.

Even though the variations are large, the differences between the two pyrene-diones are noticed. The effect of the oxygen atoms is larger for 1,6-pyrenedione (substituents are placed at different ends of the molecule) compared to 4,5-pyrenedione (the oxygen atoms are sitting next to each other). So it seems that the effect depends on both the nature of the double bound oxygen atom and its position.

6.4 Linear free energy relationships (LFER)

A common way to get reliable data for K_{oc} without experimental work, is by LFER where empirical found relationships between K_{oc} and e.g. K_{ow} or aqueous solubility (C_{sol}) is used (e.g. Schwarzenbach et al., 1993). Using this approach on the data obtained by the methods used in this work, linear correlation is found between log K_{oc} (Aldrich HA) and log K_{ow} (Fig 6.4A) and log K_{oc} vs. C_{sol} (mol l^{-1}) (Fig 6.4B) respectively.

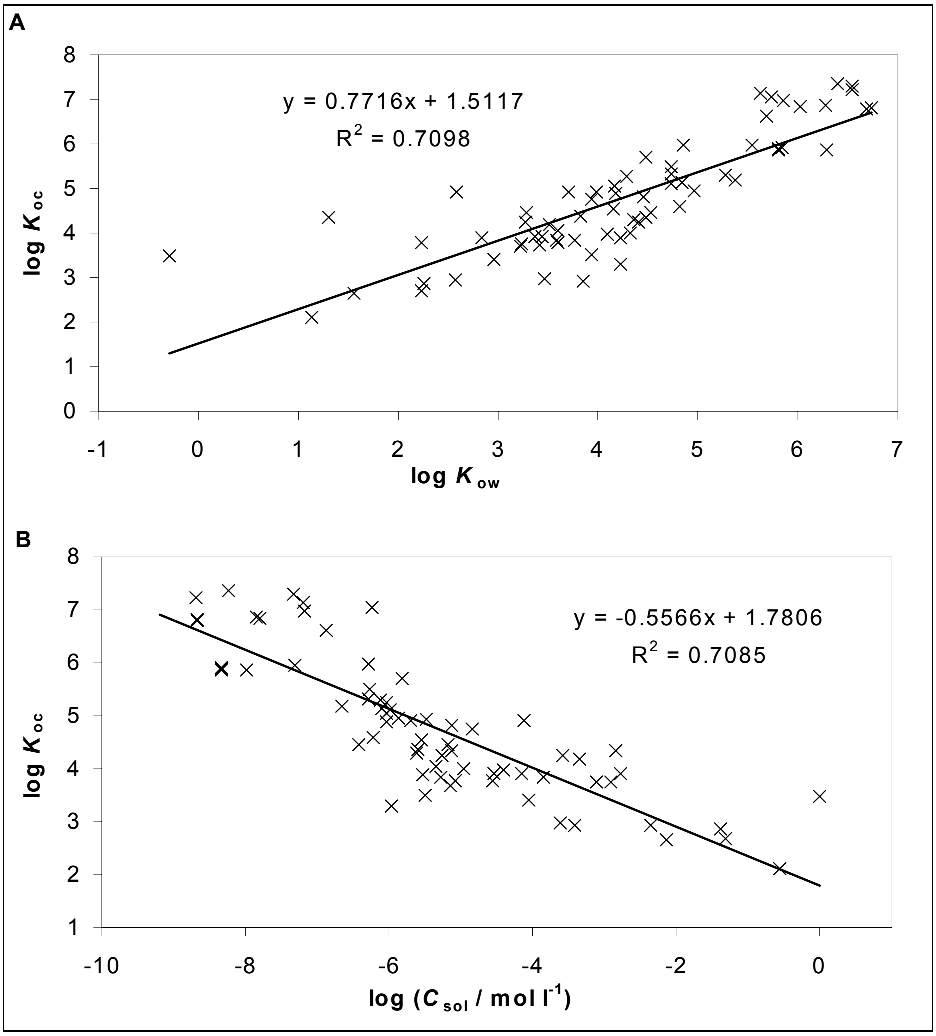


Figure 6.4. Linear free energy relationships between log K_{oc} for Aldrich HA and log K_{ow} (A) and log C_{sol} (B) respectably. The correlation is based on the 69 PAC used in this work. All data are from table 4.1 and 4.2.

These correlations are useful but obvious defective since the compounds, even though their belong to the same class of compounds, are different in size (128 to 360 g mol^{-1}), have substituents with different hydrophobic properties and therefore different aqueous solubility and affinity for humic acid. The solubility range for the compounds used here is from 2.0 nmol l^{-1} (1,2,5,6-dibenzoanthracene) to 0.97 mol l^{-1} (N-methyl quinolinium iodide), thus almost nine log units. The octanol-water partitioning coefficient, log K_{ow} covers a range from -0.29 (N-methyl quinolinium iodide) to 6.73 (1,8-dibromopyrene).

As seen in the discussion of substitution effects on anthracene (previous paragraph), efforts should be taken with regards to the chemical structure of the compounds. Thus a more precise model could be made on the data, but further work in this area lies beyond the scoop of this project.

Looking only at the unsubstituted compounds (Fig. 6.5 for Aldrich HA and Fig. 6.6 for Ulsted RSC) the LFER is better and more useful compared to figure 6.4, but the use is of course limited to a small group of compounds. Furthermore two important observations are done.

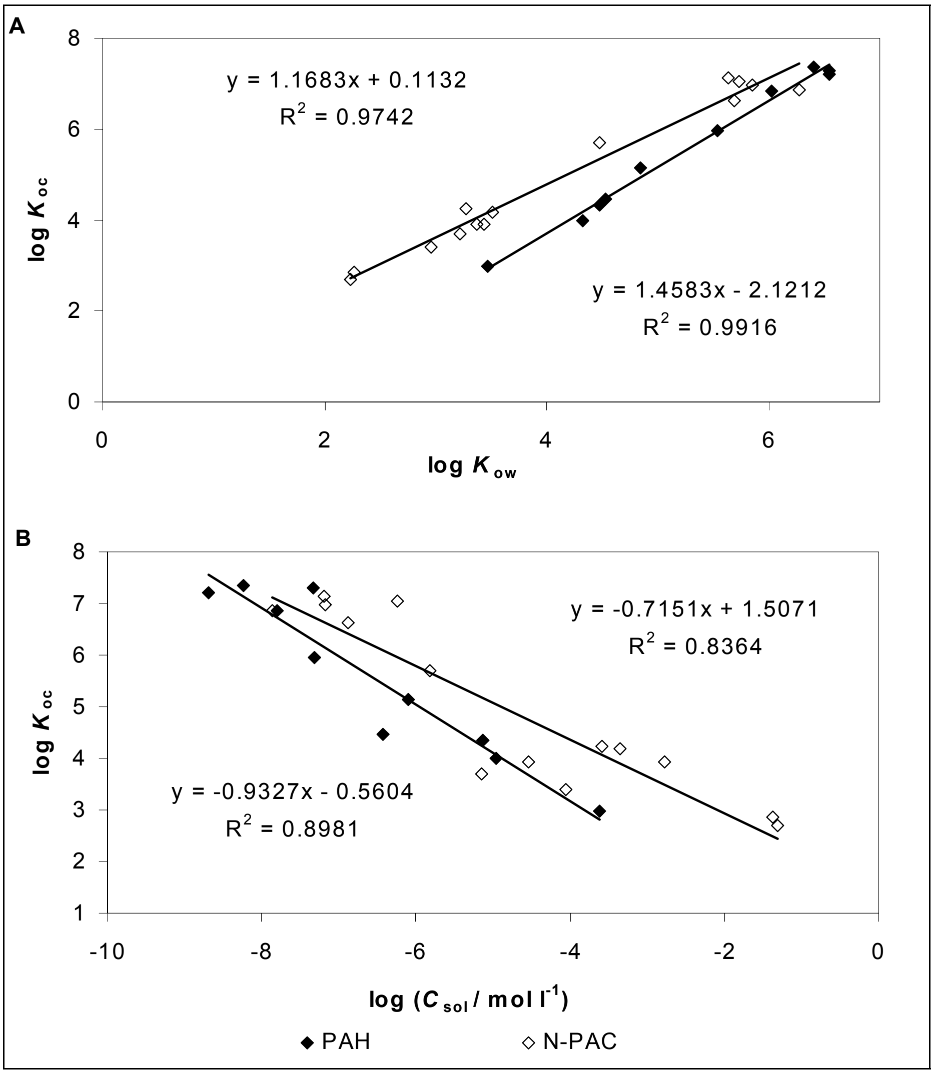


Figure 6.5. Linear free energy relationships for PAH and N-PAC between log K_{oc} for Aldrich HA and log K_{ow} (A) and log C_{sol} (B) respectably. The correlation is based on the 10 PAH (closed symbols) and 14 N-PAC (open symbols) used in this work. All data are from table 4.1 and 4.2.

The first observation is the fine relationship between log K_{ow} and log K_{oc} (Fig 6.5A) confirming the usefulness of the indirect method, at least for the unsubstituted compounds, since the correlation is made on data determined by both the direct and indirect method.

The other aspect is the differences between the two kinds of SOM. The LFER between log K_{oc} and log K_{ow} has a higher slope and lower intercept for Aldrich HA (Fig 6.5A) than ULSTED RSC (Fig. 6.6A).

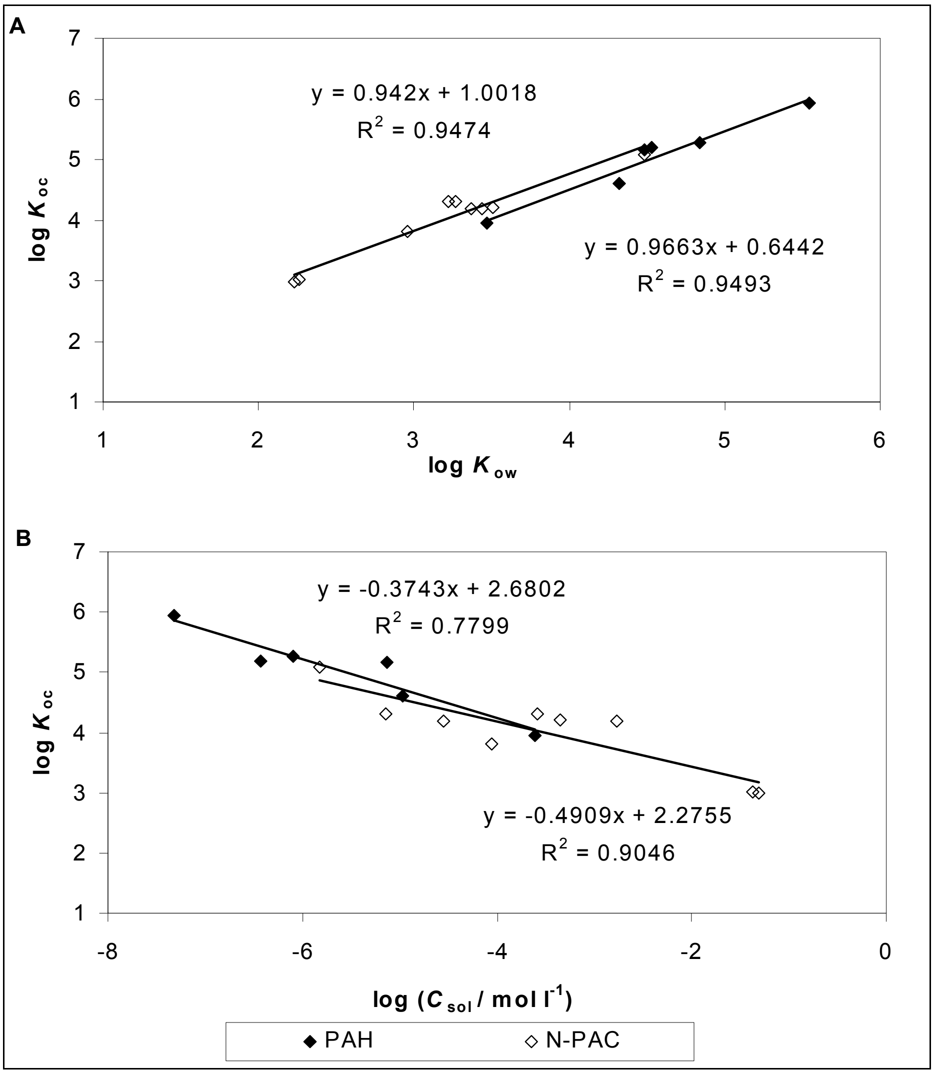


Figure 6.6. Linear free energy relationships for PAH and N-PAC between log K_{oc} for Ulsted RSC and log K_{ow} (A) and log C_{sol} (B) respectably. The correlation is based on the 6 PAH (closed symbols) and 9 N-PAC (open symbols) used in this work. All data are from table 4.1.

Analysis of the lines shows that the lines for PAH are significant different at 95 % confidence limit for the two SOM, both regarding the slope and the intercept. For N-PAC no such difference is observed. The only significant difference between LFER for log K_{oc} and log C_{sol} is the slope of the N-PAC (Fig. 6.5B and 6.6B). The observed difference between the two kinds of SOM once more emphases the need of knowledge making robust models for calculation of physical and chemical properties based on the molecular structures for different compounds.

Comparing results for PAH in this work with the literature (Karickhoff, 1981) shows similarities in the LFER with regards to solubility making a correlation based on data from both SOM (not shown). With regards to log K_{ow}, the LFER are so similar that just the smallest standard derivation on the data from Karickhoff (1981) would result in no significant differences between the two works.

The two groups of substituted compounds have also been examined separately. For the 36 X-PAH, LFER similar to those in figure 6.4 were found, but the correlation coefficients were lower. The 7 X-N-PAC alone did not show any linear relationships within a 90 % confidence limit.

6.5 Summary

Correlation was found between the sorption and parameters as molecular size, shielding effects, and hydrophobicity for the non-substituted compounds. In general the sorption was increasing with the number of fused rings. There was striking evidence for shielding effect of N in the dibenzacridines. Non-polar PAH showed a higher sorption than the polar and less hydrophobic N-PAC.
Substituent effects were evaluated and a correlation with the lipophilicity of the substituents was attempted. For substitutes on both anthracene and pyrene polar N-containing substituents shows the highest ability of formation of H-bonds. Also the position of substitution on pyrene seems to have effect on sorption, with the strongest effect on position -2.
The results indicate that the sorption is a process involving multiple interactions, including both non-specific (hydrophobic adsorption and partitioning) and more specific interactions (e.g. H-binding and van der Waals forces).

The examination of LFER shows nice linear relationships and emphasises both the usefulness and limitations of those kinds of models. The needs for reliable experimental data, or secondary use of more complicated models taking more molecular properties into accord, are of great importance.

7. Temperature variation and determination of ΔH and ΔS

The retention of a series of selected PAC was measured at different temperatures as a part of the investigation of how environmental impacts affects the sorption of PAC to Aldrich humic acid.

Besides the direct measurable results, the data gave the opportunity to go further into the thermodynamic of the sorption process. Values for changes of enthalpy and entropy (ΔH and ΔS) for the sorption process has been achieved and these values have been used in calculation of K_{oc}. By comparison of those data with the results from the direct determination of log K_{oc} (previous chapters), a validation of the usefulness of the HPLC method is made. Derivation of central thermodynamic equations is given in Appendix 4.

7.1 Enthalpy changes, ΔH_{sorp}

The values are shown in Table 4.3 calculated from the slope in the van't Hoff plot (Fig. 2.5). Standard deviations (sd) are calculated from the standard deviation on the slope of the linear fit.

The most striking feature is that all values are negative, in other words the sorption process is energetically favourable for the system. In general the PAH shows a decrease in the sorption enthalpy with increasing molecule size within each mobile phase. Between the mobile phases ΔH_{sorp} decreases with decreasing methanol content. The repeated determinations of ΔH_{sorp} in overlapping temperature intervals (using mobile phase with 65 %(V/V) MeOH) for anthracene agree extraordinary well. Similar patterns are, except for the bicyclic compounds, seen for the N-PAC both within each series (mobile phase) and between. The difference between the repeated determinations for quinoline is presently unexplained. The substituted anthracenes (X-anthracenes) will be discussed in a separate paragraph looking at the substitution effects.

The study by Wauchope et al. (1983) using naphthalene as model compound, shows values of ΔH_{sorp} between -7.1 and 3.8 kJ mol^{-1}, using different concentrations of naphthalene (10^{-4} mol kg^{-1} OM to 10^{-2} kg^{-1} OM) in slurries of soil samples (Bosket loam) with 1 % OM. Even though the method used here is different a comparison can be made. The amount of organic matter on the HPLC column is 0.054 g (calculated from V_S, ρ, and the measurements of organic carbon in Aldrich HA and on the column material). Injection volume was 20 µl using a 40 mg l^{-1} solution of naphthalene in methanol, giving a loading of $6 \cdot 10^{-9}$ mol naphthalene onto the column. Together this gives a concentration of 10^{-4} mol kg^{-1} OM. The values in table 4.3 is achieved using mixtures of water and methanol, and assuming a linear correlation it is possible to calculate a value for ΔH_{sorp} in a system without methanol to -27.7 kJ mol^{-1} (see also Table 7.1). This value is approximately four times the value found by Wauchope and co-workers, taking the different methods and kind of organic matter used in the experiments in mind. Lüers and ten Hulscher (1996) reported values of ΔH_{sorp} for sorption of six PAH with four to seven rings to natural dissolved organic matter. All values were negative and in the range of -18.28 ± 2.81 kJ mol^{-1} for fluoranthene to -40.65 ± 4.41 kJ mol^{-1} for benzo[ghi]perylene. Values in the same range are reported for pyrene in this work using high fractions of methanol (65 and 85 %(V/V)) in the mobile phase (Table 4.3). Thus ΔH_{sorp} in the pure humic acid - water system is expected to be lower than those reported by Lüers and ten Hulscher (1996). Using the same approach on pyrene as on naphthalene above (and Table 7.1) a value of ΔH_{sorp} is calculated to -54.2 kJ mol^{-1}, three times the value reported for another four ringed PAH fluoranthene.

Looking at the two examples above, the suspicion that the method used in this work gives to high (more negative) value rises. By use of pyridine as a model for N-PAC, ΔH for reactions with water respective phenol can be used as values of references. Enthalpy changes in formation of 1 to1 complexes of pyridine and phenol in CCl_4 is reported to be -31.8 kJ mol^{-1} (Mullens et al., 1985) and ΔH for dissolution of water in pyridine is -1.8 kJ mol^{-1} (Marczak et al., 2003). Using those two systems as models for the water phase and humic acid respective, the transfer of pyridine from water into phenol should result in a change of enthalpy on -30 kJ mol^{-1}, thus a value similar to what is found in this work.

7.2 Entropy changes, ΔS_{sorp}

The entropy changes (ΔS_{sorp}) are calculated from the intercept with the ordinate in the van't Hoff plot (Fig. 2.5) and shown in Table 4.4. The standard deviation on ΔS_{sorp} is calculated from standard deviations on the linear fit.

Looking at the values for ΔS_{sorp} all values are negative meaning that the degree of order in the system is increasing during the sorption process. This is not surprising as the PAC is changing state from being dissolved in a liquid (the mobile phase) to be attached to (or "dissolved" into) a solid phase (the humic acid). A similar pattern as for $\Delta H_{\mathrm{sorp},}$ that ΔS_{sorp} become more negative with decreasing methanol concentration within the series is seen for $\Delta S_{\mathrm{sorp}.}$ This is the general pattern. However, one exception is phenanthrene where ΔS_{sorp} is not changing. The two bicyclic N-PAC show the opposite trend.

Stalcup et al. (1988) found also that the relative entropies for the sorption of PAH to C_{18} HPLC column decreased (gets more negative) as the water fraction was increased.

7.3 Partitioning coefficient, K_{oc}

Knowing ΔH_{sorp} and ΔS_{sorp}, K_{oc} can be calculated at any given temperature in the range used in the experiments. Calculations of K_{oc} at 298 K (25°C) are carried out and the values of log K_{oc} are compared with data achieved from the direct determination of K_{oc} (previous chapters) in Figure 7.1. It is here necessary to remember that these new values describe a partitioning between organic carbon from SOM and mobile phases consisting of mixtures of water and methanol, hence a more favourable phase for PAC than pure water. They are therefore expected to be lower than those directly determined where the retention of PAC is estimated using a mobile phase without methanol. This can be achieved as seen in the following.

From Figure 7.1 it is obvious that there is a nearly linear relationship between log K_{oc} and the composition of the mobile phase (an assumption that was used to determine k'_{w} previous in this work and by Nkedl-Kizza et al., 1985). Taking those compounds having thermodynamic constants measured at more than one mobile phase (leaving data from 85 %(V/V) MeOH out), log K_{oc} is extrapolated in a system with only humic acid and water (Table 7.1).

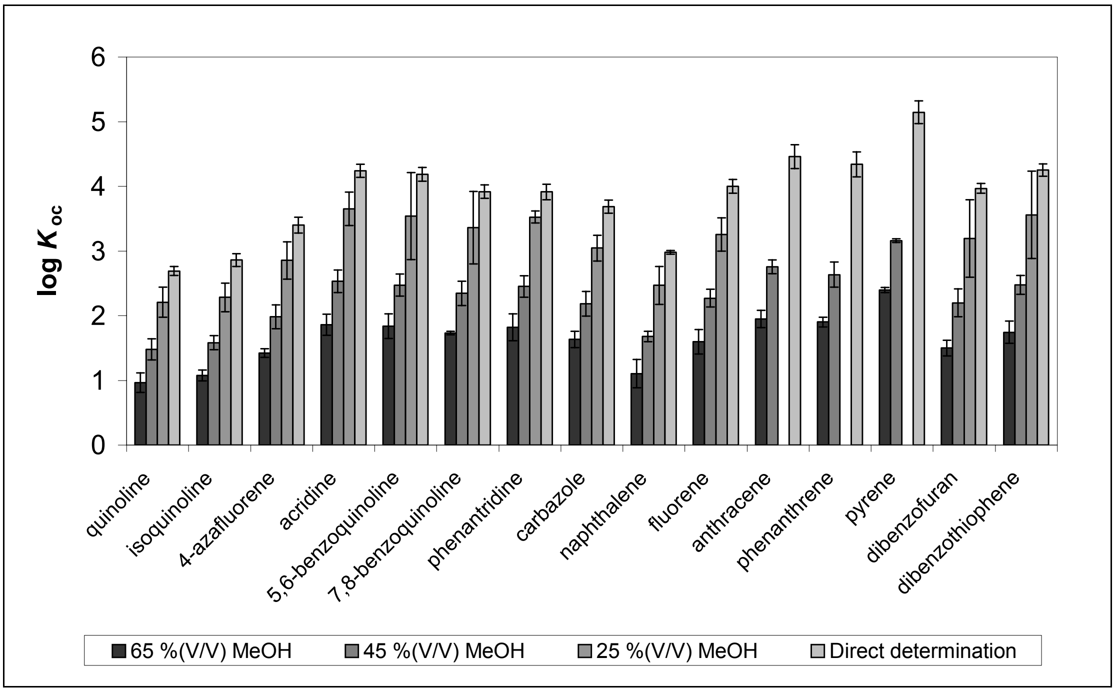


Figure 7.1. Log K_{oc} values at 298 K (25°C) calculated from ΔH_{sorp} (Table 4.3) and ΔS_{sorp} (Table 4.4). Values from a direct determination of log K_{oc} (Table 4.1) are also shown. Error bars indicate standard deviations.

Table 7.1. Estimated values of Log K_{oc}, ΔH_{sorp} and ΔS_{sorp} for a water-humic acid system (25 °C). Standard deviations are from the standard deviations on the intercept.

Compounds	**log K_{oc}**	**ΔH_{sorp} / kJ mol^{-1}**	**ΔS_{sorp} / J mol^{-1} K^{-1}**
PAH			
Naphthalene	3.29 ± 0.15	-27.7 ± 1.9	-30.1 ± 3.4
Fluorene	4.24 ± 0.21	-31.1 ± 2.4	-23.2 ± 3.9
Anthracene	4.57*	-45.6*	-65.3*
Phenanthrene	4.28*	-37.3*	-43.1*
Pyrene	4.87*	-54.2*	-88.6*
N-PAC			
Quinoline	2.95 ± 0.15	-38.8#	-70.5#
Isoquinoline	3.01 ± 0.14	-38.9#	-70.0#
4-azafluorene	3.70 ± 0.22	-34.2 ± 5.1	-43.9 ± 12.9
Acridine	4.70 ± 0.31	-57.7 ± 1.7	-103.5 ± 0.20
5,6-benzoquinoline	4.54 ± 0.30	-48.5 ± 4.2	-75.7 ± 8.5
7,8-benzoquinoline	4.31 ± 0.28	-36.7 ± 3.2	-40.5 ± 5.5
Phenantridine	4.52 ± 0.30	-47.6 ± 6.0	-73.2 ± 14.4
Carbazole	3.88 ± 0.21	-36.5 ± 3.7	-48.3 ± 8.4
O,S-PAC			
Dibenzofuran	4.20 ± 0.21	-35.1 ± 1.5	-37.3 ± 1.0
Dibenzothiophene	4.63 ± 0.24	-44.8 ± 5.2	-61.4 ± 12.8

* The linear regression is based measurements using two mobile phases (45 and 65 %(V/V)).

The linear regression is based measurements using two mobile phases (25 and 45 %(V/V)).

The values are compared and a plot containing data for eight N-PAC, five PAH, dibenzofuran, and dibenzothiophene is shown in Figure 7.2. As the two ways to determine log K_{oc} is based on the same assumption of linearity between log k' and methanol content in the mobile phase, the line representing all PAC in the plot is expected to have a slope of unity. The fact that this actually is true supports the theory behind this work and shows the robustness of the HPLC method.

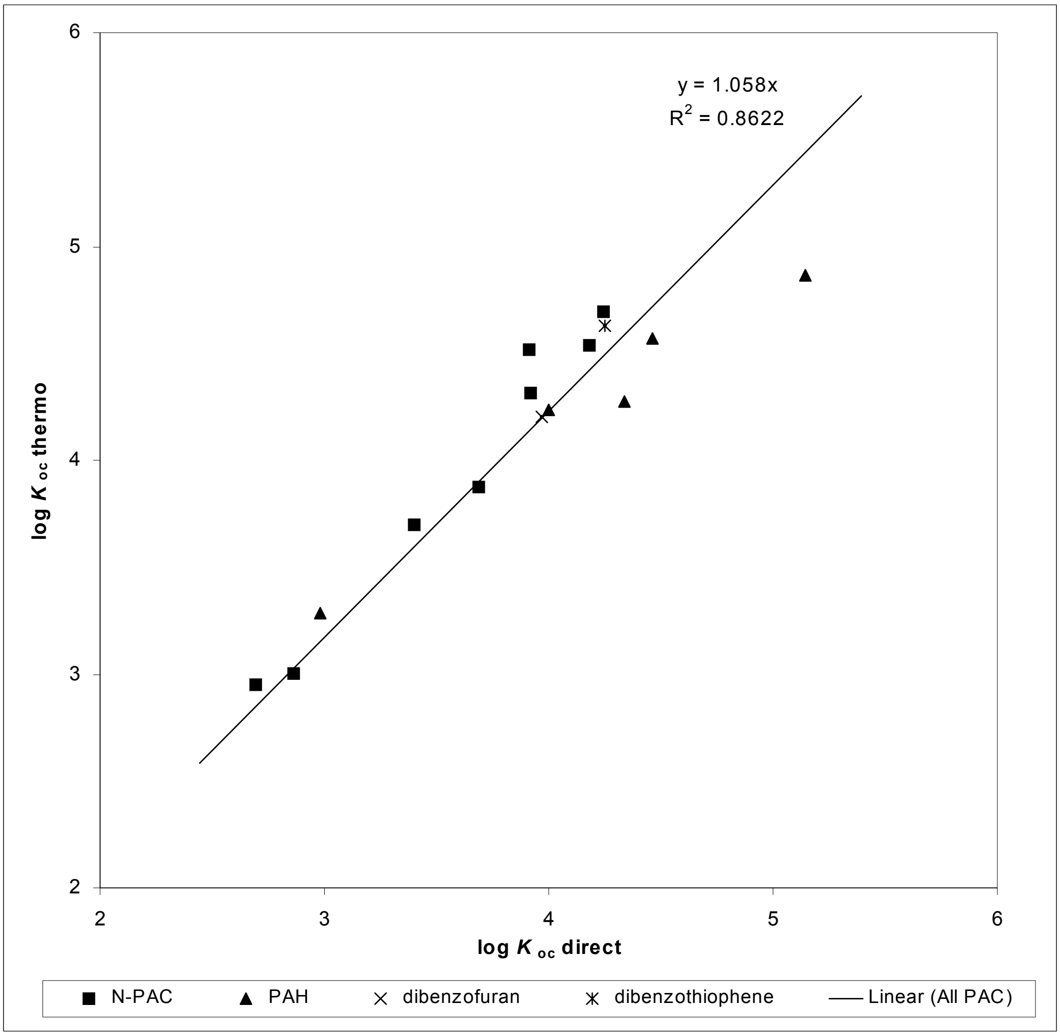


Figure 7.2. Values of log K_{oc} for 15 PAC determined by the two methods plotted vs. each other. The regression line is forced through origin. "Direct" data is from the direct method.

Looking closer at the plot (Fig. 7.2), a systematic distribution of the compounds is seen. The PAH are, except for naphthalene, all showing higher log K_{oc} values in the direct method (placed under the line), whereas the N-PAC (except isoquinoline and carbazole) show the highest values in the thermodynamic method. The reason for this observation is at the present unknown, but might be a coincidence.

In similarity to the estimation of log K_{oc} extrapolation to a liquid phase without methanol ΔH_{sorp} and ΔS_{sorp} in water are estimated by linear regression and shown in Table 7.1. The values of ΔH_{sorp} is higher (thus less negative) for PAH than N-PAC. This means that the energy achieved transferring a non-polar PAH molecule from water into the humic is less than what is achieved for the same process for a polar analogue N-PAC molecule. The solubility of PAH is lower than analogue N-PAC (e.g. Pearlman et al., 1984 and Table 4.1). So even though the transfer out of water is more energetically favourable for PAH than N-PAC, the energetic profit by formation of specific interactions such as hydrogen bonds for the N-PAC more than balances the difference. The differences in ΔS_{sorp} for PAH and N-PAC, indicates that sorption of N-PAC involving H-binding results in a higher state of order than the entire partitioning controlled sorption process. The fact that higher sorption (log K_{oc}) of PAH compared to N-PAC is observed, despite formation of H-bonds between N-PAC and humic acid, is thus a result of the higher demand of order during the sorption to humic acid.

The five compounds having "fluorene structure" show almost identical order for ΔH_{sorp} and ΔS_{sorp}. ΔH_{sorp}: fluorene > 4-azafluorene > dibenzofuran > carbazole > dibenzothiophene and ΔS_{sorp}: fluorene > dibenzofuran > 4-azafluorene > carbazole > dibenzothiophene. Dibenzothiophene has the higher energetically profit of changing phase from water to humic acid this is in good agreement with it having the lowest solubility of the five compounds. The phase transfer also causes the highest change of order indicating some kinds of specific interaction between dibenzothiophene molecules and humic acid. Dibenzofuran has the highest solubility (probably except for 4-azafluorene). So even though ΔH_{sorp} is similar to carbazole and 4-azafluorene, the higher sorption compared to the two N-PAC must be found in the lower changes of order. Table 7.2 shows the differences between ΔH_{sorp} - and ΔS_{sorp}-values for PAH and analogue N-PAC. As it is seen both $\Delta\Delta H_{sorp}$ (= $\Delta H_{sorp\ PAH} - \Delta H_{sorp\ N\text{-}PAC}$) and $\Delta\Delta S_{sorp}$ (= $\Delta S_{sorp\ PAH} - \Delta S_{sorp\ N\text{-}PAC}$) are almost constant with the exception of "phenanthrene - 7,8-benzoquinoline". Thus $\Delta\Delta H_{sorp}$ is a relative measure for the strength of the hydrogen bonds between N in N-PAC and the humic acid. $\Delta\Delta S_{sorp}$ indicates the changes of order in the system forced by formation of hydrogen bonds.

The fact that the “phenanthrene - 7,8-benzoquinoline” differences is approximately zero, indicates the absence of H-bonds formation, clearly showing the shielding effect of the nitrogen atom by the ring structure. The reason that “fluorene - 4-azafluorene” falls outside this pattern can be the presence of the five link ring in the molecules.

Table 7.2. Differences between ΔH_{sorp} - and ΔS_{sorp}-values for PAH and analogue N-PAC. ($\Delta\Delta H_{\text{sorp}} = \Delta H_{\text{sorp PAH}} - \Delta H_{\text{sorp N-PAC}}$ and $\Delta\Delta S_{\text{sorp}} = \Delta S_{\text{sorp PAH}} - \Delta S_{\text{sorp N-PAC}}$).

Compounds	**$\Delta\Delta H_{\text{sorp}}$ / kJ mol^{-1}**	**$\Delta\Delta S_{\text{sorp}}$ / J mol^{-1} K^{-1}**
Naphthalene - quinoline	11.1	40.4
Naphthalene - isoquinoline	11.2	39.9
Anthracene – acridine	12.1	38.2
Phenanthrene - 5,6-benzoquinoline	11.2	32.6
Phenanthrene - 7,8-benzoquinoline	0.6	-2.6
Phenanthrene - phenantridine	10.3	30.1
Fluorene - 4-azafluorene	3.1	20.7

[a] Based on data are from Table 7.1.

The partitioning theory is the general accepted model to explain the sorption processes to humic acid. In the pure form the theory consider the sorption as dissolution of the adsorbed compound in the humic acid phase (Wershaw, 1986; Schlauman and Morgan, 1993). The adsorption process does not affect the structure of humic acid and it is believed that the adsorbed compound is positioned in voids in humic acid. In the pure form the partitioning theory predicts that specific interactions between humic acid and the adsorbed compound, as e.g. hydrogen bonding between the nitrogen atom in N-PAC and humic acid, is non-existent and that ΔS_{sorp} should be close to zero. The present results of ΔH_{sorp} and ΔS_{sorp} thus show that the interpretation of the partitioning theory obvious should not be too rigorous.

The accuracy of ΔH_{sorp} and ΔS_{sorp} in Table 7.2 must be considered to be quite good, as the accuracy of the corresponding K_{oc} values is good. These results are interesting as no such data are reported in the literature.

Calculations of log K_{oc} at various temperatures (0-30 °C), under the assumption that ΔH_{sorp} was independent of temperature, are presented in Table 7.3, clearly showing that the partitioning coefficient is increasing with up to one log unit decreasing temperature from 30 °C to the freezing point

$$\mathrm{PAC_{water}} \Leftrightarrow \mathrm{PAC_{SOM}}$$

The changes were similar to what was observed by Lüers and ten Hulscher (1996) on PAH with larger molecular weight.

Table 7.3. Partitioning coefficients (log K_{oc}) at temperatures between 0 and 30 °C. Calculated from the data in Table 7.1 assuming that ΔH is constant within the temperature range used here.

	log K_{oc}			
Temperature / °C	**0**	**10**	**20**	**30**
PAH				
Naphthalene	3.73	3.54	3.37	3.21
Fluorene	4.74	4.53	4.33	4.15
Anthracene	5.30	5.00	4.71	4.44
Phenanthrene	4.88	4.62	4.39	4.17
Pyrene	5.74	5.37	5.03	4.71
N-PAC				
Quinoline	3.57	3.31	3.07	2.84
Isoquinoline	3.63	3.37	3.12	2.89
4-azafluorene	4.25	4.02	3.80	3.60
Acridine	5.62	5.23	4.87	4.53
5,6-benzoquinoline	5.31	4.99	4.68	4.40
7,8-benzoquinoline	4.90	4.66	4.42	4.21
Phenantridine	5.28	4.96	4.66	4.38
Carbazole	4.46	4.21	3.98	3.77
O,S-PAC				
Dibenzofuran	4.77	4.53	4.31	4.10
Dibenzothiophene	5.35	5.05	4.77	4.50

7.4 Substitution effects on anthracene

The effects of substituents on anthracene have been investigated for both ΔH_{sorp} and ΔS_{sorp}. Correlation between $\Delta\Delta S$ and the lipophilic aromatic substituent constant π_{Ar} (Hansch et al., 1973) (excluding 9-anthracene carboxamide and 9-cyanoanthracene) gives a linear regression line with a positive slope and a correlation coefficient (R^2) of 0.88 (Fig. 7.3), thus indicating weak correlation between the data.

Data for the substituted anthracenes have been modelled by multiple variance analysis using both quantum mechanical and structural descriptors such as e.g. homo, lumo, size, volume and angle of the substituted functional groups. No correlation was found expressing the substitution effects on variations between ΔS_{sorp} values for the anthracenes.

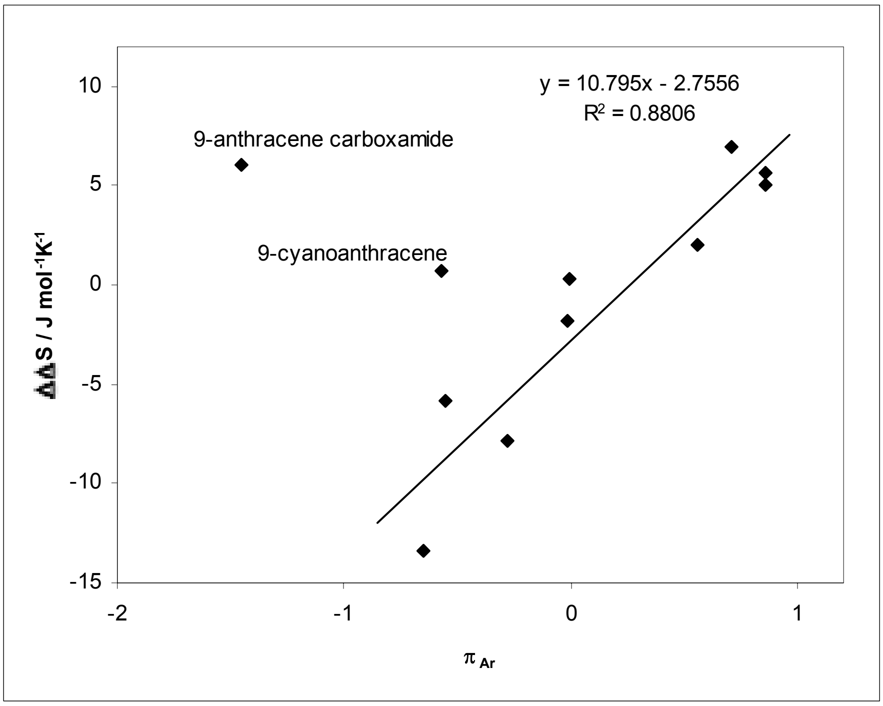


Figure 7.3. Substitution effects for ΔS for substituted anthracenes illustrated by the relationship between ΔΔS ($\Delta S_{\text{X-anthracene}} - \Delta S_{\text{Anthracene}}$) for all substituted anthracenes in Table 4.4 (excluding anthraquinone) and the lipophilic aromatic substituent constants (π_{Ar})*. The effect is split uniformly between the two bromine atoms in 9,10-dibromoanthracene. The regression line is a linear fit including all compounds except 9-anthracen carboxamide and 9-cyanoanthracene.
*From Hansch et al. (1973).

7.5 Summary

Effects of temperature variation on sorption of 27 compounds have been investigated. Only negative values were found for both ΔH_{sorp} and ΔS_{sorp}. Thus the sorption of PAC to humic acid is a spontaneous exothermic process. For ΔH_{sorp} the values become more negative as the size of the PAC and N-PAC is increasing showing that the steric requirements for binding are small and that the humic acid contain relatively large stretches of aromatic character. The negative ΔS_{sorp} means that the order is increased as the state of the molecules transfers from being dissolved in a liquid to be bound in (or unto) the humic acid. The pattern observed varies according to the type of PAH/N-PAC.

The partitioning coefficient log K_{oc} was calculated from ΔH_{sorp} and ΔS_{sorp}, representing the distribution between humic acid and a methanol-water mixture. For those compounds, for which the temperature effect was investigated at several methanol-water mixtures, values were transferred to a humic acid water system, and log K_{oc} was compared to the direct determine data (previous chapters). The agreement between values from the two methods supports the theory behind this work and shows the robustness of the HPLC method used. Values for ΔH_{sorp} and ΔS_{sorp} in a system with 100% water in the liquid phase was obtained and presented for 15 of the compounds.

There have so far been very few determinations of ΔH for sorption of PAH to organic matter. The data presented here suggests that ΔH_{sorp} should be much lower than previously observed (Wauchope et al., 1983; Lüers and ten Hulscher, 1996). This is confirmed by comparing the results with literature values for complexes of pyridine and phenol, used as a model for the sorption of N-PAC to HA.

Values of log K_{oc} is calculated at temperatures down to 0°C, showing increases of up to one log unit by decreasing the temperature by 30°C.

8. Other environmental factors

The effects of two other environmental factors (electrolyte concentration and pH) that are expected to influence the retention of PAC to the humic acid column were investigated separately by changing the conditions in the mobile phase.

8.1 Variation of pH

The sorption of six by- and tri-cyclic PAC to the humic acid column was measured decreasing pH from 7.8 to 2.5 holding other parameters constant and shown under Results in Figure 4.1.

Looking at Figure 4.1 the maximum sorption of the N-PAC (quinoline and isoquinoline) are observed at slightly acidic condition around pH 5, while the sorption of PAH increased when pH was decreased.

The explanation for the different behaviour must be found in the way pH influences both humic acid and PAC. Aldrich humic acid is affected in two ways by the pH changes; firstly it is has a high content of carboxylic groups (4.38 meq g^{-1} humic acid, Shin et al., 1999, or about 9 % of the carbon, Malcom & MacCarthy, 1986) with overall pK_a-values lying at approximately 4 (3.64 (Masisni et al., 1998) and 4.26 (Shin et al., 1999)), probably covering a broader range for the individual carboxylic groups. A decrease of pH will therefore result in a change in charge of the humic acid from negative towards neutral. Kam and Gregory (2001) show that the charge density for two humic substances has an almost linear decrease when pH was decreased by one pH unit from pH 10 to 5, further decreasing of pH (to pH 3) resulted in a more vigorous decrease of the charge density. This observation agrees with the sorption of PAH shown in this work.

Secondly, as the humic acid becomes neutral it becomes less hydrophilic and tends to curl into aggregates or micelle like structures (Kumke et al., 1994; Schlautman and Morgan, 1993; Schnitzer and Khan, 1970), hereby creating voids or pockets by witch PAC could be absorbed into the humic acid by van der Waals forces.

A decrease in pH causes a change of the overall charge of humic acid towards neutral, and PAH will therefore be more strongly attached to humic acid, as the humic phase gets more energetically favourable for neutral, non-polar compounds compared to the negatively charged humic acid at higher pH.

For the different behaviour of quinoline and isoquinoline compared to the PAH another aspect has to be taken into account. Azaarenes without pyrrole systems are in general bases with pK_a values around 5 (quinoline 4.90 and isoquinoline 5.42 at 20°C, CRC Handbook of Chemistry and Physics). This means that approximately 7% and 83 % of the quinoline molecules will be on cationic form at pH 6.0 and 4.5 respectively. For isoquinoline 21% and 89 % of the molecules will be cations under the same conditions. However only 0.1 % respective 0.4 % of the molecules of the two compounds will be cations at pH 7.8.

The higher degree of sorption of the two quinolines compared to the bicyclic PAH naphthalene in the pH-region from 3.2 to 6 is therefore probably a result of ionic binding between cationic quinoline molecules and anionic carboxylic groups in humic acid (Nielsen et al., 1997). Even though a larger fraction of the two compounds becomes cations at decreased pH, the degree of sorption is highest between pH 3.8 and 4.5, witch means that the changes in the total charge of humic acid towards neutral affects sorption of cationic N-PAC in a negative direction. The differences in pK_a values between the two quinolines explain the higher retention of isoquinoline compared to quinoline at pH values above 4 as a larger part of the isoquinoline molecule are cations. At lower pH almost all molecule of both compounds will be on cationic form.

8.2 Electrolyte concentration

In order to investigate the influence of electrolyte concentration on sorption of the small N-PAC and PAH, the sorption was measured under the conditions described in Experimental (Chapter 2) and log k' was calculated and presented on the diagrams in Figure 4.2-4.4 (Chapter 4).

From Figure 4.2 it is seen that sorption of the N-PAC (Fig. 4.2A) shows an increase in sorption (especially quinoline and isoquinoline), while PAH (Fig. 4.2B) is almost unaffected by the ionic strength in the mobile phase (pH 6.0) when lowering the total phosphate concentration from 35 to 10mM. The highest sorption at pH 6.0 for all the investigated PAC is seen about 1mM phosphate. In the nonbuffered mobile phase, the sorption decreases to the lowest point for all compounds.

At the higher pH value (8.0) the same pattern is seen for the N-PAC (Fig. 4.3A) and naphthalene, whereas the other PAH (Fig. 4.3B) shows maximum sorption at 2.5 mM phosphate. The importance of hydrogen bonds between N-PAC and HS appears to be increasing with decreasing electrolyte concentration. In general a lower retention is seen at pH 8.0 than at 6.0, a trend that was more distinct for N-PAC than PAH, this observation agrees with the results from the pH variation discussed above.

For the three bicyclic compounds (quinoline, isoquinoline, and naphthalene) the retention, measured using a mobile phase with only 10 %(V/V) MeOH at pH 8.0 (Fig. 4.4) is higher than at 35 %(V/V) MeOH as expected referring to the chapter dealing with the direct determination of log K_{oc}. Again the same pattern is seen. The sorption is increasing as the phosphate concentration decreases.

The variation in sorption could be the result of several factors. Firstly the solubility of organic compounds like PAC decreases as a function of increasing salt concentration as a result of the “salting out” effect (Schwarzenbach et al., 1993 and Xie et al, 1997). Secondly, the cationic concentration affects the structure of humic acid, as humic acid tends to curl as the concentration of (hydrated) cations is raised (Tombacz and Meleg, 1990; Schwarzenbach et al., 1993), and hereby creating voids or pockets by which PAC could by absorbed into the humic acid by van der Waals forces. Polyvalent hydrated cations are also capable to act as “cation bridges” in creating complexes involving humic acid and anionic compounds (Senesi, 1993), but as K^+ being the only cation in the system (besides H^+) this mechanism can be neglected as K^+ is a monovalent cation. However, cations can occupy the same sites in humic acid as PAC, and hereby act as competitors to protonated N-PAC (Chorover et al., 1999; Kollist-Siigur et al., 2001).
It is obvious that these factors cause different effects on the degree of sorption, and that one should not expect a clear pattern. The higher variation in retention between N-PAC and PAH at the lowest concentrations can be explained by the polarity of the N-PAC molecules compared to PAH.

Schwarzenbach et al. (1993) states, that the most important parameter in the changes of electrolyte concentrations affecting the sorption is the changes in activity coefficients of the compounds dissolved in water (γ_w). In other words the "salting out" effect should be the main factor of those mentioned above. If this were true an increasing sorption raising the electrolyte concentration would have been observed. As the opposite is the fact, the conformation of humic acid must be a central part of the sorption process (Chorover et al., 1999; Lassen and Carlsen, 1997; Schlautman and Morgan, 1993).

8.3 Summary

Effect of pH and electrolyte concentration on the sorption of bi- and tri-cyclic compounds has been investigated using the SOM HPLC column method.

A decrease in pH from pH 7.8 to 2.5 resulted in an almost linear increase of the sorption (measured as the retention by the capacity coefficient, k') of PAH by a factor 2.5 to 3. For the bicyclic N-PAC the maximal retention was observed at pH values between pH 3.8 and 4.5, approximately one pH unit below their pK_a values, but in the range of the pK_a value for the humic acid. The retention at maximal sorption was between 5.5 and 6 times as high as at pH 7.8. At pH 2.5 the sorption was approximately twice the value at pH 7.8. Those observations indicates that some kind of specific interaction (ionic bonding) is present in the sorption process, as the sorption is highest when the carboxylic groups in humic acid are anionic and the N-PAC is cationic.

The sorption of all compounds were general increased when the electrolyte concentration was decreased, indicating that not only the behaviour in water but also the conformation of humic acid affects the sorption.

9. Conclusion and perspectives

9.1 Conclusion

Improvements were achieved during optimisation of the preparation procedure for column materials. The amount of SOM has been multiplied almost three times and the cross-linking material (carrier carbon) has been reduced by a factor 25, leading to fewer artefacts. The new column material had the same properties as the free humic acid when investigating the pH effect on the sorption of N-PAC. This was not the case for the old column material. Similarities in sorption of PAC onto column materials fabricated by different procedures, indicates that the properties of humic acid in general are retained after being immobilised.

The HPLC method using column material modified by soil organic matter has been evaluated. The method has shown to be a useful tool to determine the sorption (as the partitioning coefficient, log K_{oc}) of several polycyclic aromatic compounds in a time saving way. The “direct method” was found to be useful within the limits regarding solubility and/or lipophilic properties of the compounds. For compounds not fulfilling these limits, the “indirect method” must be considered to be a good alternative.

Log K_{oc} values was measured for 69 PAC, for some of them no value has been reported in the literature before. In general there is good agreements between values found by the column method and from literature.

Changes in enthalpy (ΔH) and entropy (ΔS) during the sorption process were measured. For all compounds negative values were found for both thermodynamic parameters, emphasising that sorption of PAC to humic acid is a spontaneous exothermic process and that the order of the system is increased as the solutes are transferred from the mobile phase into humic acid.

Partitioning coefficients calculated from ΔH and ΔS agreed with values achieved by the direct determination. Values of log K_{oc} are calculated at temperatures down to 0°C, showing increases of up to one log unit by decreasing the temperature 30°C.

A decrease in pH from pH 7.8 to 2.5 resulted in an almost linear increase of the sorption (measured as the retention by the capacity coefficient, k') of PAH by a factor 2.5 to 3. For the bicyclic N-PAC the maximal retention was observed at pH values between pH 3.8 and 4.5. The retention at maximal sorption was between 5.5 and 6 times as high as at pH 7.8.

Correlations were found between log K_{oc} and molecular properties such as size (number of fused rings), shielding effects for the azo-heterocycles, and substituent effects. For entropy changes linear correlations with the lipophilicity of substituents were found.

The results indicate that the sorption is a process involving multiple mechanisms, including both non-specific (hydrophobic adsorption and partitioning) and more specific interactions e.g. hydrogen bonding, ionic bonding, dipole-dipole, and van der Waals forces.

9.2 Perspectives

The fact that the OECD has already accepted the use of HPLC in determination of K_{oc} emphasises the lack of (and needs for) knowledge of physico-chemical characteristics of the huge amounts of chemicals that are spread into the environment as a result of human activity. By use of HPLC partitioning coefficients can be obtained in a time saving and cost reducing way.
The method used in this thesis is a refinement compared to the method recommended by the OECD, as it is the direct sorption to soil organic matter, which is measured. Thus the results are more realistic and provide a more reliable foundation for environmental risk assessments.
A small study on the sorption of more polar and hydrophilic compounds (pesticides and degradation products, not yet published) shows that the use of this method in determining partitioning coefficients is not restricted to the polycyclic aromatic compounds. As the method can be used on both different kinds of chemicals and soil organic matter, the potential use of the method is huge in generating knowledge of the sorption process. The ability of easy modelling environmental conditions increases the applicability of the method.

This thesis only deals with a limited number of compounds in one group of chemicals and it is the hope of the author, that the work can be continued in some way as the needs of more knowledge is crucial in understanding how our activities affect the environment surrounding us.

10. References

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Appendices

Appendix 1: Table of PAC used in this work

Appendix 2: Characterisation of Humic Materials

Appendix 3: Derivation of equations for log K_{oc}

Appendix 4: Derivation of thermodynamic equations

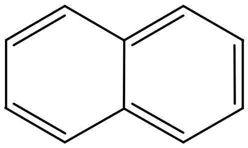
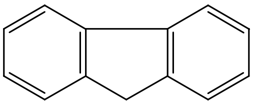
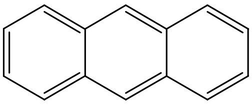
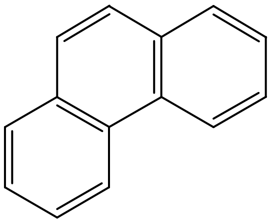
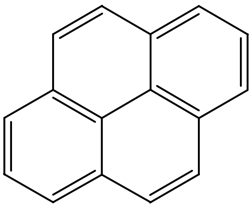
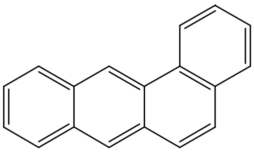
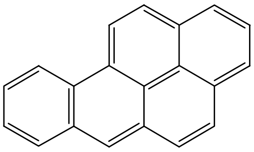
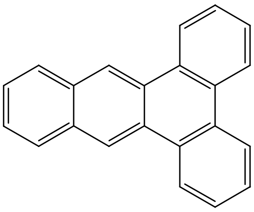
Appendix 5: List of publications and reprints of two publications:

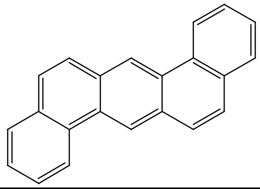
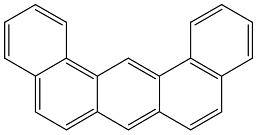
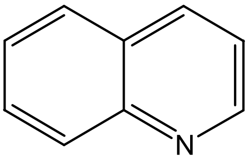
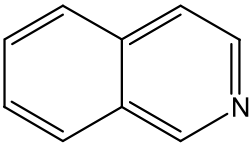
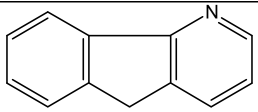
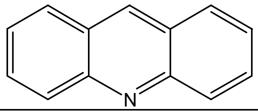
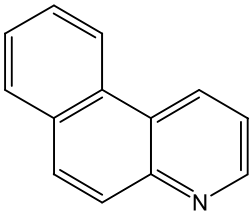
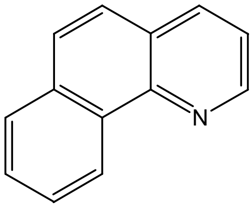
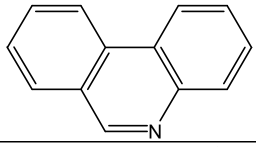
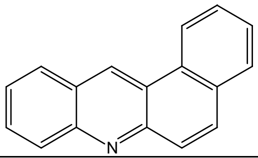
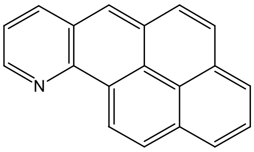
Kollist-Siigur, K., Nielsen, T., Grøn, C., Hansen, P.E., Helweg, C., Jonassen, K.E.N., Jørgensen, O. and Kirso, U.; Sorption of polycyclic aromatic compounds to humic and fulvic acid HPLC column materials. *J. Environ. Qual.* (2001) 30, 526-537.

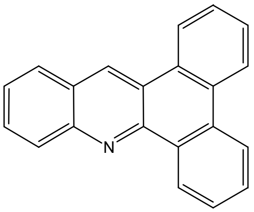
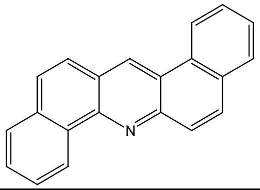
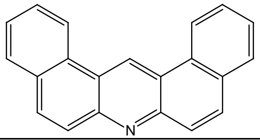
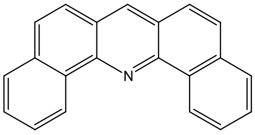
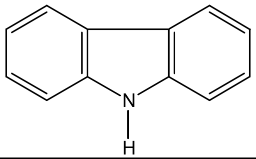
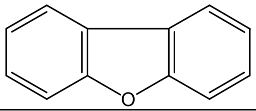
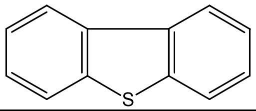
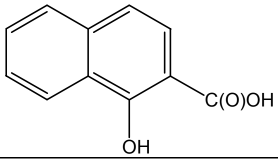
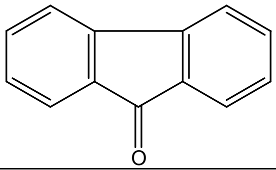
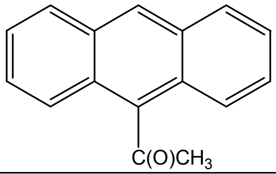
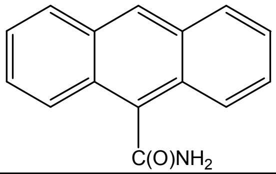
Jonassen, K.E.N., Nielsen, T. and Hansen, P.E.; The application of high-performance liquid chromatography humic acid columns in determination of Koc of polycyclic aromatic compounds. *Environ. Toxicol. Chem.* (2003) 22, 741-745.

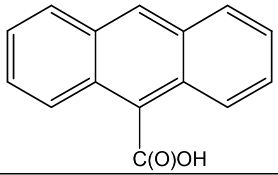
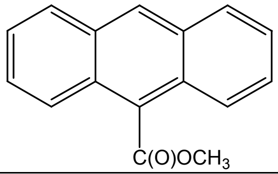
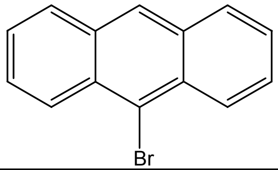
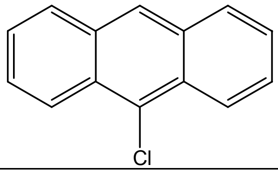
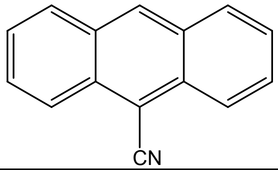
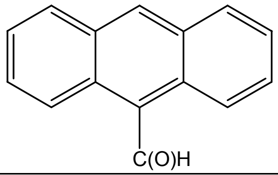
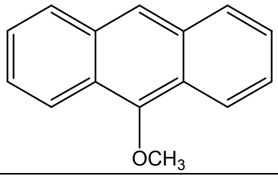
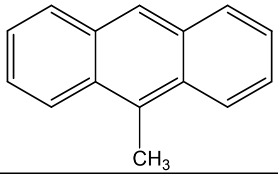
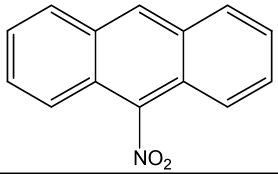
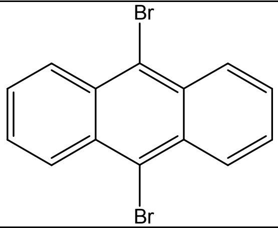
Appendix 1

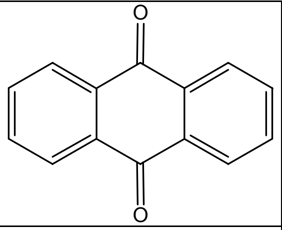
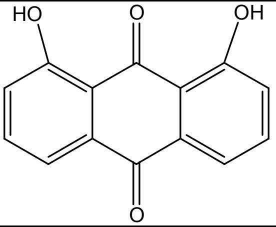
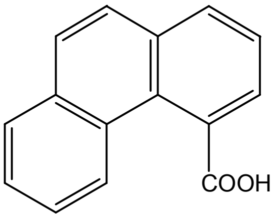
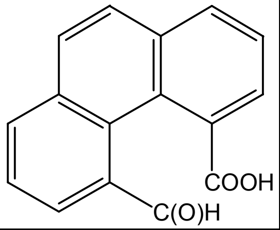
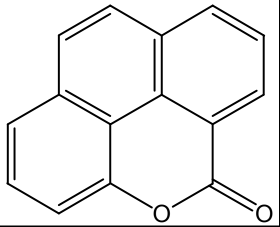
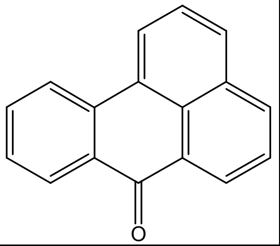
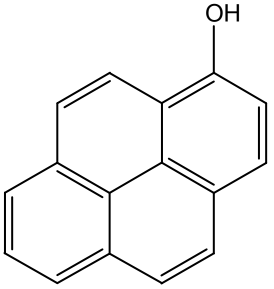
List of polycyclic aromatic compounds (PAC) used in this work. CAS # is the numbering of compounds according to Chemical Abstract. When more than the used nomenclature is common in literature, alternative names are included. In general "quinoline" is used instead of "chinoline". IUPAC-nomenclature is not used systematically.

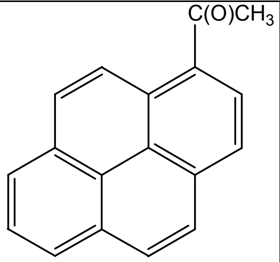
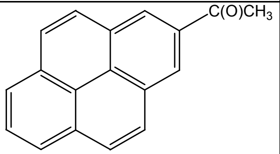
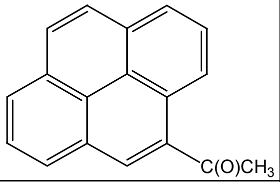
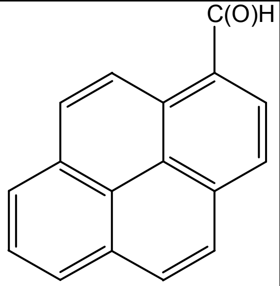
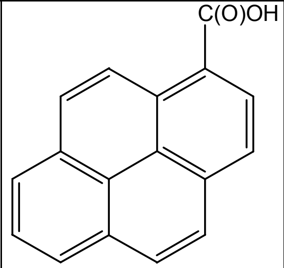
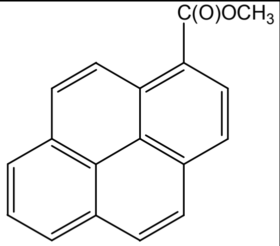
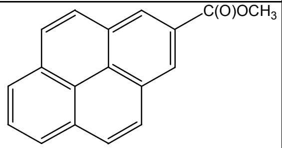
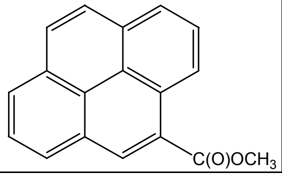
Compound	Structure	CAS #	Alternative name	Source
PAH				
Naphthalene		91-20-3	Naphthalin	Merck
Fluorene		86-73-7		Mikrolab, Aarhus
Anthracene		120-12-7		Fluka
Phenanthrene		85-01-8		Commission of the European communities, Community bureau of reference materials.
Pyrene		129-00-0		Fluka, 95%
1,2-benzanthracene		56-55-3	Benz[a]anthracene	Commission of the European communities, Community bureau of reference materials.
Benzo[a]pyrene		50-32-8	1,2-benzpyrene Benzo[def]chrysene	Commission of the European communities, Community bureau of reference materials.
1,2,3,4-dibenzanthracene		215-58-7	Dibenz[a,c]anthracene Benzo[b]triphenylene	Commission of the European communities, Community bureau of reference materials.

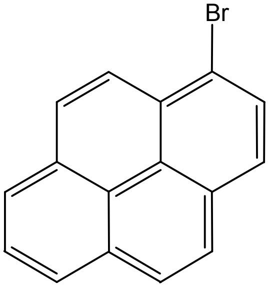
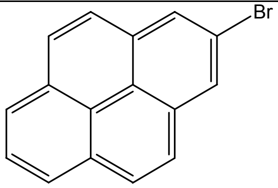
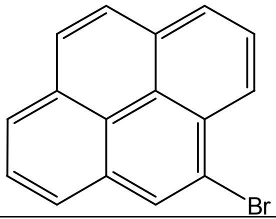
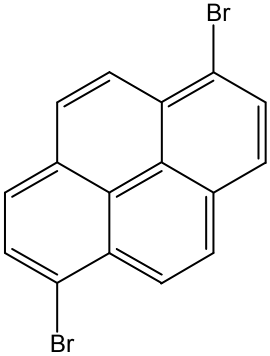
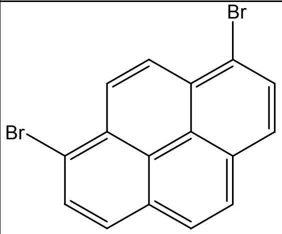
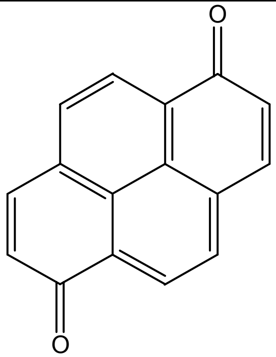
1,2,5,6-dibenzanthracene		53-70-3	Dibenz[a,h]anthracene	Dr. Ehrenstorfer GMBH, 97.3%
1,2,7,8-dibenzanthracene		224-41-9	Dibenz[a,j]anthracene	Commission of the European communities, Community bureau of reference materials.
N-PAC				
Quinoline		91-22-5		Fluka
Isoquinoline		119-65-3		Aldrich, >98%
4-azaflourene		244-99-5	5H-indeno[1,2-b]pyridine	Fluka, purum
Acridine		260-94-6		Fluka, >97%
5,6-benzoquinoline		85-02-9	Benzo[f]quinoline	Aldrich, >99%
7,8-benzoquinoline		230-27-3	Benzo[h]quinoline	Aldrich, >97%
Phenantridine		229-87-8	2,3-benzo-quinoline Benzo[c]quinoline	Fluka, purum
1,2-benzacridine		225-11-6	Benz[a]acridine	Commission of the European communities, Community bureau of reference materials.
10-azabenz[a]pyrene		189-92-4	Phenaleno[1,9-gh] quinoline	Commission of the European communities, Community bureau of reference materials.

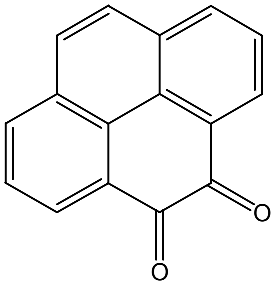
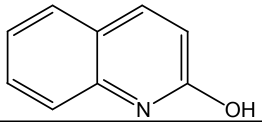
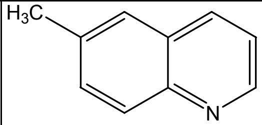
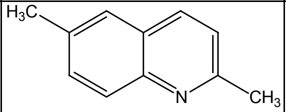
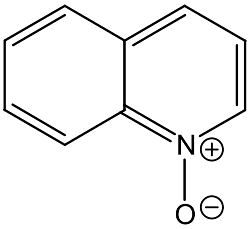
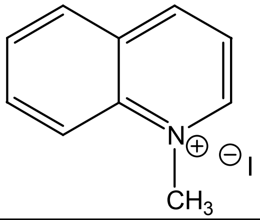
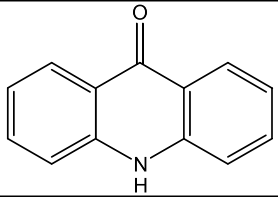
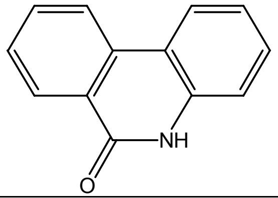
1,2,3,4-dibenzacridine		215-62-3	Dibenz[a,c]acridine	Commission of the European communities, Community bureau of reference materials.
1,2,5,6-dibenzacridine		226-36-8	Dibenz[a,h]acridine	Commission of the European communities, Community bureau of reference materials.
1,2,7,8-dibenzacridine		224-42-0	Dibenz[a,j]acridine	Ferak, Berlin
3,4,5,6-dibenzacridine		224-53-3	Diben[c,h]zacridine	Commission of the European communities, Community bureau of reference materials.
Carbazole		86-74-8	9H-carbazole	The British Drug house, >98%
O,S-PAC				
Dibenzofuran		132-64-9		Merck, >97%
Dibenzothiophene		132-65-0		Aldrich, >95%
X-PAH				
1-hydroxy-2-naphthoic acid		86-48-6	1-hydroxy-naphthalene-2-carboxylic acid 1-hydroxy-[2]naphthoic acid 1-hydroxy-2-naphthalene-carboxylic acid	Aldrich
Fluorenone		486-25-9	9-fluorenone 9H-fluoren-9-one	Aldrich, >98%
9-acetylanthracene		784-04-3	1-[9]anthryl-ethanone 9-anthryl methyl ketone 1-(9-anthracenyl)-ethanone 1-anthracen-9-yl-ethanone	Aldrich, >95%
9-anthracene carboxamide		34810-13-4	Anthracene-9-carboxamide Anthracene-9-carboxylic acid amide	Provided from Ole Hammerich and Tove Thomsen, University of Copenhagen

9-anthracene carboxylic acid		723-62-6	Anthracene-9-carboxylic acid	Merck, 98%
9-anthracene carboxylic acid methyl ester		1504-39-8	Anthracene-9-carboxylic acid methyl ester	Provided from Ole Hammerich and Tove Thomsen, University of Copenhagen
9-bromoanthracene		1564-64-3		Provided from Ole Hammerich and Tove Thomsen, University of Copenhagen
9-chloroanthracene		716-53-0		Aldrich
9-cyanoanthracene		1210-12-4	9-Anthracene carbonitrile	Provided from Ole Hammerich and Tove Thomsen, University of Copenhagen
9-formylanthracene		642-31-9	Anthracene-9-carbaldehyde 9-anthraldehyde Anthracene-9-carboxaldehyde Anthracen-carbaldehyd-(9)	Provided from Ole Hammerich and Tove Thomsen, University of Copenhagen
9-methoxyanthracene		2395-96-2	[9]-anthryl-methyl ether	Provided from Ole Hammerich and Tove Thomsen, University of Copenhagen
9-methylanthracene		779-02-2		Aldrich, >99%
9-nitroanthracene		602-60-8		Provided from Ole Hammerich and Tove Thomsen, University of Copenhagen
9,10-dibromoanthracene		523-27-3	Anthracene-dibromid-(9,10)	Aldrich, >98%

Anthraquinone		84-65-1	Anthracene-9,10-dione 9,10-anthraquinone	Aldrich
1,8-dihydroxy-anthraquinone		117-10-2	1,8-dihydroxy-9,10-anthraquinone 1,8-dihydroxy-9,10-anthracenedione Chrysazin Dantron Danthron	Aldrich, 96%
Phenanthrene-4-carboxylic acid		42156-92-3		Sigma-Aldrich
4-carboxyl-5-phenanthrene carboxaldehyde		5684-15-1	5-formylyl-4-phenanthrene carboxaldehyde	Aldrich
5H-phenanthro [4,5-bcd]pyran-5-one		23702-49-0	Phenanthren-4-hydroxy-5-carbonsaeurelacton 4-oxa-pyren-5-one	Prepared according to Chapter 2.3.1
Benzanthrone		82-05-3	Benzo[*de*]anthracen-7-one	Aldrich
1-hydroxypyrene		5315-79-7	Pyren-1-ol 1-pyrenol	Aldrich, 98%

1-acetylpyrene		3264-21-9	1-pyren-1-yl-ethanone	Prepared at Roskilde University according to Vollman et al. (1937)
2-acetylpyrene		789-06-0	1-pyren-2-yl-ethanone	Prepared at Roskilde University (Helweg et al., 1997)
4-acetylpyrene		22245-47-2	1-pyren-4-yl-ethanone	Prepared at Roskilde University (Helweg et al., 1997)
1-formylpyrene		3029-19-4	Pyrene-1-carbaldehyde Pyrene-1-carboxaldehyde Pyren-carbaldehyd-(1)	Prepared at Roskilde University according to Vollman et al. (1937)
1-pyrene carboxylic acid		19694-02-1	Pyrene-1-carboxylic acid	Aldrich
1-pyrene carboxylic acid methyl ester		6145-35-3	Pyrene-1-carboxylic acid methyl ester	Prepared at Roskilde University according to Vollman et al. (1937)
2-pyrene carboxylic acid methyl ester		36373-11-2	Pyrene-2-carboxylic acid methyl ester	Prepared at Roskilde University according to Vollman et al. (1937)
4-pyrene carboxylic acid methyl ester		78751-67-4	Pyrene-4-carboxylic acid methyl ester	Prepared at Roskilde University from the corresponding acid

1-bromopyrene		1714-29-0	1-bromo-pyrene	Prepared at Roskilde University according to Vollman et al. (1937)
2-bromopyrene		1714-27-8	2-bromo-pyrene	Prepared at Roskilde University according to Vollman et al. (1937)
4-bromopyrene		1732-26-9	4-bromo-pyrene	Prepared at Roskilde University according to Vollman et al. (1937)
1,6-dibromopyrene		27973-29-1	1,6-dibromo-pyrene	Prepared at Roskilde University according to Vollman et al. (1937)
1,8-dibromopyrene		38303-35-4	1,8-dibromo-pyrene	Prepared at Roskilde University according to Vollman et al. (1937)
1,6-pyrene-dione		1785-51-9	pyrene-1,6-dione	Sigma-Aldrich

4,5-pyrene-dione		6217-22-7	pyrene-4,5-dione	Sigma-Aldrich
X-N-PAC				
2-hydroxyquinoline		59-31-4	quinolin-2-ol 2-(1H)-quinolinone	Fluka, purum >99%
6-methylquinoline		91-62-3		Reagent grade, kindly donated by Department of Chemistry, University of Copenhagen
2,6-dimethylquinoline		887-43-0		Reagent grade, kindly donated by Department of Chemistry, University of Copenhagen
Quinoline-N-oxide		1613-37-2	Quinoline-1-oxide	Janssen Chimica
N-methyl quinolinium iodid		3947-76-0		Pepared at Risø (Kollist-Siigur et al., 2001)
Acridone		578-95-0	10H-acridin-9-one 9(10H)-acridone 9-acridone	Fluka, >98%
6[5H]-phenanthridon		1015-89-0	5H-phenanthridin-6-one Phenanthridon	Aldrich

References: Helweg et al. (1997): Helweg, C., Nielsen, T. and Hansen, P.E.; *Chemosphere*, 34, 1673-1680.

Kollist-Siigur et al. (2001): Kollist-Siigur, K., Nielsen, T., Grøn, C., Hansen, P.E., Helweg, C., Jonassen, K.E.N., Jørgensen, O. and Kirso, U.; *Journal of Environmental Quality*, 30, 526-537.

Vollmann et al. (1937): Vollmann, H., Becker, H., Corell, M. and Streek, H.; *Liebigs Ann. Chem.*, 531, 1-159.

Appendix 2

Characterisation of Humic Materials

As an important part in understanding the properties of the humic materials bound to the HPLC column materials, the soil organic materials (SOM) were characterised by laboratory work and by available information in the literature. The main efforts concentrated on the characteristics of the commercially available Aldrich humic acid (HA), which has been the main SOM in this work. The Ulsted refractory soil carbon (RSC) was isolated by a demineralisation (modified from Bender Koch and Christiansen, 1993) of soil from Ap horizon from a field in northern Jutland, Denmark. The Ulsted RSC constituted approximately 30 %wt of the total organic carbon in the soil sample.

A2.1 Results and Discussion

A2.1.1 Organic carbon and sulphur

The combustion of soil organic matter gave an organic carbon content in Aldrich humic acid of 42.5 %wt. and 60 %wt in Ulsted refractory soil carbon. For Aldrich HA the literature gives values of 38.13 (Marshall et al.1998), 38.23 (Gauthler et al. 1987), 39.03 (Sigma-Aldrich 2003), 55.08 %wt. (Koopal et al., 1998), and 69 %wt. (Malcom and MacCarthy 1986), the last one for purified HA. Measurements on Aldrich HA also showed a sulphur content of 2.71 %wt., literature values are between 0.957 %wt. (Sigma-Aldrich, 2003) and 4.24 %wt. (purified HA) (Malcom and MacCarthy, 1986).

A2.1.2 Infrared spectroscopy

Spectra of the two kinds of soil organic matter were measured using KBr pellet technique (Fig. A2.1). Figure A2.1 shows the two spectra, the differences at frequency above 1900 cm^{-1} are modest. The two peaks around 2900 cm^{-1} (C-H stretch from CH_2 and CH_3) are a bit more intensive in Ulsted RSC than Aldrich HA, the same is seen for O-H stretch (3000-3600 cm^{-1}) as the Ulsted RSC was not dried, water probably also contribute to the observed difference. At lower frequencies the most characteristic peaks Ulsted RSC are at 1730 cm^{-1} (C=O stretch from carbonyl), 1620 cm^{-1} (aromatic C=C stretch), and 1450 cm^{-1} (CH_2

deformation). In the same range Aldrich HA shows highest absorption around 1600 cm^{-1} (C=C and/or C=O from quinones) and 1375 cm^{-1} (C-O stretch from COO^{-}). A shoulder is observed at approximately 1700 cm^{-1} witch partly may be due to carbonyl C=O. Around 800 cm^{-1} there is a broad band that might be from out of plane bending NH_2.

Figure A2.1. FTIR spectra of the two SOM, Aldrich humic acid (lower thick trace) and Ulsted RSC (upper thin trace). See the text for interpretation of the spectra.

Shin et al. (1999) reported the IR-spectra of Aldrich HA. Spectra of different size fractions (made by ultrafiltration) show that the larger humic molecules have an aliphatic nature, while the smaller fractions have a relatively higher content of aromatic and carboxylic parts.

The analysis indicates that the backbone of the two SOM consists of both aromatic and aliphatic parts, and that both samples of SOM contain carbonyl groups and carboxylic acids.

A2.1.3 Size exclusion chromatography

Dextrans of different sizes yielded SEC chromatograms with well-defined peaks. Calibration of the size exclusion chromatography yielded a linear correlation of log MW vs. retention time for dextrans of MW´s higher than 9890 Da, e.g. having retention times between 11.9 and 13.6 min. The two standards of smallest PAMW

(4440 and 1080 Da) lies outside of the range of the linear correlation. They are used as standards for peaks having similar retention times. Acetone, used as internal standard in all samples, has a retention time of 24.1 min.

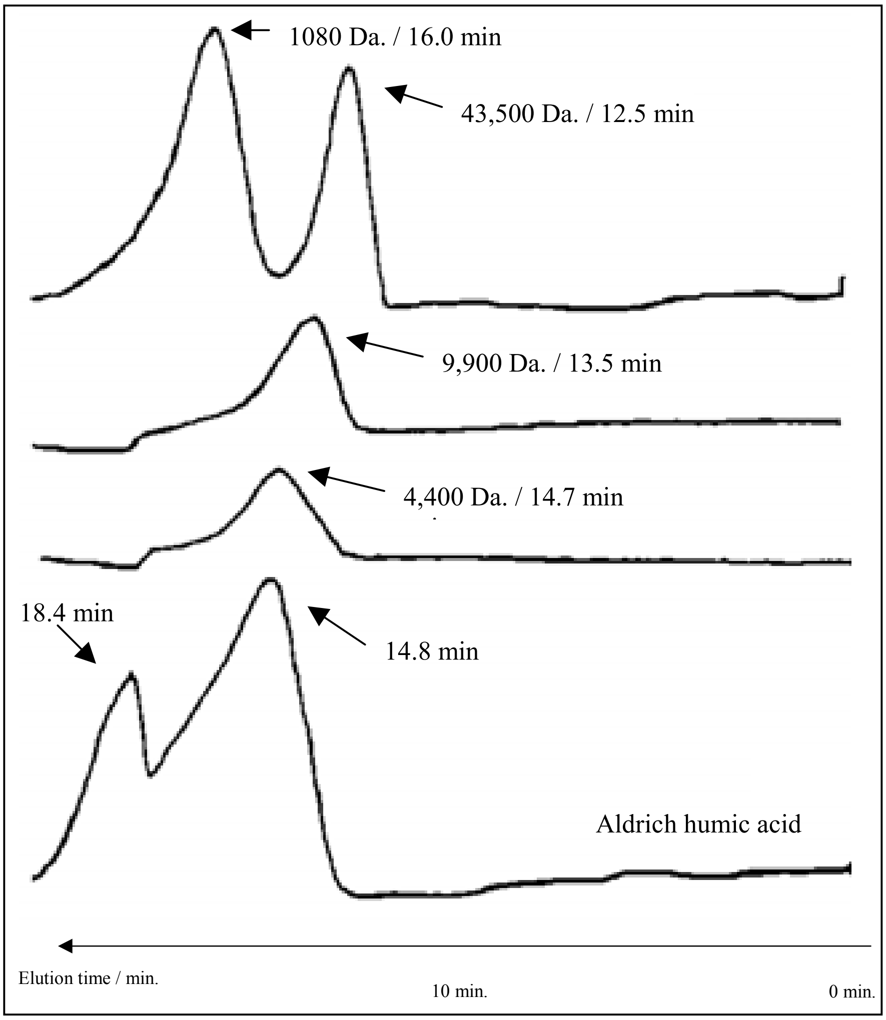


Figure A2.2. Size exclusion chromatograms of standard dextrans (upper three traces) and Aldrich humic acid (lower trace). Elution time is cut at 20 min, not showing the internal standard (acetone) with an elution time at 24.1 min.

For Aldrich humic acid two peaks are noted in the chromatogram: a dominant peak with retention times at 14.8 min which tails into a minor peak at 18.4 min (Fig. A2.2). Both peaks are outside the linear calibration range, but the mass can be compared directly to the individual standards. The first peak corresponds in retention time to the dextran standard with PAMW of 4440, whereas the other peak has a retention time falling out of scale of the standards, e.g. MW below 1000 Da.

Chin and co-workers (1994) have by the same technique (HPSEC) determined the weight-averaged molecular weight to 4100 using sodium polystyrene sulfonates (PSS) as standards, which normally are expected to result in lower averaged molecular weight because of their less globular structure than polysaccharides standards. A small amount of organic cosolvents as methanol in the elution solvent results in a dispersion of humic acid aggregates into smaller humic acid molecules (Conte and Piccolo, 1999). This could be a reason for the relatively small molecule size found in this work.

Size exclusion chromatography of surplus HA from the synthesis of column material give two more distinct peaks (chromatogram not shown) than those seen for the Na-salt. The retention times were 16.3 and 18.4 min. This indicates a smaller molecule size of the surplus HA suggesting that a fractionation by size occurred during the column synthesis. A more likely explanation for the differences between the two samples is that the surplus HA was dissolved in a phosphate buffer (pH 7.5, from the synthesis) with a concentration of 0.4 mol l^{-1}. This affects the pH and therefore the secondary structure of the humic acid (Tombacz and Meleg 1990, Maurice and Namjesnik-Dejanovic 1999, Piccolo et al., 1996) and hereby the elution time through the columns as the spherical size of the molecules changes. Also the ionic strength affects the secondary structure, Varga et al. (2000) noted a significant increase in retention time of Aldrich humic acid on gel chromatography adding phosphate to the system.

Investigations show that there is a heterogeneous distribution of carboxyl groups between the size fractions separated by ultrafiltration of the Aldrich humic acid, where the concentration by mass of carboxyl groups is decreasing with increasing molecular masses (Shin et al., 1999).

The best resolved peak in the SEC chromatogram for the Ulsted refractory soil carbon (Fig. A2.3) has a retention time of 12.0 min corresponding to a MW of 110.000 Da. For longer retention times a broad featureless hump with maximum at 13.7 min is noted. The broad hump may represent an actual distribution of molecular weights peaking at approximately 10.000 Da, but smearing of the peak(s) due to specific interactions between the refractory soil carbon and the column material might probably also contribute.

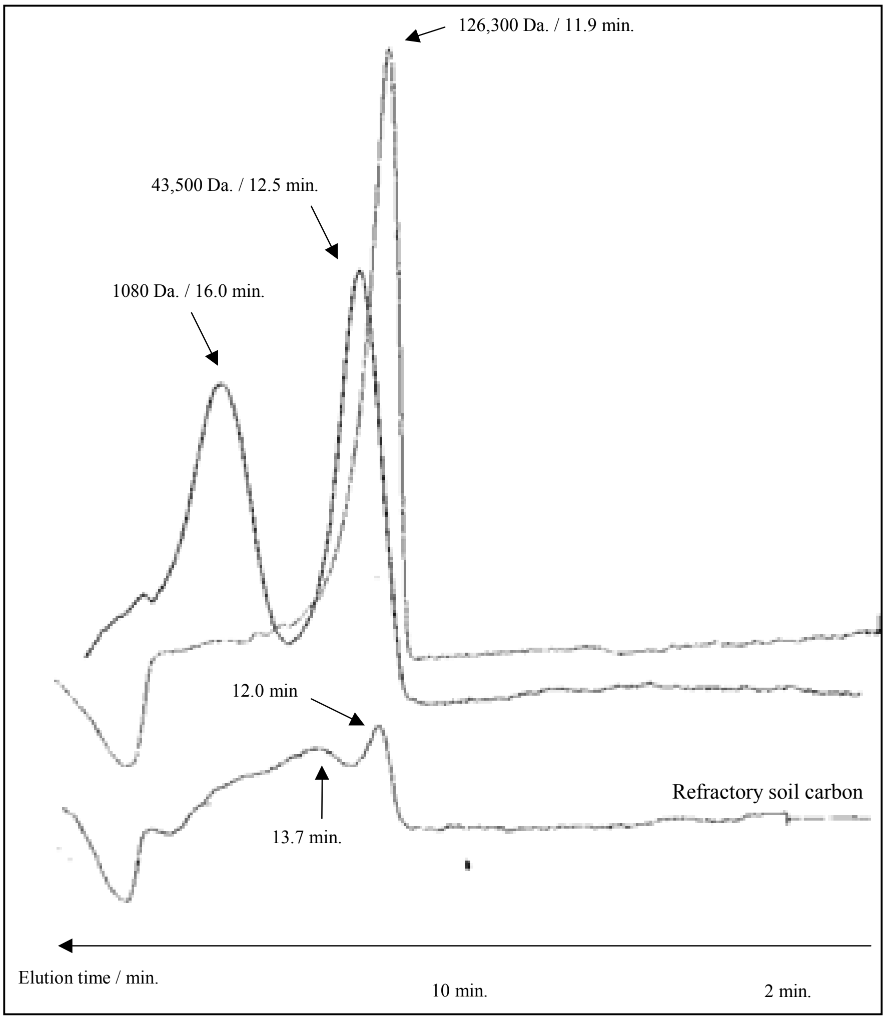


Figure A2.3. Size exclusion chromatograms of standard dextrans (upper two traces) and Ulsted refractory soil carbon (lower trace). Elution time is cut at 20 min, not showing the internal standard (acetone) with an elution time at 24.1 min.

Thus the SEC analysis indicates that the Ulsted RSC is characterised by a maximum molecular weight of 110.000 Da and a broad distribution of units having smaller molecular weights.

A2.1.5 Primary amino-groups in Aldrich Humic Acid

In Aldrich HA the amount of reactive amino groups was determined by its ability to remove crotonaldehyde. Determination of the crotonaldehyde concentration before and after the reaction was performed as described in Experimental

$CH_3\text{-}CH\text{=}CH\text{-}CHO + H_2N\text{-}HA \rightarrow CH_3\text{-}CH\text{=}CH\text{-}CH\text{=}N\text{-}HA$

The reduction of the crotonaldehyde concentration corresponded to a content of reactive amino groups in Aldrich HA of 1.04 ± 0.03 g N/kg or 25% of the total content of N on 4.2 g N/kg (Gauthler et al. 1987).

A2.1.6 Pyrolysis GC/MS of Ulsted RSC

A chromatogram showing a full scan of all ions from the pyrolysis of Ulsted RSC with m/z between 45 and 550 is shown in Figure A2.4A. Aliphatic fragments (represented by m/z 55 and 57) are identified from Figure A2.4B. Further separation into individual chromatograms yielded no extra information with regard to differences in intensity and distribution of the two ions.

The presence of aromatic structures are indicated by identification of PAH, specifically naphthalene (m/z 128), methylnaphthalene (m/z 142), acenaphthylene (m/z 152), anthracene and phenanthrene (both having m/z 178) (Fig. A2.4C). Phenol moieties are indicated by m/z 94 and 108 (phenol and methyl-phenols) in Figure A2.4D. The intensity of the ions decreases both with increasing time and mass.

No other significant fragments were detected by further analysis of the MS data. Based on the relative peak intensities, the refractory soil carbon consists dominantly of aliphatic chains with a minor contribution of aromatic and phenolic parts.

The data from pyrolysis at 694 °C were very similar to those obtained from pyrolysis at 804 °C.

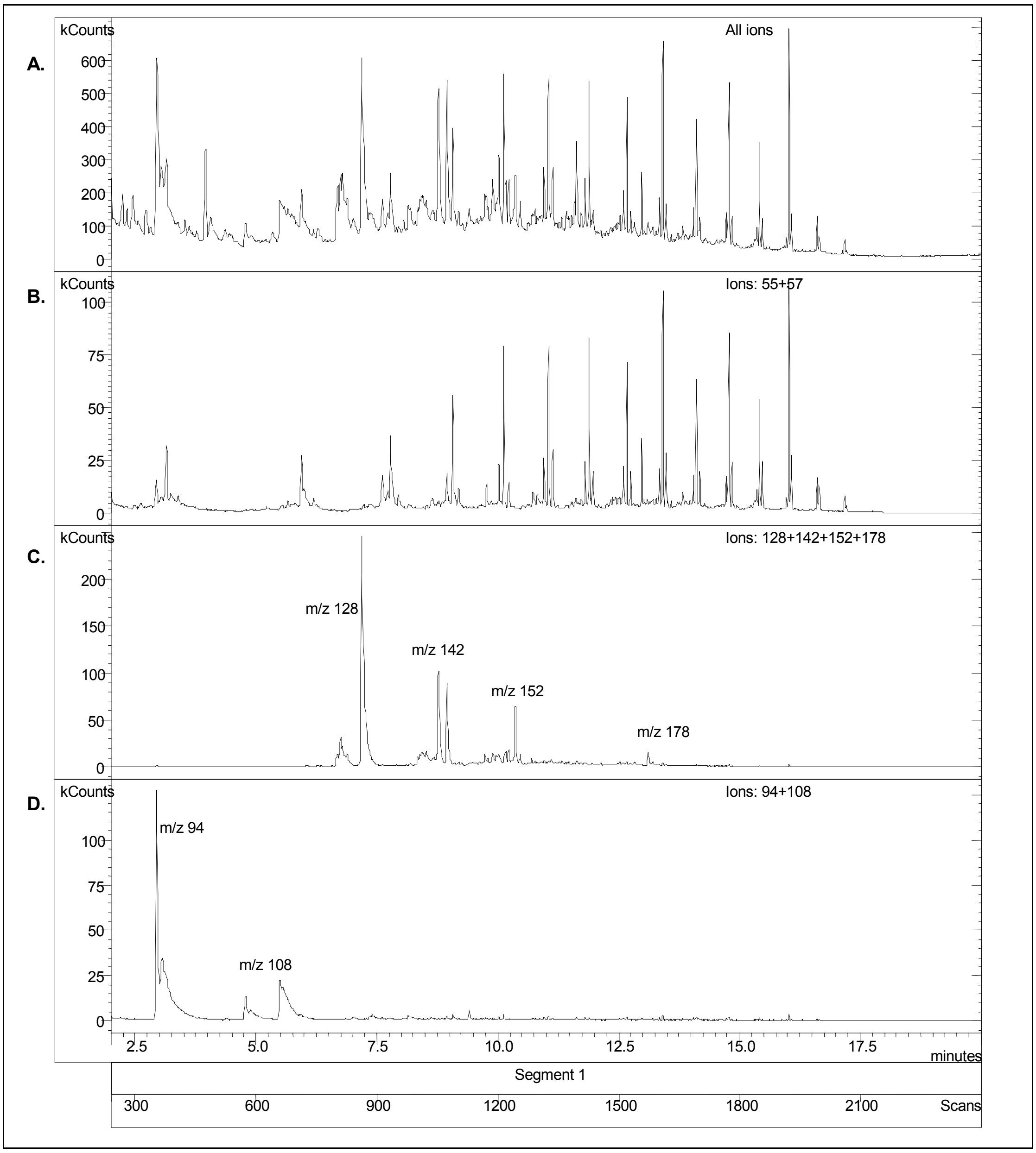


Figure A2.4. GC/MS chromatograms from pyrolysis of Ulsted refractory soil carbon sample at 804 °C. (A) Total scan of all ions with m/z between 45 and 550, (B) the aliphatic part of the material represented by m/z 55 and 57. Aromatic parts represented by PAHs (m/z 128, 142, 152 and 178) in (C) and phenols (m/z 94 and 108) in (D). The time scale is cut off at 20 min., no other peaks were observed up to 30 min.

A2.2 Summary

The elemental composition, functional groups and structural relationships of the Aldrich humic acid, and partially the Ulsted refractory soil carbon, have been characterised.

Ulsted RSC has a higher content of organic carbon (60 %wt.) than Aldrich HA (42.5 %wt.). Both samples contain aliphatic and aromatic parts having varying functional groups dominated by carbonyl groups. Aldrich HA, which is well characterised in the literature, consists of smaller molecules (approximately 4500 Da), having a higher content of aromatic carbon in comparison to the Ulsted RSC, that has molecular size up to 110,000 Da. This latter characteristic probably is the reason for the higher sorption of PAH to Ulsted RSC compared to Aldrich HA (Chapter 6).

Appendix 3

Derivation of Equation 2.1 for calculation of log K_{oc}

The distribution coefficient K is given by the distribution of a compound (as concentration, c) between a solid (c_S) and a mobile (c_M) phase:

$$K = \frac{c_S}{c_M} . \qquad (i)$$

By this definition the unit will be in volume pr. mass, usually l/kg. If the distribution coefficient is to be dimensionless ($K^\dagger$), K must be multiplied by the density of the stationary phase (ρ):

$$K^\dagger = K \cdot \rho = \frac{c_S \cdot \rho}{c_M} . \qquad (ii)$$

By introduction of the column coefficient k', it is possible to describe $K^\dagger$ by the volumes of the two phases:

$$K^\dagger = k' \cdot \frac{\mathrm{V}_M}{\mathrm{V}_S} , \qquad (iii)$$

$$\text{where } k' = \frac{t_R - t_M}{t_M} . \qquad (iv)$$

From (ii) and (iii) K is given by

$$K = k' \cdot \frac{\mathrm{V}_M}{\mathrm{V}_S \cdot \rho} . \qquad (v)$$

As K_{oc} only describes the sorption to organic carbon from soil organic matter, V_S is replaced by V_{SOC}, the volume of soil organic carbon on the stationary phase.

$$K_{OC} = k' \cdot \frac{\mathrm{V}_M}{\mathrm{V}_{SOC} \cdot \rho} , \qquad (vi)$$

where V_{SOC} is given by the multiplication of V_S by the content of organic carbon from SOM in the column material ($\mathrm{V}_{SOC} = \mathrm{V}_S \cdot OC_{SOM}$).

To avoid errors in K_{oc} from retention to binder carbon and silica gel, k' must only be a result of sorption to SOM. By achieving data from two columns, one contains

SOM, and a blank column, k' can be calculated as the difference between column coefficients from both columns.

So from this K_{oc} can be calculated from:

$$K_{OC} = \frac{\left(k'_{SOM} - k'_{blank}\right) \cdot \mathrm{V}_M}{\mathrm{V}_S \cdot \rho \cdot OC_{SOM}} \qquad (vii)$$

$$\Rightarrow \log K_{OC} = \log \frac{\left(k'_{SOM} - k'_{blank}\right) \cdot \mathrm{V}_M}{\mathrm{V}_S \cdot \rho \cdot OC_{SOM}} \quad . \qquad (viii)$$

Which is the expression that is used to calculated log K_{oc} in the "direct determination" method.

References: Atkins, P.W.: *Physical Chemistry,* 4th Ed., Oxford University Press, Oxford, UK, 1994.

Skoog, D.A. and Leary, J.J.: *Principles in Instrumental Analysis,* 4th Ed., Saunders College Publishing, Fort Worth, Texas, USA, 1992.

Appendix 4

Derivation of thermodynamic equations

In thermodynamics *K* designates the equilibrium constant for a reaction. In this work, a compound can be either dissolved in the mobile phase or bounded to the stationary phase, so for the reaction

$$\mathrm{PAC}_{\mathrm{mobile}} \Leftrightarrow \mathrm{PAC}_{\mathrm{stationary}} \qquad (i)$$

the equilibrium constant (in concentrations) is given by

$$K = \frac{c_S}{c_M} \quad . \qquad (ii)$$

At equilibrium the changes in the free Gibbs energy is zero ($\Delta G = 0$), so the equilibrium constant can be described from standard sorption free energy (ΔG_{sorp}), the gas constant (R) and the absolute temperature (T).

$$\ln K = -\frac{\Delta G_{sorp}}{R \cdot T} \quad . \qquad (iii)$$

As changes in the Gibbs energy are simply related to changes in enthalpy (ΔH) and entropy (ΔS):

$$\Delta G = \Delta H - T \cdot \Delta S \qquad (iv)$$

K is easily expressed by:

$$\ln K = -\frac{\Delta H_{sorp}}{R \cdot T} + \frac{\Delta S_{sorp}}{R} \quad \text{, or} \qquad (v)$$

$$K = \exp\left(-\frac{\Delta H_{sorp}}{R \cdot T} + \frac{\Delta S_{sorp}}{R} \right) \quad . \qquad (vi)$$

Using this equation and Eq. (*vi*) from Appendix 3, we have an expression (*viii*) describing the capacity coefficient by changes in enthalpy and entropy during the sorption process. There are some reservations during this; 1) K_{oc} can freely replace *K* in the equation above, and 2) k' is equal to k'_{SOM} neglecting k'_{blank} (other sorption than the one that takes place to SOM). This latter assumption is not of major concern, as k'_{blank} in general are less than 1% of k'_{SOM}, and in most cases below the standard derivation on k'_{SOM}.

$$k' \cdot \frac{\mathrm{V}_M}{\mathrm{V}_{SOC} \cdot \rho} = \exp\left(-\frac{\Delta H_{sorp}}{R \cdot T} + \frac{\Delta S_{sorp}}{R} \right) \qquad (vii)$$

$$\Rightarrow \qquad \ln k' = -\frac{\Delta H_{sorp}}{R \cdot T} + \frac{\Delta S_{sorp}}{R} + \ln\left(\frac{\mathrm{V}_{SOC} \cdot \rho}{\mathrm{V}_M} \right). \qquad (viii)$$

By introduction of $\varphi_{SOM} = \frac{\mathrm{V}_{SOC} \cdot \rho}{\mathrm{V}_M}$, (*viii*) can be rewritten as

$$\ln k' = -\frac{\Delta H_{sorp}}{R \cdot T} + \frac{\Delta S_{sorp}}{R} + \ln \varphi_{SOM} \qquad (ix)$$

Differentiation of ln k' with respect to T^{1} makes it possible to determinate ΔH_{sorp} by a linear plot of (*viii*).

$$\frac{d \ln k'}{d\left(T^{-1}\right)} = -\frac{\Delta H_{sorp}}{R} \qquad (x)$$

$$\Rightarrow \qquad \Delta H_{sorp} = -\frac{d \ln k'}{d\left(T^{-1}\right)} \cdot R \qquad (xi)$$

The entropy changes (ΔS_{sorp}) can be calculated from the intercept with the ordinate in the same plot.

$$\ln k'_{\mathrm{int}} = \frac{\Delta S_{sorp}}{R} + \ln \varphi_{SOM} \qquad (xii)$$

$$\Rightarrow \qquad \Delta S_{sorp} = R \cdot \left(\ln k'_{\mathrm{int}} - \ln \varphi_{SOM} \right) \qquad (xiii)$$

Having ΔH_{sorp} and ΔS_{sorp}, K_{oc} is calculated from (*vi*)

$$K_{oc} = \exp\left(-\frac{\Delta H_{sorp}}{R \cdot T} + \frac{\Delta S_{sorp}}{R} \right) . \qquad (vi)$$

References:

Atkins, P.W.: *Physical Chemistry,* 4th Ed., Oxford University Press, Oxford, UK, 1994.

Skoog, D.A. and Leary, J.J.: *Principles in Instrumental Analysis,* 4th Ed., Saunders College Publishing, Fort Worth, Texas, USA, 1992.

Appendix 5

List of publications

International publications:

Jonassen, K.E.N.; Nielsen, T. and Hansen, P.E., Determination of enthalpy- and entropy-changes in sorption of polycyclic aromatic compounds to humic acid. To be submitted for *Environmental Science and Technology*.

Hübner, M.; Jonassen, K.E.N.; Nielsen, T., Interactions and Conversions of Polycyclic Aromatic Compounds in the Process of Humification. Proceeding Humic substances seminar 6, Boston, MA (US), 27 July 2002. In Humic Substances: Nature's Most Versatile Materials" Taylor and Francis, N.Y. In print.

Jonassen, K.E.N.; Nielsen, T.; Hansen, P.E., The application of high-performance liquid chromatography humic acid columns in determination of Koc of polycyclic aromatic compounds. *Environ. Toxicol. Chem.* (2003) **22**, 741-745

Kollist-Siigur, K.; Nielsen, T.; Grøn, C.; Hansen, P.E.; Helweg, C.; Jonassen, K.E.N.; Jørgensen, O.; Kirso, U., Sorption of polycyclic aromatic compounds to humic and fulvic acid HPLC column materials. *J. Environ. Qual.* (2001) **30**, 526-537

Ziechmann, W.; Hübner, M.; Jonassen, K.E.N.; Batsberg Pedersen, W.; Nielsen, T.; Hahner, S.; Hansen, P.E.; Gudmundson, A.-L., Humic substances and humification. In: Humic substances: Versatile components of plants, soil and water. Humic substances seminar 4, Boston, MA (US), 22-24 Mar 2000. In: Ghabbour, E.A.; Davies, G. (eds.), (Royal Society of Chemistry, London, 2000) (Royal Society of Chemistry Special Publication, 259) p. 9-20

Posters:

Jonassen, K.E.N.; Nielsen, T.; Hansen, P.E., Predicting the sorption of Polycyclic Aromatic Compounds (PAC) to organic material by HPLC Humic Acid columns. SETAC Europe: Organic Soil Contaminants 2001, 2-5 Sep 2001, Copenhagen, Denmark.

Jonassen, K.E.N.; Nielsen, T.; Hansen, P.E., Thermodynamic Constants (Enthalpy and Entropy) in Sorption of PAC to Soil Organic Material. SETAC Europe: Organic Soil Contaminants 2001, 2-5 Sep 2001, Copenhagen, Denmark.

Nielsen, T.; Hansen, T.M.; Hestbjerg, H.; Andersen, M.P.; Jonassen, K.E.N., The lactone 5H-phenanthro[4,5-bcd]pyran-5-one. A new type of metabolite from degradation of pyrene by *Pleurotus ostreatus*. SETAC Europe: Organic Soil Contaminants 2001, 2-5 Sep 2001, Copenhagen, Denmark.

Jonassen, K.E.N.; Nielsen, T.; Hansen, P.E., Sorption of 42 different Polycyclic Aromatic Compounds to Humic Acid HPLC Column materials using wide-pore Silica. An improvement of the Method. Poster at ”Environmental science day” at Roskilde University, November 1999.

Jonassen, K.E.N.; Nielsen, T.; Hansen, P.E., Sorption of 55 different Polycyclic Aromatic Compounds to Humic Acid HPLC Column materials using wide-pore Silica. 17th. International Symposium On Polycyclic Aromatic Compounds, Bordeaux, France, 22-26 Oct 1999.

Oral presentations:

Jonassen, K.E.N.; Nielsen, T.; Hansen, P.E., Predicting Koc and Thermodynamic Constants (Enthalpy and Entropy) in Sorption of PAC to Soil Organic Material by HPLC Humic Acid columns. 19th. International Symposium On Polycyclic Aromatic Compounds, Amsterdam, The Netherlands, 22-25 Sep 2003.

Jonassen, K.E.N.; Nielsen, T., Sorption of PAH to Humic Acid, measured by use of HPLC. Department seminar, Risø, Denmark, 30 March 2001. Unpublished.

Ziechmann, W.; Hübner, M.; Jonassen, K.E.N.; Batsberg Pedersen, W.; Nielsen, T., The structure of humic substances - limits and potential. Humic substances seminar 5, Boston, MA (US), 21-23 Mar 2001. Unpublished.

Jonassen, K.E.N.; Nielsen, T.; Hansen, P.E., The influence of structural factors on the partitioning of Polycyclic Aromatic Compounds (PAC) in soils. 1st French Meeting on Environmental Chemistry, Nancy, France, 13-15 Dec 2000. Unpublished.

Jonassen, K.E.N.; Nielsen, T.; Hansen, P.E., Sorption of PAH and other PAC to humic acid. BIOPRO Workshop - Bioremediation and risk assessment of tar-contaminated soil, Copenhagen, Denmark, 11 Dec 2000. Unpublished.

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Sorption of Polycyclic Aromatic Compounds to Humic and Fulvic Acid HPLC Column Materials

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Christian Helweg, Kristoffer E. N. Jonassen, Ole Jørgensen, and Uuve Kirso

ABSTRACT

Two different humic acids (HA) and a fulvic acid (FA) were chemically immobilized to a high performance liquid chromatography (HPLC) silica column material. The immobilization was performed by binding amino groups in HA/FA to the free aldehyde group in glutardialdehyde attached to the silica gel. The HPLC column materials were compared with a blank column material made by applying the same procedure but without immobilizing HA or FA. Also, a column was made by binding carbonyl groups in HA to amino groups attached to the silica gel. The humic substances were selected to secure appropriate variation of their structural features. The retention factors of 45 polycyclic aromatic compounds (PAC) to the four columns were determined by HPLC. The advantage of the technique is a large number of compounds can easily be studied. The binding procedure does not appear to cause a drastic selection between the HA molecules. The k' values obtained for the two Aldrich HA columns agree in general reasonably. The retention or sorption of the compounds increased with the size of the PAC and the number of lipophilic substituents, but decreased when polar substituents were present. The PAC retention was much stronger to the two HA columns than to the FA and blank column, both for hydrophobic polycyclic aromatic hydrocarbons (PAH) and the polar PAC. Other factors impacting the PAC binding may be specific interactions with HA and the ionic strength of the aqueous phase. The technique has been applied to do direct determinations of K_{oc}.

Among the organic pollutants in the environment, the polycyclic aromatic compounds (PAC) have caused major concern because many are considered to be carcinogenic (IARC, 1983). Besides, PAC with nitrogen atoms in the structure (N-PAC) have recently been demonstrated to be phytotoxics (Gissel-Nielsen and Nielsen, 1996). A number of different PAC has been identified in the environment. The best known group is the hydrophobic polycyclic aromatic hydrocarbons (PAH), but also N-, S-, O-PAC, a number of different oxygenated derivatives, phenols, quinones, ketones, aldehydes and carboxylic acid derivatives, and nitro and chloro substituted PAC have been found (Blanco et al., 1992; Haglund et al., 1987; Nielsen et al., 1983; Youngblood and Blumer, 1975).

Transport, fate, and bioavailability of organic pollutants in the aquatic and terrestrial environment depend on the partitioning of these compounds between water, dissolved humic matter, and soil or sediment humic matter (Calvet, 1989; Schwarzenbach et al., 1993; Totsche et al., 1997). Binding of nonionic organic compounds to soils and sediments depends on the concentration of organic carbon (OC) in the solid (de Maagd et al., 1994; Means et al., 1980). The distribution coefficient, K_{oc} (mol kg^{-1} OC/mol L^{-1} water), is a measure for the binding of the compound. Generally, a simple linear relationship between binding and total OC of the solid is assumed, but recently, a dependency also upon the properties of the solid organic matter has been demonstrated (Chiou et al., 1998; Gauthier et al., 1987). Humic substances constitute a large fraction of the solid organic matter in soils and sediments. The molecular size appears to be an important factor determining the binding of solids to organic matter (Chiou et al., 1986; Engebretson et al., 1996; Engebretson and Wandruszka, 1997). Gauthier et al. (1987) found that K_{oc} for pyrene binding to four different humic acids increased from 0.9×10^5 to 2.0×10^5 L kg^{-1} as the humic acid aromatic fraction varied from 0.20 to 0.34 of total C. One explanation for the changes in binding with the properties of the solid organic matter is that the binding may be either by adsorption or by partitioning into the surface organic phase of the humic acids (LeBoeuf and Weber, 1997; Murphy et al., 1990). The process appears to shift from partitioning to adsorption with the diagenetic alteration of the materials and thus with decreasing O/C and increasing H/C elemental ratios (Huang and Weber, 1997).

The complexity and inhomogenity of humic substances precludes determination of their exact structures for evaluation of the impact of their structural variability upon the binding capacity for organic compounds. The substances exhibit a great variation in their content of aromatic and aliphatic C, N, and S, and phenolic and carboxylic acid groups (Aiken et al., 1996; Garcia et al., 1994; Gauthier et al., 1987; Malcolm and MacCarthy, 1986; Schulten, 1995; Wershaw, 1986). The inhomogenity of the humic samples is also reflected in their broad molecular weight distributions and also the chemical structures of the different molecular size fractions vary (Tanaka and Senoo, 1995). As an example, the fraction of aromatic C varied from 0.14 to 0.51 and the content of carboxylic C from 0.06 to 0.30 for humic substances isolated from Danish ground water systems (Grøn et al., 1996).

Humic acid chemically bonded to HPLC column ma-

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Abbreviations: HA, humic acid; FA, fulvic acid; HPLC, high performance liquid chromatography; PAC, polycyclic aromatic compounds; PAH, polycyclic aromatic hydrocarbons; t_r, retention time; t_0, dead volume; $k' = (t_r - t_0)/t_0$, capacity coefficient; OC, organic carbon; K_{oc}, organic carbon partition coefficient; K_{ow}, octanol-water partition coefficient; NMR, nuclear magnetic resonance.

terial has been used as a new and cost efficient method for the study of the binding of organic compounds to solid organic matter (Nielsen et al., 1997; Szabo and Bulman, 1994). In this investigation the effects of different humic (HA) and fulvic acids (FA) have been studied with this technique. Two different binding procedures were compared for the Aldrich HA. The impact of the humic substance properties on the binding of 45 PAC was determined and compared, as were the ionic strength and cosolvent effects on the binding. The method was tested for its applicability in direct K_{oc} determinations. To span as broad a range of compounds as possible, 45 PAC were evaluated, representing PAH, substituted PAH, N-, O- and S-PAC, neutral as well as charged compounds.

MATERIALS AND METHODS

Chemicals

The test compounds listed in Table 1 along with results discussed later were dissolved in methanol (Lichrosolv 99.8%, Merck, Darmstadt) with a typical concentration of 0.04 g L^{-1}. The preparation of the bromopyrenes is described elsewhere (Hansen and Berg, 1981; Nielsen et al., 1997). *N*-methylquinolinium iodide was prepared from mixing quinoline and methyl iodide in ethanol. 9-anthracenecarboxamide, -carboxylic acid methyl ester, 9-formyl-, 9-methoxy-, 9-nitro- and 9-cyano-anthracene were provided by Ole Hammerich and Tove Thomsen, University of Copenhagen. All other compounds were obtained from commercial sources.

The humic acid was extracted from ground water in Western Jutland (Fjand) and the fulvic acid from ground water in Northern Jutland (Skagen), Denmark. Ground water was pumped through 0.3-μm filters (200-35-AAH borosilicate glass microfibers with inorganic resin binder from Balston, UK). The samples were stored and transported in glass containers to the laboratory for isolation of the humic substances according to the method applied by Krog and Grøn (1995). In short, humic acids were isolated from the Fjand ground water by precipitation with nitric acid, purification by dialysis, and hydrogen saturation by cation exchange. Fulvic acids were isolated by concentration from the acidified Skagen ground water on XAD-8 column material, further purified and desalted by repeated XAD-8 column treatments and finally hydrogen saturated by cation exchange. The humic substances were lyophilized and stored dry until used.

Glassware was cleaned with detergents, followed by nitric acid rinse, several rinses with low OC laboratory water (MilliQ water purification system from Millipore) and was heated to 450°C for 18 h while flushing with air filtered through activated C. For Teflon tubing, rinses with 0.01 *M* sodium hydroxide, low OC water, 0.1 *M* hydrochloric acid, and again low OC water were used.

Preparation of the Chemically Bonded Humic and Fulvic Acid Silica Gel for HPLC Columns [Amino (NH_2) Procedure]

The silica gel with chemically bonded humic substances was prepared in a number of stages as described elsewhere (Nielsen et al., 1997; Szabo and Bulman, 1994). The blank column material was the same as the FA and the HA column materials, the only exception being that neither HA nor FA was applied in the synthesis. The method in short is as follows: A suspension of the silica gel (Nucleosil-Si-50-10, surface area [BET] 450 000 $m^2\ kg^{-1}$, Macherey-Nagel) was refluxed with 3-aminopropyl triethoxysilane (Lancaster, 97%) dissolved in toluene. A suspension of the purified aminopropyl silica gel was stirred at room temperature with a 5% aqueous solution of glutardialdehyde under argon. The activated silica gel was added to a solution of humic or fulvic acid (HA/FA) in water. Finally, the HA/FA silica gel was deactivated with a 0.1 *M* aqueous solution (pH = 7.5) of 2-aminoethanol and isolated. The gels were packed in methanol at 35 MPa into the HPLC columns. The dimensions for the Aldrich HA, Fjand HA, and the blank column were 12.5 cm by 4.6 mm (i.d.) and for the Skagen FA column 5 cm by 4.0 mm (i.d.).

The immobilization of macromolecules to silica gel or glass beds by means of (3-aminopropyl)triethoxysilane and glutardialdehyde have been applied for several years within enzymology (Andres and Narayanaswamy, 1995; Suleiman et al., 1993). Analogous methods have also been used for the preparation of chiral HPLC column materials (Haginaka et al., 1994). In the first step the silanol groups in the silica gel are alkylated with 3-aminopropyl triethoxysilane:

$$\begin{aligned}&3\mathrm{Si{-}OH} + \mathrm{H_2N(CH_2)_3Si(OEt)_3}\\&\quad\rightarrow \mathrm{(Si{-}O)_3Si(CH_2)_3NH_2} + 3\mathrm{EtOH}\end{aligned} \qquad [1]$$

In the second step, a 10-fold excess of glutardialdehyde implies that only one of the carbonyl groups is transformed to an imine:

$$\begin{aligned}&\mathrm{(Si{-}O)_3Si(CH_2)_3NH_2} + \mathrm{OHC(CH_2)_3CHO}\\&\quad\rightarrow \mathrm{(Si{-}O)_3Si(CH_2)_3N{=}CH(CH_2)_3CHO} + \mathrm{H_2O}\end{aligned} \qquad [2]$$

In the third step humic or fulvic acid is immobilized to the silica gel by imine formation between the aldehyde group and one or more amino groups in the HA/FA molecules:

$$\begin{aligned}&\mathrm{(Si{-}O)_3Si(CH_2)_3N{=}CH(CH_2)_3CHO} + \mathrm{H_2N{-}HA/FA}\\&\quad\rightarrow \mathrm{(Si{-}O)_3Si(CH_2)_3N{=}CH(CH_2)_3CH{=}N{-}HA/FA}\end{aligned} \qquad [3]$$

Only amino groups are expected to be attached. Residual aldehyde groups are inactivated by imine formation with 2-aminoethanol in the fourth step:

$$\begin{aligned}&\mathrm{(Si{-}O)_3Si(CH_2)_3N{=}CH(CH_2)_3CHO}\\&\quad + \mathrm{H_2NCH_2CH_2OH}\\&\quad\rightarrow \mathrm{(Si{-}O)_3Si(CH_2)_3N{=}CH(CH_2)_3CH{=}}\\&\quad\mathrm{NCH_2CH_2OH}\end{aligned} \qquad [4]$$

The latter, *N*-5-(2-hydroxyethylimino)pentyleno-3-aminopropyl, is the structure of the substituted group in the blank column material. Thus, the blank column contains OC.

Preparation of the Chemically Bonded Aldrich Humic Acid Silica Gel for HPLC Columns [Carbonyl (C=O) Procedure]

This silica gel with chemically bonded humic acid was prepared in a number of stages modified from the way listed above, leaving the stage with glutardialdehyde out and using formaldehyde instead of 2-aminoethanol in the last step.

A suspension of the silica gel (Nucleoprep-Si-300-20, surface area (BET) 100 000 $m^2\ kg^{-1}$, Macherey-Nagel) was stirred under N_2 with 3-aminopropyl triethoxysilane (Merck, p.a.) dissolved in toluene. The product was filtered, washed and dried, and the purified aminopropyl silica gel was added to a solution of humic acid (Aldrich) in water and stirred at room

Table 1. PAC log K_{ow}† and capacity coefficients (k') on the three humic acid coated columns and the blank column. Mobile phase (pH = 8): Methanol (MeOH) + buffered water.

Column:	Fjand Ha	Aldrich HA†	Aldrich HA	Skagen FA	Blank	log K_{ow}†
HA/FA attachment site:	Amino	Amino	Carbonyl	Amino		
Compound/mobile phase:	0.75 MeOH	0.65 MeOH	0.65 MeOH	0.50 MeOH	0.50 MeOH	
X-N-PAC						
2-hydroxyquinoline	0.22	0.21	0.16	0.51	0.36	1.56
Quinoline-N-oxide	0.12	0.10	0.14	0.26	0.08	1.13
N-methylquinolinium	30.48	8.73	4.66	2.07		−0.29
N-PAC‡						
Quinoline	0.08	0.14	0.06	0.57	0.39	2.07
Isoquinoline	0.13	0.19	0.08	0.57	0.36	2.08
4-azafluorene	0.36	0.44	0.18	1.18	0.92	2.96
Acridine	0.74	0.78	0.35	1.96	1.47	3.27
Benzo[f]quinoline	0.74	0.87	0.42	2.16	1.77	3.40
Benzo[h]quinoline	0.79	0.95	0.33	2.52	2.10	3.60
Phenanthridine	0.76	0.86	0.36	2.05	1.61	3.44
Benz[a]acridine	3.93	3.67	2.23	6.85	5.57	4.48
10-azabenzo[a]pyrene	18.14	13.79	8.35	25.68	22.73	5.53
Dibenz[a,c]acridine	23.99	21.96	14.51	39.27	33.38	5.66
Dibenz[a,h]acridine	23.50	18.35	16.41	34.32	29.64	5.73
Dibenz[a,j]acridine	15.54	13.78	17.10	26.02	19.50	5.63
Dibenz[c,h]acridine	24.28	25.24	13.44	52.52	43.38	6.45
Carbazole	1.79	2.46	0.29	7.89	7.90	3.51
X-PAH						
9-acetylanthracene	0.88	1.09	0.33	3.05	2.66	3.60
9-anthracenecarboxamide	0.76	0.90	0.32	1.64	1.55	2.23
9-anthracenecarboxylic acid methylester	1.15	1.61	0.42	4.70	4.41	3.83
9-bromoanthracene	3.57	4.58	1.11	14.76	12.78	5.37
9-chloroanthracene	3.10	3.40	1.05	12.23	8.74	5.27
9-cyanoanthracene	2.40	2.31	1.16	5.07	4.12	3.98
9-formylanthracene	0.56	0.82	0.17	2.64	1.60	3.77
9-methoxyanthracene	1.09	1.53	0.37	4.58	4.06	4.36
9-methylanthracene	2.02	2.95	0.81	8.00	7.46	4.96
9-nitroanthracene	1.76	2.25	0.71	6.92	6.43	3.70
9,10-anthraquinone	1.74	1.27	0.90	2.03	1.51	3.28
1-bromopyrene	8.72	10.24	4.46	27.39	23.65	5.81
2-bromopyrene	9.01	10.06	5.64	26.61	23.54	5.80
4-bromopyrene	9.47	10.95	5.93	28.48	23.18	5.84
1,3-dibromopyrene	24.27	23.13		45.13	37.92	6.85
1,6-dibromopyrene	19.63	17.94	16.53	41.49	33.24	6.69
1,8-dibromopyrene	19.72	22.59	16.42	42.16	33.39	6.73
PAH‡						
Naphthalene	0.34	0.53	0.05	1.68	1.36	3.40
Fluorene	1.76	2.23	0.26	3.21	2.90	4.32
Anthracene	1.48	2.12	0.50	6.61	5.84	4.48
Phenanthrene	1.47	2.14	0.45	6.75	5.77	4.46
Benz[a]anthracene	8.03	9.49	2.80	26.84	22.56	5.54
Benzo[a]pyrene	20.87	25.76	14.06	58.14	56.08	6.02
Dibenz[a,c]anthracene	40.46	39.25	24.96	95.51	88.74	6.54
Dibenz[a,h]anthracene	30.13	33.40	20.74	78.53	75.22	6.40
Dibenz[a,j]anthracene	38.39	41.66	21.40	88.29	82.58	6.54
O, S-PAC						
Dibenzofuran	0.67	0.99	0.21	3.07	2.88	4.12
Dibenzothiophene	1.24	1.93	0.38	6.13	5.21	4.49

† k' from Nielsen et al. (1997). K_{ow} (octanol–water partition coefficient) from Nielsen et al. (1997) and Helweg et al. (1997a,b).

‡ Listed with increasing molecular weight with the exception of carbazole.

temperature under N_2 and finally deactivated with 0.1 *M* aqueous solution of formaldehyde. The gel was packed in methanol at 35 MPa into a 12.5 cm by 4.6 mm (i.d.) HPLC column.

The first step is the same as that in the amino procedure. In the second step, humic acid is immobilized to the silica gel by imine formation between the amino group and carbonyl groups in the HA molecules:

$$\begin{aligned}&(\text{Si–O})_3\text{Si}(\text{CH}_2)_3\text{NH}_2 + \text{ORC–HA}\\ &\quad\rightarrow (\text{Si–O})_3\text{Si}(\text{CH}_2)_3\text{N=RC–HA}\end{aligned} \qquad [5]$$

The basic residual amino groups are inactivated by imine formation with formaldehyde:

$$\begin{aligned}&(\text{Si–O})_3\text{Si}(\text{CH}_2)_3\text{NH}_2 + \text{O=CH}_2\\ &\quad\rightarrow (\text{Si–O})_3\text{Si}(\text{CH}_2)_3\text{N=CH}_2\end{aligned} \qquad [6]$$

Determination of Reactive Primary Amino-Groups in Aldrich Humic Acid

A 2.0 m*M* crotonaldehyde (Fluka, tech.) solution was prepared by dissolving 0.2 mmol (16.5 μL) crotonaldehyde in 1 mL methanol (Lab-Scan, HPLC-grade) and adding purified water (MilliQ) to a total volume of 100 mL. A suspension of 0.5 g Aldrich Humic Acid in 50 mL of the crotonaldehyde solution was stirred under N_2 at room temperature for 7.5 h.

Two aliquots of the suspension, each of a few milliliters, were centrifuged for 15 min at 500 × *g*, and filtrated through a 45-μm PTFE filter before analyses.

The concentration of crotonaldehyde in the solution before and after the reaction was determined by HPLC, using a C-18 HPLC column [25.0 cm by 4.6 mm (i.d.) column packed with Nucleosil 100-5 C_{18} (Macherey-Nagel) at 35 MPa]. A mixture of equal amounts (volume) of purified water (MilliQ) and methanol (Lab-Scan, HPLC-grade) was used as mobile phase. The crotonaldehyde was measured at 214 nm on a photodiodearray detector.

Mobile Phases

Methanol (Lichrosolv 99.8% from Merck) and low OC laboratory water (MilliQ water purification system from Millipore) were used as mobile phase components. The eluent water (phosphate buffer 0.01 *M*, pH = 7) to methanol ratio was adjusted depending of the properties of the applied humic and fulvic acids to keep approximately the same retention times of the PAC. Thus, the methanol fraction in the eluent was 0.65 for the Aldrich HA column, 0.75 for the Fjand HA column, and 0.50 for the Skagen FA column and the blank column. pH in the methanol water mixtures was about 8 (Roses et al., 1996).

The fraction of phosphate buffer varied from 0.35 to 0.95 when investigating the influence of increasing water content in the mobile phase to the capacity coefficients of the bicyclic aromatic compounds. The pH in the methanol water mixtures was 7.

When investigating the salt effect on the retention of tricyclic aromatic compounds on the Aldrich HA column, an eluent fraction of 0.01 *M* $Na_2B_4O_7$ in water of 0.65 and of methanol of 0.35 was used. The salt ($Ca(NO_3)_2$) concentration was changed from 0 to 0.07 *M*.

HPLC Instrumentation

A low pressure gradient Shimadzu LC-10 HPLC system with photodiodearray detector, thermostatted (30°C) column oven and autoinjector was used. Appropriate mixtures of the compounds were injected and the retention time of each compound was recorded. The injected amount was typical 2 μg of each component. The correct identification of the peaks was controlled by means of the UV spectrum. All measurements were repeated at least two times. The dead time (t_0) of the system, used for calculating the capacity coefficient ($k' = (t_r - t_0)/t_0$), was determined by injecting and chromatographing water six times. The reproducibility of t_0 was 1.3%, and that of the retention time, t_r, for the 45 PAC was 0.9 ± 0.6% for the Aldrich HA column as determined by the expression ($100 \times \Sigma(t - t_{mean})/(n \times t_{mean})$). The reproducibilities of t_0 and t_r were 0.5% and 0.6 ± 0.5% for the Fjand HA column, 0.5% and 0.5 ± 0.3% for the Skagen FA column, and 0.1% and 1.4 ± 1.3% for the blank column.

Dialysis Experiments

The influence of pH on the sorption of quinoline and acridine on dissolved humic acid was also studied with the dialysis method. The method is described in details elsewhere (Nielsen et al., 1997). In short, the equilibrium dialysis experiments to measure the binding of N-PAC to HA were performed by placing 5 mL of a HA solution in dialysis tubing and clamping the ends. The dialysis bag (Spectra Por 6; molecular weight cut off of 1000) was then placed in a 100-mL glass bottle containing a buffered (0.01 *M* phosphate) solution of the N-PAC. The bottle was shaken in the dark for 24 h at 25°C. Control experiments demonstrated that this was sufficient to achieve a steady state. The described procedure was done for a series of different HA concentrations. In each series two blank experiments without HA were performed to correct for sorption of the N-PAC to the surfaces. The N-PAC concentration in the outer solution was determined by HPLC. The K_{oc} value was derived from the relationship between the ratio of the concentration of the N-PAC in the blank experiment to that in the sorption experiments and the HA total OC concentration at different pHs in the range 2 to 12.

RESULTS AND DISCUSSION

Immobilization of Humic Substances to Silica Gel HPLC Column Material

Humic substances are a complex mixture of different molecules and hardly two identical humic acid molecules exist in nature. The same is true for fulvic acid. Humus compounds are made up of a skeleton of aromatic rings and aliphatic chains. The skeleton may contain different substituents, e.g., phenolic OH and carboxylic acid. Some groups of the aromatic systems (phenols) are partially oxidized to quinones. The humic substances also contain residues of biomolecules, proteins, and carbohydrates. The amino procedure for preparing HPLC column materials utilizes the content of protein residues. The advantage of the technique is that it does not change the aromatic structures in HA. However, the content of amino groups may not be homogeneous in the humic substance material. Therefore, the binding procedure may modify the humic substance composition. Unless otherwise stated, the Aldrich HA column mentioned here and in the following sections refers to the column material prepared by the amino procedure. The relative loading of the column materials can be assessed from their C content. The total content of C in the blank column material was 125 ± 2 g kg^{-1}, in the Skagen FA column material 137 ± 0.7 g kg^{-1}, in the Aldrich HA column material 132 ± 0.5 g kg^{-1}, and in the Fjand HA column material 146 ± 3 g kg^{-1}. Thus, the loading appears to follow the range: Fjand HA > Skagen FA > Aldrich HA. An approximate value of the loading of the column material with humic substance OC can be achieved by comparing the column OC content with that of the blank column. This implies that the content of the C from the binding material, the substituted aminopropyl part, in the HA and FA columns should be approximately 125 g kg^{-1}. The error in doing this should in the worst case be the following: Fjand HA 3.6%, Aldrich HA 1.5%, and Skagen FA 0.55%. Thus, the content of humic OC is estimated to be the following with the number in parentheses indicating the worst case maximum value: Fjand HA 21 (26) g kg^{-1}, Aldrich HA 7 (9) g kg^{-1}, and Skagen FA 12 (13) g kg^{-1}. The worst case number of aldehyde groups bonded to HA amino groups was estimated by comparing the OC content of the HA-loaded column with that of the blank column and accounting for the C/N ratio of the HA using the data in Table 2. The estimation requires that all the N in the three humic/fulvic acids are amino N and that all the amino groups have reacted with a carbonyl group. However, this does not appear plausible. The amount of hydrolyzable amino acids was 0.098 g N kg^{-1} (0.7% of total N) in Fjand HA and 1.8 g N kg^{-1} (16%) in Skagen FA (Grøn et al., 1996). These amounts appear to represent an upper limit for the part of N being amino groups (Anderson et al., 1989). In Aldrich HA the amount of reactive

Table 2. Properties of the free and the bonded humic and fulvic acids.

	Fjand HA	Aldrich HA	Skagen FA
Free acid—elemental composition, g kg^{-1}			
C	590	385†	546
H	42	31†	44.5
N	15	4†	11
Ash	20	310†	20
H/C atomic ratio	0.85	0.97	0.98
O/C atomic ratio‡	0.42	0.53	0.52
Free acid—fraction§ of different types of organic C			
Aliphatic	0.16	0.44†	0.27
Aromatic	0.61	0.26†	0.25
Carbohydrates	0.02	0.12†	0.06
Carboxylic	0.17	0.09†	0.29
		Aldrich HA (amino)	
Bonded acid—fraction¶ of different types of organic C			
Aliphatic	0.32	0.62 (0.47)	0.23
Aromatic	0.44	0.10 (0.17)	0.40
Carbohydrates	0.04	0.27 (0.41)	0.22
Carboxylic	0.11	0.08 (0.08)	0.12

† From Gauthier et al. (1987) and Malcolm and MacCarthy (1986), our measurement of TOC (Grøn et al., 1996) showed a content of elemental C of 390 g kg^{-1}.

‡ It is assumed that the residue is oxygen.

§ From ^{13}C NMR: Skagen FA and Fjand HA: Aliphatic 0–65 ppm, Carbohydrates: 65–90 ppm, Aromatic: 90–165, Carboxylic: 165–190 ppm. Aldrich HA: Aliphatic 0–50 ppm, Carbohydrates: 50–95 ppm, Aromatic: 108–165 ppm, Carboxylic 165–190 ppm. The wide range (50–95 ppm) for carbohydrates is believed to lead to a too large content.

¶ From ^{13}C NMR: As for Skagen FA and Fjand HA in footnote§. In brackets for Aldrich HA is also shown the numbers applying the C assignment for Aldrich HA in the ref. in footnote†. Fraction of C type i = (((Column carbon content × Ar$_i$)/Sum Ar$_i$) − ((125 × Ar$_{i(blank)}$)/Sum Ar$_{i(blank)}$))/(Column carbon content − 125).

amino groups was determined by its ability to remove crotonaldehyde and determination of the crotonaldehyde concentration before and after reaction as described in Materials and Methods:

$$\mathrm{CH_3{-}CH{=}CH{-}CHO + H_2N{-}HA} \rightarrow \mathrm{CH_3{-}CH{=}CH{-}CH{=}N{-}HA} \qquad [7]$$

The reduction of the crotonaldehyde concentration corresponded to a content of reactive amino groups in Aldrich HA of 1.04 ± 0.03 g N/kg or 26% of the total content of N. In the Aldrich HA carbonyl column material, the content of OC from humic acid was 19 g kg^{-1} and that from the post- and pretreatment was 5 g kg^{-1} (Jonassen et al., 1999). The Skagen FA had a narrow molecular weight distribution below 5000 D, while the Fjand HA had broad molecular weight distributions up to 100 000 D (Grøn et al., 1996). The Aldrich HA also had a broad molecular weight distribution but with contributions >100 000 D (Tanaka and Senoo, 1995).

Column Composition

Table 2 shows further details of the composition of the free and bounded humic and fulvic acids. The free acids have a medium range of O/C ratio and a low H/C ratio, indicating that partitioning should be an important mechanism for the binding of nonionic organic compounds to these humic substances according to Huang and Weber (1997). Characteristics for the Fjand HA column material are a medium aliphatic and high aromatic content, the Aldrich HA material showed high aliphatic and low aromatic contents, and the Skagen FA material low aliphatic and high aromatic content. Fourier Transformation Infrared Spectroscopy of the bonded Fjand HA and Skagen FA showed a strong peak at 1610 cm^{-1} and a high content of aromatic C, but higher in the bonded Fjand HA than in Skagen FA. The C composition of the bonded acids was also estimated by means of ^{13}C NMR (see Table 2). These numbers should be interpreted with caution, as it is not possible to do an exact correction for the C originating from the pre- and posttreatment procedure, i.e., non-HA/FA C, (see the end of the section on preparation of the chemically bonded humic and fulvic acid silica gel for HPLC columns [amino (NH$_2$) procedure]). The numbers are based on the assumption that this procedure contributes with the same amount of C in the three humic substance columns as in the blank column. The silica gel used for the Aldrich HA (amino) column was the same product but not the same batch as that used for the other columns (Fjand HA, Skagen FA, and blank), but the different batches should be very similar in their properties. The difficulties doing an exact estimate for the pre- and posttreatment C is also illustrated by the fact that the carbonyl C fraction in the bonded Aldrich HA (amino) gave a negative value. Thus, the sum of the fraction of the other types of C is higher than 1.0 (see Table 2). The bonded Fjand HA has a high content of aromatic C and a medium content of carboxylic C. Both appeared to be reduced compared with the free humic acid, while the content of aliphatic C had increased. The bonded Aldrich HA (amino) has a lower content of aromatic C than the free humic acid, whereas the amount of carboxylic C appeared to be the same. The bonded Skagen FA appeared to be enriched in aromatic C and depleted in carboxylic C compared with the free acid.

Variation of the Capacity Coefficients on Different Columns

The HPLC capacity coefficient, $k' = (t_r - t_0)/t_0$, is a relative measure for the distribution coefficient, $K = C_s/C_w$, of the compound, where C_s = sorbed compound and C_w = dissolved compound. The expression $(t_r - t_0)/t_r$ is a measure for the proportion of the compound being adsorbed to the column material and t_0/t_r is a measure for the part being dissolved in the eluent (Antworth et al., 1989). The capacity coefficients of all 45 PAC on the Aldrich HA, the Fjand HA, the Skagen FA and the blank columns are presented in Table 1 and compared with K_{ow} (octanol–water partition coefficients). In addition, results for the Aldrich HA using the carbonyl procedure are also presented. A higher amount of methanol was used in the eluent for Fjand HA and Aldrich HA than for Skagen FA and the blank column to keep the retention times approximately the same on the five columns (see Table 1). An increase of the methanol content causes k' to decrease. This should be borne in mind comparing the log k' for the five columns. The need for different methanol content implied that Fjand HA was the strongest sorbent (highest affinity binding) and Skagen FA and the blank column was the weakest ones. The Fjand and Aldrich HA results do not appear to have been significantly affected by the eventual swelling of the column material with increasing methanol content as discussed later, although some minor effects cannot be ruled out.

The column material is probably linked to residues of carbohydrates and aromatic quinones in the humic substance when prepared by the carbonyl procedure and to peptide residues by the amino procedure. Thus, a comparison of the retention on the two types of column materials should make it possible to determine if the attachment procedure causes major changes in the humic acid composition. Figure 1 shows a comparison of the log k' values on the two types of column materials applying the same eluent composition. In general, there is a good accordance between the results. However, as discussed later, e.g., in the section on retention of substituted PAH, there are significant differences for some compounds. The retention of a compound is dependent both on the column surface area of the column material (BET) and its humic acid content, as expressed as humic acid organic carbon (HAOC):

$$k' = \text{constant} \times \text{BET} \times \text{HAOC}$$

The ratio $(\text{BET} \times \text{HAOC})_{\text{C=O}}/(\text{BET} \times \text{HAOC})_{\text{NH2}} = (100 \times 19)/[450 \times (132 - 125)] = 0.60$ is very close to the slope of 0.62 ± 0.02 ($y = 0.62x$, $R^2 = 0.92$) for the regression line plotting $k'_{\text{C=O}}$ vs. k'_{NH2} (data not shown). Figure 1 shows the corresponding log k' to log k' plot. The log BET × HA ratio of 0.94 is also reasonably close to the slope in Fig. 1 of 1.10 ± 0.05 considering the negative intercept value. Thus, in general terms there was fairly good agreement in the properties of the humic acid bonded by the two methods. This also means that the properties of the bonded humic acid should not be very different from those of the free humic acid, at least for the Aldrich humic acid.

The K_{ow} value of a compound is often applied to estimate its K_{oc} value. Figure 2 uses an approach corresponding to that suggested by Seth et al. (1999), applying log K_{ow} as a reference indicator (x axis) and the expression log (k'/K_{ow}) as the y axis. This is a way to show if other factors than hydrophobicity contribute to the partitioning. The regression line for the hydrophobic

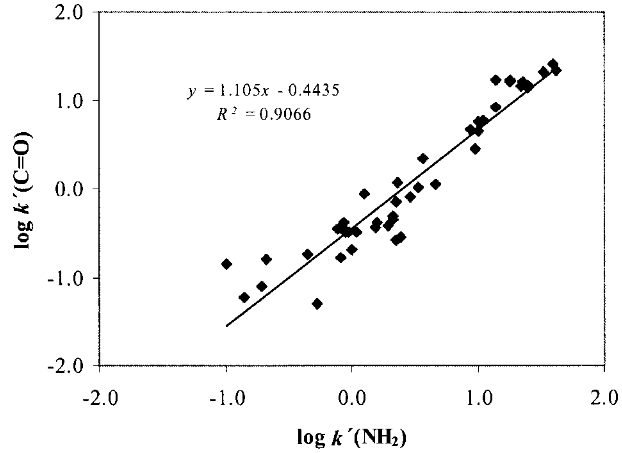

Fig. 1. The relationship between log k' for the two Aldrich HA columns prepared by the amino procedure (NH_2) and the carbonyl procedure (C=O). The methanol fraction in the eluent was 0.65 (see Table 1).

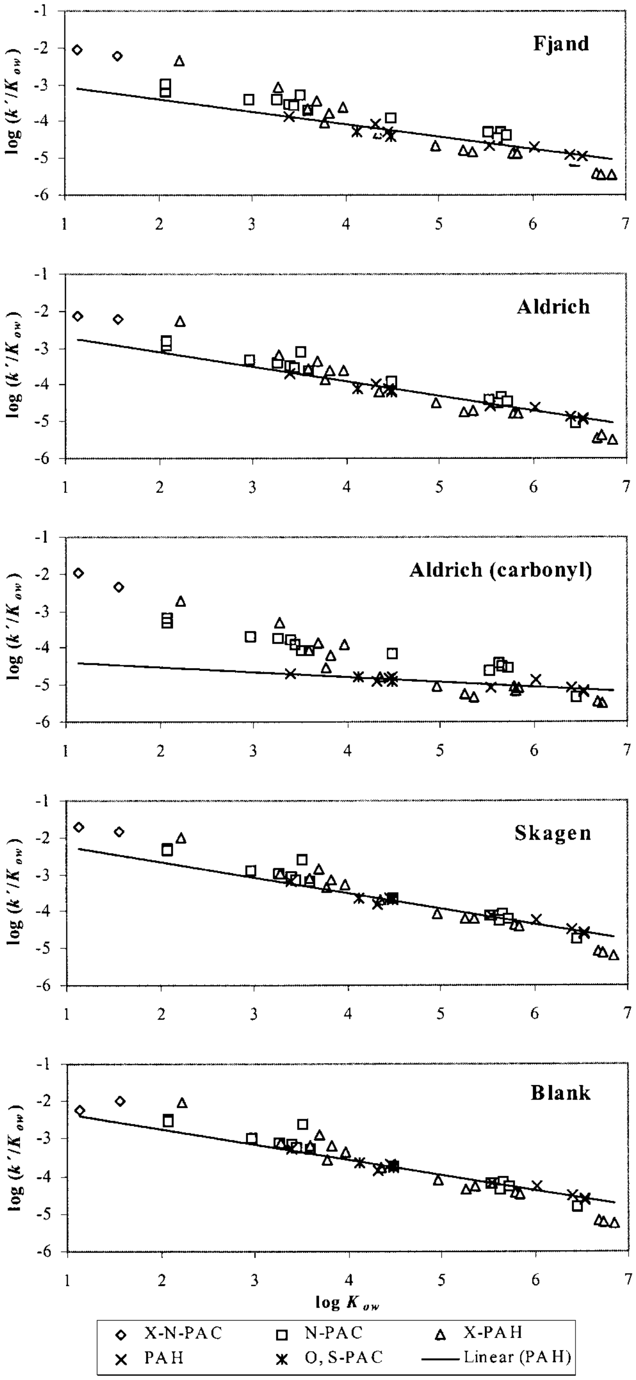

Fig. 2. The relationship between log (k'/K_{ow}) and log K_{ow} for the humic acids Fjand HA and Aldrich HA, a fulvic acid, Skagen FA (all attached using amino-groups for the binding), a blank column using the same procedure for attachment without adding HA or FA and Aldrich HA (carbonyl) using carbonyl in HA for the attachment. The methanol content in the eluent was as shown in Table 1. PAH are polycyclic aromatic hydrocarbons, X-PAH-substituted PAH, N-PAC-azaarenes, and X-N-PAC- substituted azaarenes.

PAH is incorporated in the figures as a reference line. Data for *N*-methylquinolinium iodide is excluded in these plots, because its behavior is very different from those of the other compounds. The *N*-methylquinolinium is a cation in contrast to the other neutral compounds. In general the figure indicates a good correlation between the log k' values [plotted as log (k'/K_{ow})] and log K_{ow} values. However, some systematic variations can be observed comparing Fig. 2 with the values in Table 1. These variations are most pronounced for the humic acid columns, Fjand and Aldrich (both the amino and the carbonyl version). The sorption to the column materials is relatively stronger for the polar compounds (N-PAC, substituted N-PAC and polar substituted PAH), when the K_{ow} values are used as a relative reference. The relative increase is particularly strong for those polar PAC having low K_{ow} values. Thus, the comparison indicates that for polar compounds chemical interactions contribute to the partitioning process in addition to the hydrophobicity. The sorption of PAH with lipophilic substituents, especially the three dibromopyrenes, is relatively weaker than the sorption of the unsubstituted PAH. The reasons for this are not known, but an explanation might be that the substituents cause some steric hindrance in the sorption process to the column material. The same type of deviations from the PAH line was observed for columns prepared by either the amino method or the carbonyl method, although the PAC containing polar substituents are adsorbed relatively stronger on the *carbonyl* column than on the *amino* column.

Retention Dependency of the Mobile Phase Composition

The capacity factors (log k') of bicyclic aromatic compounds were determined changing the methanol/water ratio in the mobile phase (see Fig. 3). For most of the bicyclic aromatic compounds the retention increased with increasing eluent water content. When the methanol content of the mobile phase increases, the polarity of the mobile phase and the retention of the solute decreases. The increase in retention with decreasing methanol content is found for all other compounds than *N*-methylquinolinium iodide as shown in Fig. 3. The elution order of the other compounds was independent

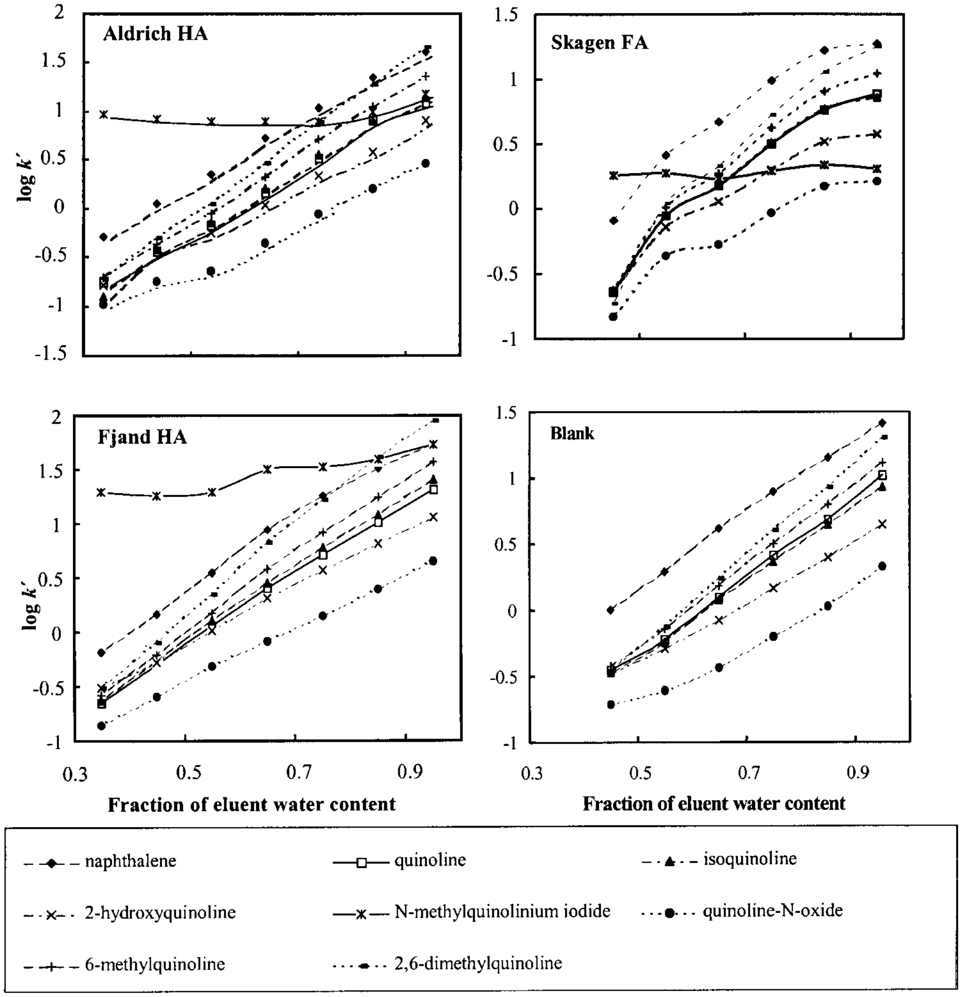

Fig. 3. The influence of the water content of the mobile phase on the retention of bicyclic aromatic compounds on the Aldrich HA (amino procedure), Skagen FA, Fjand HA, and blank column.

of the methanol content in the mobile phase on the Skagen FA and the blank columns as indicated by the lines on Fig. 3 not crossing. On the Aldrich HA (amino) and the Fjand HA columns 2,6-dimethylquinoline and naphthalene changed their elution order at high water content (for the Aldrich HA column >0.85 and for the Fjand HA column >0.75 of water). On the Skagen FA column there was no significant change in the capacity coefficient of *N*-methylquinolinium iodide when the water fraction of the eluent was changed from 0.45 to 0.85. A different behavior is observed for the Aldrich HA and the Fjand HA columns. With increasing water content in the mobile phase, the retention of *N*-methylquinolinium iodide decreases slightly. This decrease may be caused by a decrease in the size of the humic acid or an increase in the rigidity of the structure of the humic structure. When water fraction reaches ~0.50 (Fjand HA column) and ~0.70 (Aldrich HA column) the capacity coefficient started to increase. However, the increase is not large compared with those for the other compounds.

Values of log k' for seven of the eight bicyclic aromatics were linearly dependent on the eluent water concentration for the Fjand HA, Aldrich HA, and Blank column, although there are some minor deviations from the linearity in the low end of the eluent water content (Fig. 3). This suggests that changes in the eluent composition did not cause major changes in the sorption mechanism. The ranges of the correlation coefficients were as follows: $r = 0.995$ to 0.999 (Fjand HA), $r = 0.995$ to 0.9998 (Aldrich HA), and $r = 0.982$ to 0.999 (Blank). For Skagen FA a better relation between log k' and the eluent water concentration was achieved using a second-order relation: $\log k' = ax^2 + bx + c$, where x is the eluent water proportion, $r = 0.987$ to 0.998.

The k' data was extrapolated to an eluent composition of pure buffered water (water fraction = 1.0) for seven of the eight bicyclic aromatics (not *N*-methylquinolinium iodide). Figure 4 compares the extrapolated k' values on the three HA/FA columns with that on the blank column. The relative extrapolated k' values are Fjand HA 3.5, Aldrich HA 1.8, Blank 1.0, and Skagen FA 0.6. The comparison suggests a decrease in the sorption in the range Fjand HA > Aldrich HA > Blank ~ Skagen FA. The retention of the bicyclic aromatics was greater ($k'_{\mathrm{Skagen}}/k'_{\mathrm{Blank}} = 1.26$ to 1.48) on the Skagen FA column than on the blank column when the eluent water fraction was in the range 0.55 to 0.85, but lower when the proportion was 0.45 or 0.95 (see Fig. 3). The major difference between Skagen FA and Fjand HA and Aldrich HA is the size of the molecules. Thus, the results indicate that the molecule size of the humic substances is the dominant factor affecting the sorption. The higher sorption capacity of the Fjand HA column relative to the Aldrich HA column appears to be caused by its higher loading with the humic acid. As discussed in the section Direct Determination of K_{oc}, the determined K_{oc} values (which account for loading) of bicyclic aromatics are almost the same for the Fjand and the Aldrich HA columns. The Fjand HA and Aldrich HA column materials have very different C composition. Thus, our results did not corroborate the observations of Gauthier et al. (1987), that the type of the humic acid C is an important factor affecting the binding of PAC.

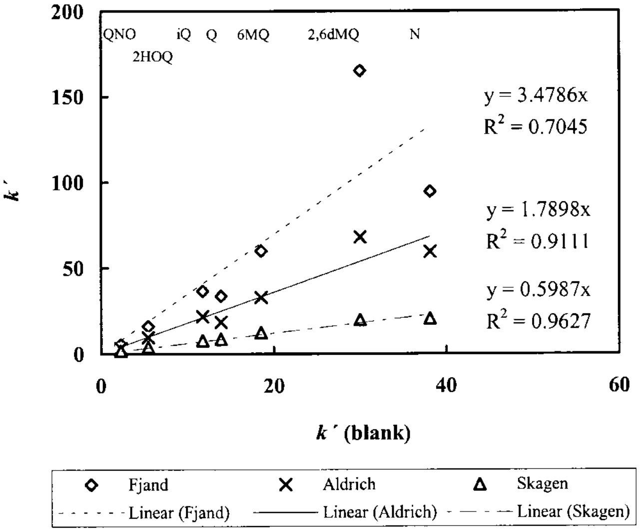

Fig. 4. Comparison of the capacity coefficient, k' (achieved by extrapolation), for bicyclic aromatics at buffered water (without methanol) of the three humic/fulvic acid bounded columns, Fjand HA, Aldrich HA (amino), and Skagen FA, with the corresponding k' values for the blank column. Legends: QNO, quinoline-*N*-oxide; 2HOQ, 2-hydroxyquinoline; iQ, isoquinoline; Q, quinoline; 6MQ, 6-methylquinoline; 2,6-dMQ, 2,6-dimethylquinoline; N, naphthalene.

Retention of Unsubstituted PAH and N-PAC on Different Columns

The capacity coefficients (k') of unsubstituted PAH and N-PAC increased with the molecular size of the PAH and N-PAC for all columns as exemplified by comparison of napthalene, anthracene, benz[a]anthracene, and benzo[a]pyrene (Table 1); likewise for quinoline, acridine, and 10-azabenzo[a]pyrene. The N-PAC has smaller k' than the corresponding PAH. For both types of compounds the increase with molecular weight was larger in the range Fjand HA > Aldrich HA > Blank ~ Skagen FA. Thus, for the Fjand HA column the capacity coefficients varies by a factor of 119 from the bicyclic naphthalene to the pentacyclic dibenz[a,c] anthracene. For the Aldrich HA column the increase was a factor of 74, for the Skagen FA column the factor was 57 and for the blank column without any humic/fulvic acid it was 65. For the corresponding N-PAC, isoquinoline and dibenz[a,c]acridine, the increase of k' on the four columns was by a factor of 178 for the Fjand HA column, 113 for the Aldrich HA column, 68 for the Skagen FA column, and 92 for the blank column. For all the five column materials, k' as well as log K_{ow} for the various dibenzacridines decreased in the order: dibenz[c,h]acridine > dibenz[a,c]acridine, ≈ dibenz[a,h] acridine > dibenz[a,j]acridine, reflecting the shielding of the N atom by the benzene rings. The shielding causes the behavior of the shielded N-PAC to be more like that of PAH (Helweg et al., 1997a; Nielsen et al., 1997). The relatively stronger sorption of polar compounds on Aldrich HA (carbonyl) column material is perhaps the

Table 3. Ranking of the effect of substituents on the sorption of anthracene derivatives on five different column materials and the octanol–water partition coefficient, K_{ow}.†

	Increase →											
Fjand HA	CHO	$CONH_2$	$COCH_3$	OCH_3	$COOCH_3$	H	*AQ*	NO_2	CH_3	CN	Cl	Br
Aldrich HA (amino)	CHO	$CONH_2$	$COCH_3$	*AQ*	OCH_3	$COOCH_3$	H	NO_2	CN	CH_3	Cl	Br
Aldrich HA (carbonyl)	CHO	$CONH_2$	$COCH_3$	OCH_3	$COOCH_3$	H	NO_2	CH_3	*AQ*	Cl	Br	CN
Skagen FA	$CONH_2$	*AQ*	CHO	$COCH_3$	OCH_3	$COOCH_3$	H	CN	NO_2	CH_3	Cl	Br
Blank	*AQ*	$CONH_2$	CHO	$COCH_3$	OCH_3	CN	$COOCH_3$	H	NO_2	CH_3	Cl	Br
log K_{ow}	$CONH_2$	*AQ*	$COCH_3$	NO_2	CHO	$COOCH_3$	CN	OCH_3	H	CH_3	Cl	Br

† *AQ* = anthraquinone.

reason that the order of sorption of the four dibenzacridines is the opposite on this column.

Retention of Substituted PAH Compounds on Different Columns

Generally the capacity coefficients, k', increased with the addition of lipophilic substituents and decreased with the addition of polar substituents; K_{ow} and k' increased for the substituted anthracenes on the five columns as seen in Table 3. Processes other than partitioning contribute to the sorption, as the order is not the same on the five different columns as in K_{ow}. The largest difference is the position of anthraquinone (AQ) in these rows. Anthraquinone elutes relatively faster in the order: Blank > K_{ow} ~ Skagen FA > Aldrich HA (amino) > Fjand HA > Aldrich HA (carbonyl). A similar pattern can be seen for cyano substituent, although the changes are smaller. 9-Cyanoanthracene is adsorbed stronger on Fjand HA and Aldrich HA than one should expect, if the sorption process solely was a partitioning phenomenon. Two factors are likely to contribute to a high k': (i) partitioning, and (ii) specific interactions with the humic acid. Examples of specific interactions are H bonding and charge transfer or dipole–dipole interactions. In the case of anthraquinone probably both types of specific interactions are active. In the case of 9-cyanoanthracene, only charge–transfer or dipole–dipole interactions seems likely. 9-Nitroanthracene shows also variations in its positions in the elution sequences. Its position in the blank column sequence compared with others indicates that H bonding is the dominant specific interaction. The specific interactions between some of the 9-substituted anthracenes and humic acid are plausible, considering that humic acid is assumed to consist of quinoid and phenolic structures.

Retention of Substituted N-PAC on Different Columns

The most significant variation of capacity coefficients on the three columns can be noticed in the case of polar substituted N-PAC. Figure 4 compares the extrapolated capacity coefficients for the three HA/FA columns with those of the blank column with buffered water (no methanol). The capacity coefficients increased in the order:

Fjand
quinoline-N-oxide < 2-hydroxyquinoline
< quinoline < *N*-methylquinolinium iodide
($k' = 44$)

Aldrich (amino)
quinoline-N-oxide < 2-hydroxyquinoline
< *N*-methylquinolinium iodide ($k' = 11$)
< quinoline

Skagen
N-methylquinolinium iodide ($k' = 2.1$)
< quinoline-N-oxide < 2-hydroxyquinoline
< quinoline

The sorption of *N*-methylquinolinium ion is very dependent of the type of humic substance, as it increases a factor of 20 from the Skagen FA column to the Fjand HA column (Fig. 3). The eluent composition has also some influence on its sorption, but the influence is much less than that on the neutral bicyclic aromatics (see Fig. 3). On the blank column no peak of *N*-methylquinolinium iodide was observed in the chromatograms. The reason for this is not known. The *N*-methylquinolinium ion is expected to bind to negatively charged groups on the column material. This may be carboxylate and phenolate ions or residual silanols having a pK_a of about 5 to 7 in the silica surface column material (Dorsey and Cooper, 1994). The residual silanols do not, however, appear to be important as k'_{Fjand} and k'_{Aldrich} for *N*-methylquinolinium ion is much higher than k'_{Skagen}. The number of residual silanols is probably almost the same in the two HA materials as in the Skagen FA material as all columns initially are treated the same way (see Materials and Methods).

Experiments with *N*-methylquinolinium iodide at varying pH showed a pH effect on the sorption of *N*-methylquinolinium iodide (see Fig. 5) with a linear dependency of pH in the range 5.5 to 7.5. The sorption of quinoline and acridine to the free Aldrich HA and Fjand HA at varying pH (see Fig. 6) also clearly illustrates the effect of a binding between a positively charged N-PAC and negatively charged carboxylate group in the humic substance as discussed previously in Nielsen et al. (1997). The sorption coefficients had a maximum at pH = 4 to 5 and K_{oc} decreased strongly when pH fell below 4. However, the retention of *N*-methylquinolinium ion on the three HA/FA columns may also be more complex than a simple ionic interaction. The sorption of the ion is stronger to the Aldrich HA than the Skagen FA column even though the Skagen FA column material has a higher content of carboxylic C.

The sorption order of 2-hydroxyquinoline, quinoline-*N*-oxide and quinoline was different on the two Aldrich columns, confirming that polar PAC are relatively

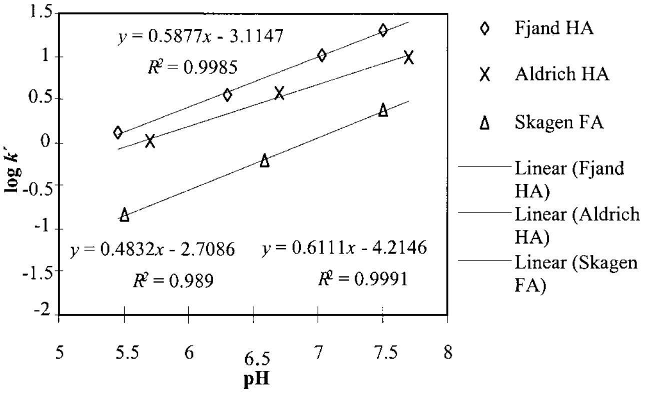

Fig. 5. The influence of pH on the retention of *N*-methylquinolinium iodide on Skagen FA (eluent: methanol fraction 0.25 + buffered water fraction 0.75), Aldrich HA (amino procedure) (eluent: methanol 0.35 + buffered water 0.65), Fjand HA columns (eluent: methanol 0.45 + buffered water 0.55). pH was determined in the eluent mixtures (see Roses et al., 1996).

stronger adsorbed to the carbonyl column than to the amino column. Thus 2-hydroxyquinoline and quinoline-*N*-oxide eluted later than quinoline on the carbonyl column.

Direct Determination of K_{oc}

The HPLC method can be applied to do direct determination of K_{oc} in case of a linear relationship between log k' and the eluent water content as found for the Fjand and Aldrich HA (amino) columns. The results for this part of the study are presented in Table 4. This appears to be an important aspect considering the variety of humic acid at different locations and the variety of organic pollutants. Although the bonded humic acid has been modified compared with the free one, the achieved K_{oc} values should be more useful than the K_{ow} values (Seth et al., 1999). Differences between K_{oc} values for the Fjand HA and the Aldrich HA were minor, indicating that sorption was influenced more by the size (molecular weight) of the HA molecules than by the content of aromatic C (Table 2). The major uncertainty in the K_{oc} determination is the assessment of the content of humic acid C on the two columns. However, in the worst case this should only affect the log K_{oc} values with an uncertainty of 0.1. For Fjand HA there is a good agreement with the K_{oc} HPLC value for quinoline and the estimated one for quinoline using the K_{oc} acridine data from the batch experiment (see Fig. 6). The direct log K_{oc} values for naphthalene and quinoline for the Aldrich HA column deviate with 0.2 to 0.6 from the literature batch values, but this is also a good agreement considering the different techniques. Work is in progress to test a large number PAC for direct K_{oc} determinations on Aldrich HA (carbonyl) (Jonassen et al., 1999). The present study was limited because the relation between log k' and the water eluent only was investigated for the bicyclic PAC. The method for the direct determination was not applied for the results on the Skagen FA column, as the k' values were almost the same as for the blank column.

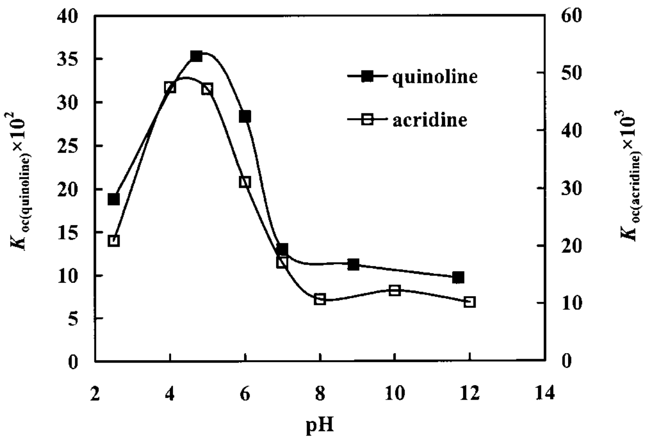

Fig. 6. The influence of pH on the binding of acridine to dissolved Fjand humic acid and quinoline to dissolved Aldrich HA from Nielsen et al., 1997.

Influence of Addition of Salt [$Ca(NO_3)_2$] to the Mobile Phase

The method using bonded HA is also useful and fast in the study of salt effects. The influence of salt addition on retention of bi- and tricyclic PAC to the Aldrich humic acid column was investigated, because inorganic ions may compete with organic compounds on sorption sites or change the structure of humic substances. Figure 7 depicts the salt effect on a few representative compounds, but the total data set includes all the bi- and

Table 4. The K_{oc} of bicyclic aromatics determined directly from k' on the bonded HA columns or indirectly and literature values (Nielsen et al., 1997).

Compound/HA	Fjand HA column log K_{oc} direct†	Fjand HA log K_{oc} (batch)	Aldrich Ha column (amino) log K_{oc} direct†	Aldrich HA column (amino) log K_{oc} (correlation)	Aldrich HA log K_{oc} literature values
Naphthalene	3.73		3.91	3.74§	3.32
Quinoline	3.27	3.28‡	3.24	2.89§	3.05
Isoquinoline	3.37		3.58	3.09§	
2-hydroxyquinoline	3.00		3.19	2.7¶	
Quinoline-*N*-oxide	2.47		2.56	2.0¶	
6-methylquinoline	3.59		3.74		
2,6-dimethylquinoline	4.11		4.16		

† log K_{oc} = log $\{[(k'_{HA}-k'_{Blank})*v_0]/[(cv-v_0)*0.01*(oc_{HA}-oc_{Blank})]\}$, where k' is the extrapolated value for a water fraction of 1.0 on either the HA column or the blank column, v_0 is the dead volume, *cv* the inner column volume and *oc* the content of organic C on either the HA column or the blank column.
‡ log K_{oc} (quinoline, Fjand) = log K_{oc} (acridine, Fjand) + log K_{oc} (quinoline, Aldrich) − log K_{oc} (acridine, Aldrich).
§ log K_{oc} = 1.5 * log k' + 4.16 (Nielsen et al., 1997).
¶ log K_{oc} = log K_{oc} (naphthalene(N)) + 1.5* (log $k'(X)_{0.95water}$ − log $k'(N)_{0.95water}$) (Nielsen et al., 1997).

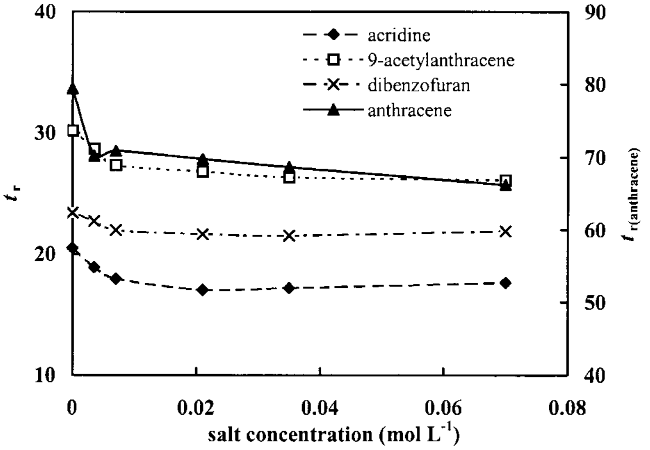

Fig. 7. The influence of salt [$Ca(NO_3)_2$] concentration (*M*) on the retention time, t_r (min) of selected tricyclic aromatic compounds on the Aldrich HA column (amino procedure).

tricyclic compounds mentioned in Table 1. Addition of 0.007 *M* $Ca(NO_3)_2$ to the mobile phase decreased the retention slightly, but if the salt concentration was increased further, little or no change on the retention was noticed. The salt effect seems to be strongest for substituted PAH. Increasing the salt concentration in the mobile phase from 0 to 0.007 *M* decreased the retention for substituted PAH (nine anthracene derivatives) 1.17 ± 0.13 times; for PAH (four compounds) 1.10 ± 0.05 times; for O, S-PAC (two compounds) 1.09 ± 0.04 times; for N-PAC (eight compounds) 1.07 ± 0.04 times; and for substituted N-PAC (three compounds) 1.00 ± 0.03 times. The results from a representative of the four former classes are depicted in Fig. 7. The negative salt effect on the sorption of quinoline to a surface-bound humic acid, of phenanthrene to Aldrich humic acid, and of anthracene to Suwannee River humic and fulvic acid was observed by others (Chorover et al., 1999; Lassen and Carlsen, 1997; Schlautman and Morgan, 1993). The possible causes of electrolyte effects on t_r include conformation of the bonded humic acid, e.g., increased coiling with increasing Ca^{2+} concentration (Tombacz and Meleg, 1990) and decreasing PAC solubility with increasing electrolyte concentration (Schwarzenbach et al., 1993). If the only mechanism was a salting out effect, it is estimated that t_r for anthracene should have increased 1.01 times at the 0.007 *M* Ca^{2+} concentration and 1.05 times at the concentration on 0.07 *M*. The estimate is based on data from Xie et al. (1997). It is also interesting that the effects in general almost counterbalanced each other at salt concentrations above 0.007 *M*.

CONCLUSION

A technique involving bonding of humic substances to HPLC columns allowed rapid determination of relative binding affinity of a wide range of PAC to HA. The K_{oc} values determined for selected compounds on humic acid by this method were comparable to values determined on the free (nonbonded) humic acid and to values reported in the literatue. In addition, similar results were achieved for columns when Aldrich HA was bonded by two different methods. Thus, the possible modification of the humic substances during the bonding was in general not a problem, although it is not negligible. Column studies on two HAs and a FA showed that sorption of PAC was determined by the size (molecular wt.) of the humic molecules. Data indicated that the predominant mechanism for PAC bonding to HA and FA was partitioning, but with contributions of other mechanisms such as H bonding, ionic bonding (for ionic PAC), dipole–dipole interactions, or steric effects. Sorption of PAC to humic substances increased with size (molecular wt.) of the PAC. Lipophilic substituents at the PAC molecule caused sorption to increase while substitution of polar substituents caused sorption to decrease. However, the sorption of PAC with polar substituents is stronger than indicated by their K_{ow} values. This is especially distinct for nitro and cyano-substituents or the presence of a quinone structure. Sorption of PAC was also affected by aqueous $Ca(NO_3)_2$ concentration in the eluent, particularly at Ca^{2+} between 0 and 0.007 *M*.

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THE APPLICATION OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY HUMIC ACID COLUMNS IN DETERMINATION OF K_{oc} OF POLYCYCLIC AROMATIC COMPOUNDS

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Abstract—An improvement of a method for determination of the distribution coefficient for polycyclic aromatic compounds (PAC) between organic carbon and water (K_{oc}) by high-performance liquid chromatography (HPLC) is presented in this paper. By use of silica-based HPLC columns with chemically immobilized humic acid (Aldrich, Zigma-Aldrich, Taufkirchen, Germany), the retention of PAC to humic acid can be measured, and the results can, when they are compared to those from a blank column, be used to a direct determination of K_{oc}. These values agree quite well with literature K_{oc} values for two- to four-ringed PAC. Our work has shown that it is possible to use this method to investigate how environmental parameters like electrolyte concentration, pH, and temperature affect the sorption of PAC to humic acid. Thermodynamic parameters can be estimated from variable temperature experiments. The advantage of the method is its cost and manpower effectiveness.

Keywords—Distribution coefficients Polycyclic aromatic compounds Humic acid High-performance liquid chromatography

INTRODUCTION

Polycyclic aromatic compounds (PAC) are ubiquitous environmental pollutants. About 10% of the total PAC pollution is N-PAC (nitrogen-containing polycyclic aromatic compounds). These are more water soluble than the most abundant group of PAC, polycyclic aromatic hydrocarbons (PAH) [1].

Knowing the physicochemical properties of pollution for bioremediation and risk assessment is important. The distribution of the contaminants between the solid phase and the pore water in the soil matrix is, for example, a key parameter in predicting to what extent the compound is bioavailable, that is, dissolved in the pore water [2]. The partitioning coefficient between organic carbon and water (K_{oc}) is the key factor describing the distribution since organic carbon, such as humic acid, is the main sorbent for nonionic compounds, such as PAC.

Normally, K_{oc} values are estimated in batch experiments, where K_{oc} is calculated from the changes in aqueous concentration of the compound during the experiments, including both experimental and analytical work. The method presented in this work enables K_{oc} to be calculated directly from the retention of the compounds on a HPLC humic acid column. It is generally accepted to estimate relative K_{oc} values by HPLC. However, instead of using a matrix of silica and cyanopropyl [3], it is much more realistic to use silica HPLC columns with chemically immobilized humic acid (HA) as the sorbent.

This work presents some improvements of earlier work [4], mentioned as the carbonyl procedure in [5]. The most important improvement is the higher content of humic acid in the HPLC column together with a lowering of carrier carbon. Besides that, direct measurements of organic carbon and sulfur in the column material allow us to calculate the amount of organic carbon from the humic acid using sulfur as internal standard.

The influence of environmental parameters, such as electrolyte concentration, pH, and temperature, on the sorption of PAC to humic acid is investigated in addition to the K_{oc} determination.

MATERIALS AND METHODS

Column material

The humic acid HPLC column material was prepared in a number of stages modified from [4] and described as the carbonyl procedure in [5]. A suspension of the silica gel (Nucleoprep-Si-300-20, surface area [Brunauer-Emmett-Teller] 100 m^2/g, Macherey-Nagel Düren, Germany) was stirred under nitrogen with 3-aminopropyl triethoxysilane (pro analysis, Merck-Schuchardt, Hohenbrunn, Germany) dissolved in toluene (pro analysis, Merck, Darmstadt, Germany). The product was filtrated on glass filter (pore size 10–16 μm), washed, and dried, and the purified aminopropyl silica gel was added to a solution of humic acid in water and stirred at room temperature under nitrogen for 7 h. Finally, the free amino groups from nonreacting 3-aminopropyl triethoxysilane were deactivated with 0.1M water solution of formaldehyde (pro analysis, Merck), and the column material was washed with water before drying. The HPLC columns were packed in methanol at 35 MPa in a 12.5-cm × 4.6-mm (i.d.) column using a pneumatic HPLC pump from Knauer (Berlin, Germany). A blank column was made in a similar way but without the humic acid step.

The content of organic carbon and sulfur in the column materials was determined with a Leco CS 200 (St. Joseph, MI, USA) by combustion in pure O_2 and subsequent infrared analysis of the amount of CO, CO_2, and SO_2 (relative standard

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derivation of 0.5% for carbon and 1.5% for sulfur). Control of this method has been made by measurements of residual humic acid from the synthesis of column materials by means of spectroscopy. Ultraviolet/visible-spectroscopic measurements have been made at 360 nm with a Phillips PU 8800 Ultraviolet/Visible spectrophotometer (Philips, Eindhoven, The Netherlands) to measure the residual humic acid in an aqueous solution with known volume. Standard aqueous solutions between 0.5 and 5mg/L of the humic acid were used to obtain a linear regression line.

Chromatographic work

Polycyclic aromatic compounds were dissolved in methanol as suitable mixtures of three or four PAC in concentrations at approximately 10 mg/L and chromatographed through the humic acid column on a high-pressure-gradient Shimadzu LC-10 HPLC (Shimadzu, Kyoto, Japan) at 25°C. Because of short retention time on the blank column, the PAC was injected separately on this column to avoid problems with poor separation of the compounds.

Mobile-phase methanol/water mixtures were used with different methanol volume fractions (0.35–0.85 [v/v] for the humic acid column and 0.05 to 0.50 [v/v] for the blank column). Mobile phases were buffered to pH 7.0 with KH_2PO_4/NaOH, having a total phosphate concentration of 35 mM. For mobile phases with 0.75 and 0.85 [v/v] fractions of methanol, the phosphate concentration was 20 mM, to avoid salt precipitation. Tests have shown that this has negligible effects on the sorption as long as the total phosphate concentration is >10 mM.

Retention times (t_r) for each compound were measured at least twice, and the dead volumes (t_0), determined from the retention time of water, was measured at least 10 times for each mobile phase.

Electrolyte concentration

For some selected nonsubstituted two- and three-ring PAH and N-PAC, the total phosphate concentration (HPO_4^{2-} and $H_2PO_4^-$) was varied in a mobile phase having a methanol fraction content at 0.35 (v/v). At pH 7.9, the total phosphate concentration was between 1 and 35 mM and at pH 5.9 from no electrolyte to 35 mM.

Variation of pH

The pH of the mobile phase, containing a methanol fraction of 0.35 (v/v) and a phosphate concentration at 35 mM (H_3PO_4 and $H_2PO_4^-$), was changed from 7.9 to 2.6. The retention time was again measured twice for quinoline, isoquinoline, naphthalene, fluorene, anthracene, and phenanthrene.

Temperature variation

Effects of temperature on the sorption of two- to four-ring PAH and N-PAC, together with dibenzofuran and dibenzothiophen, have been investigated at column temperatures between 17 and 30°C. The mobile phases containing a methanol fraction of 0.35 (v/v) were buffered to pH 7.0 by total phosphate concentration of 35 mM and NaOH. All retention times were measured at least twice.

RESULTS AND DISCUSSION

Humic acid content on the columns

Total organic carbon measurements of the humic acid column materials give a carbon content by mass of 2.4% (w/w) and 0.12% (w/w) sulfur. Similar measurements on Aldrich humic acid showed 42.5% (w/w) and 2.71% (w/w) of carbon and sulfur, respectively. Measurements of blank column material show an organic carbon content of 0.54% (w/w) and no sulfur. By use of sulfur as internal standard, the total organic carbon from humic acid on the humic acid column material is calculated to 1.95% (w/w); the rest (0.51% [w/w]) is carrier carbon.

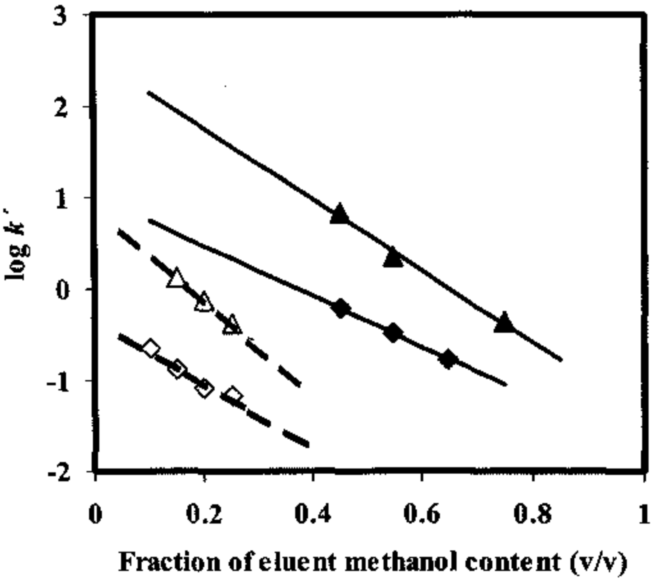

Fig. 1. Examples of estimating log *k'* for two polycyclic aromatic hydrocarbons in buffered water. Temperature: 25°C; pH: 7.0; total phosphate concentration: 35 mM. ▲ anthracene and ◆ naphthalene. Filled symbols represent the humic acid high-performance liquid chromatography column and open symbols the blank column. Regression lines show the linear relationship between log *k'* and the methanol content in the mobile phase.

Calculations made on the ultraviolet measurements of aqueous solutions of residual humic acid from synthesis of column material show that the use of sulfur as internal standard gives reliable data for the humic acid carbon content in the column material.

The advantage of the carbonyl method is a much higher column content of humic acid carbon and lower content of carrier carbon compared with earlier work [4,5]. The material presented as the amino procedure in Kollist-Siigur et al. [5] had a total organic carbon content of 13.2% (w/w), of which only 0.7% (w/w) was estimated to originate from humic acid. On the column material used in this work, humic acid contributes, with 1.95% (w/w) organic carbon of a total organic carbon content of 2.4% (w/w). This improvement of the method not only gives a higher content of humic acid on the column material but also provides a humic acid organic carbon–carrier carbon ratio of almost 4, whereas the earlier method had a ratio of 0.06, hereby decreasing the errors on the method caused by sorption to carrier carbon.

Direct determination of K_{oc}

The capacity coefficient ($k' = (t_r - t_0)/t_0$) is calculated, and log *k'* in buffered water is extrapolated by a linear fit of the calculated values of log *k'* in the different mixtures of methanol and water used as mobile phases (see examples for selected PAH in Fig. 1). For the three bicyclic compounds, this linear relationship is afterward controlled in mobile phases with a methanol content down to 0.05 (v/v). The logarithms to the

Table 1. Directly determined partitioning coefficients, log K_{oc} between organic carbon and water for eight nitrogen-containing polycyclic aromatic compounds (N-PAC), six polycyclic aromatic hydrocarbons (PAH), and two polycyclic aromatic compounds containing either oxygen (O-PAC) or sulfur (S-PAC) using Aldrich humic acid as organic material

Compound	Direct determination[a]	Indirect determinations[b]	Batch experiments		
N-PAC					
Quinoline	2.5 ± 0.11	2.89	3.05[b]		
Isoquinoline	2.6 ± 0.13	3.09			
4-Azaflourene	3.3 ± 0.16	3.63			
Acridine	4.1 ± 0.12	4.00			
5,6-Benzoquinoline	4.1 ± 0.15	4.07			
7,8-Benzoquinoline	3.8 ± 0.16	4.13			
Phenantridine	3.8 ± 0.18	4.06			
Carbazole	3.6 ± 0.17	4.74			
PAH					
Naphthalene	2.7 ± 0.17	3.74	3.32[b]		
Flourene	3.9 ± 0.12	4.68			
Anthracene	4.3 ± 0.24	4.65	4.87 ± 0.26[b]	4.64[c]	5.0[d]
Phenanthrene	4.2 ± 0.19	4.65	4.81 ± 0.16[b]	4.33[c]	
Pyrene	5.0 ± 0.18		5.20[b]	5.05[c]	5.4[d]
1,2-Benzanthracene	5.1 ± 0.62	5.62	5.48[b]		
O,S-PAC					
Dibenzofuran	3.9 ± 0.11	4.15			
Dibenzothiophene	4.15 ± 0.09	4.59			

[a] Log K_{oc} is calculated directly on an estimated value of k' in pure water (log K_{oc} = log{[(k'_{HA} − k'_{blank})·V_0]/(V_s·ρ·0.01·%OC_{HA})}. Measurements were made at 25°C, with KH_2PO_4 as electrolyte at a total concentration of 0.035M in the mobile phase. pH was adjusted to 7.0 by use of NaOH. The methanol content was between 0.05 and 0.85 (v/v).

[b] Data from Nielsen et al. [4]. "Indirect determination" means an empirical correlation between log K_{oc} and log k'.

[c] Data from Laor et al. [6] using Aldrich humic acid.

[d] Data from Perminova et al. [7] using Aldrich humic acid.

partitioning coefficients (log K_{oc}) are then calculated by Equation 1:

$$\log K_{oc} = \log\left[\frac{(k'_{HA} - k'_{blank})V_0}{(V_S)\cdot\rho\cdot 0.01\cdot\%OC_{HA}}\right] \quad (1)$$

where k'_{HA} is the estimated capacity coefficient on the humic acid column and k'_{blank} is the capacity coefficient on a blank column. The dead volume of the column (V_0) is calculated as t_0 times the flow velocity of the mobile phase, V_S is the volume of the solid phase (total volume minus V_0) of the column, ρ is the density of the column material, and %OC_{HA} is the content of organic carbon from humic acid in the column material (w/w).

The absolute standard deviation on values of log K_{oc} is calculated from standard deviations on the intercept with the ordinate of the linear fits made when estimating log k' in buffer without methanol.

Examples of directly determined values of log K_{oc} are shown in Table 1 together with data from [4,6,7], also using Aldrich humic acid, confirming the validity of the method. Good agreement exists between the values in this work and those of others, even though a tendency was observed toward lower sorption, especially for the bicyclic compounds. This minor difference perhaps reflects that batch experiments have time enough to achieve sorption at sites requiring a long process time in contrast to our column method. One should be aware that PAH has a generally higher degree of sorption to Aldrich humic acid compared with other soil humic and fulvic acids as well as aquatic and peat humic substances [7].

Polycyclic aromatic hydrocarbons have slightly higher distribution coefficients than the N-PAC having the same ring structure. This might be due to the fact that N-PAC consists of polar molecules having an electron lone pair, which causes higher water solubility than PAH. The hydrophobic effect [8] is therefore larger for nonpolar compounds such as PAH than for N-PAC.

Environmental parameters

The influence of electrolyte concentration and pH on log k' are calculated and presented in Figures 2 and 3. Each point is the mean value of at least two measurements; standard errors on each point are marked as error bars. The temperature variation (Fig. 4) is shown as ln k' versus the inverse absolute temperature.

Figure 2 shows that sorption of PAH (Fig. 2A) is almost unaffected by the ionic strength in the mobile phase (pH 5.9) when lowering the total phosphate concentration from 35 to 10 mM, while the N-PAC (especially quinoline and isoquinoline) shows an increase in sorption (Fig. 2B). The highest sorption for all the investigated PAC is seen about 1 mM phosphate. In the nonbuffered mobile phase, the sorption decreases to the lowest point for all compounds. The same trend was seen for phenanthridine, 5,6-benzoquinoline, 7,8-benzoquinoline, and phenanthrene.

This variation in sorption could be the result of three factors. First, the solubility of organic compounds such as PAC decreases as a function of increasing salt concentration as a result of the salting-out effect [9,10]. Second, the cation concentration affects the structure of humic acid, as humic acid tends to curl as the concentration of (hydrated) cations is raised, thereby increasing the number of possible sorption sites as voids are formed as the humic acid curls up. However,

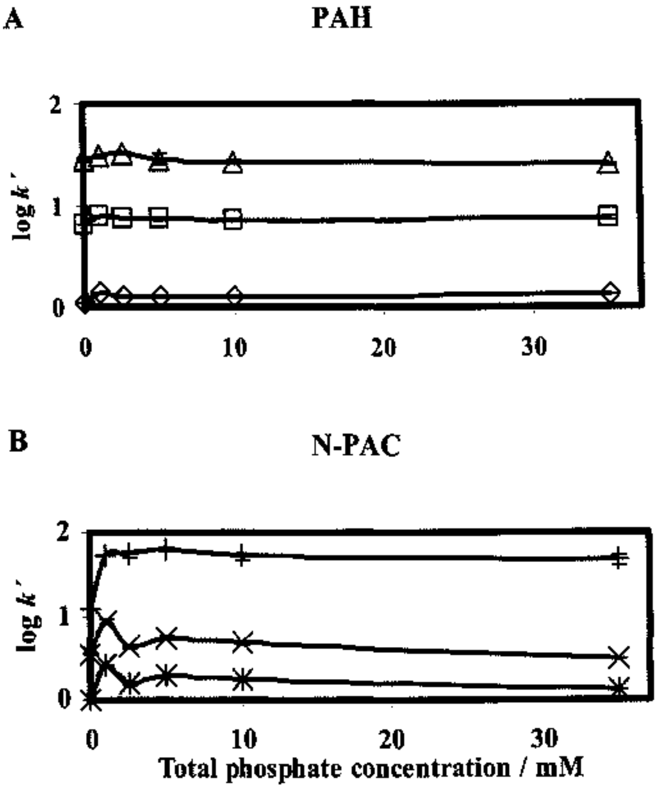

Fig. 2. Variation of the sorption (measured as log *k*′) of six polycyclic aromatic compound (PAC) with total phosphate concentration. Methanol fraction in the mobile phase: 0.35 (v/v); pH: 5.9; temperature: 25°C. (A) Polycyclic aromatic hydrocarbon (PAH) (△ anthracene, □ fluorene, and ◇ naphthalene). (B) N-PAC (+ acridine, × isoquinoline, and * quinoline). Error bars indicate standard error on mean values of log *k*′.

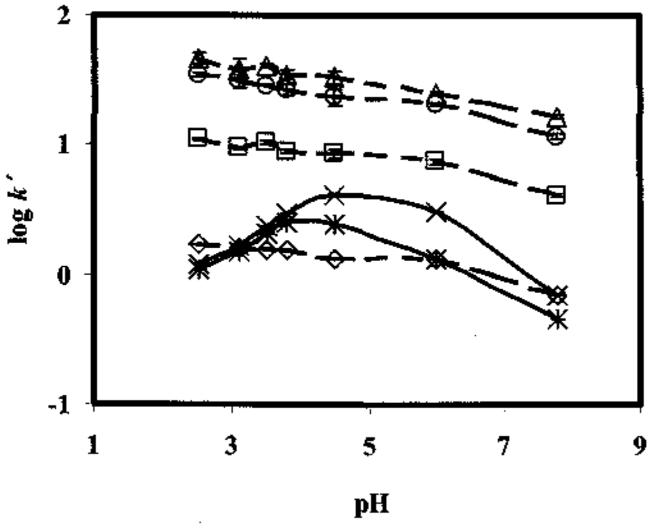

Fig. 3. Variation of the sorption (measured as log *k*′) to a humic acid high-performance liquid chromatography column of four polycyclic aromatic hydrocarbon and two N-polycyclic aromatic compound with pH. Methanol fraction in the mobile phase: 0.35 (v/v). Total phosphate concentration: 35 mM; temperature 25°C. △ anthracene, ○ phenanthrene, □ fluorene, ◇ naphthalene, × isoquinoline, and * quinoline. Error bars indicate standard error on mean values of log *k*′.

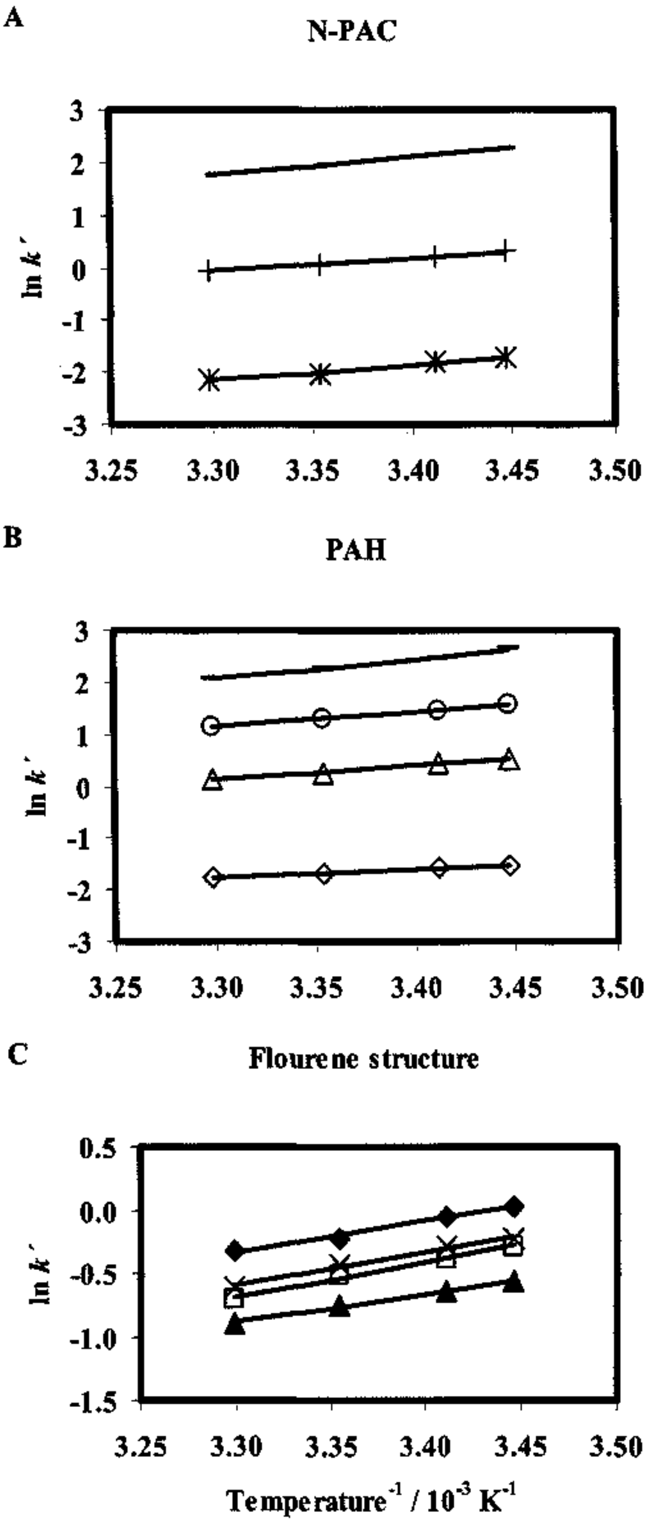

Fig. 4. Temperature variations of polycyclic aromatic compound (PAC) sorption (ln *k*′) to humic acid high-performance liquid chromatography column (note different scales on the ordinates). Methanol fraction in the mobile phase: 0.35 (v/v); total phosphate concentration: 35 mM and pH 7.0. (A) N-PAC (——— 1,2-benzacridine, + acridine, and * quinoline); (B) polycyclic aromatic hydrocarbon (PAH) (——— 1,2-benzanthracene, ○ pyrene, △ anthracene, and ◇ naphthalene); (C) fluorene structures (◆ dibenzothiophene, × carbazol, □ fluorene, and ▲ dibenzofuran).

cations can at the same time act as competitors to PAC, occupying the same sites in humic acid. Third, an increase in ionic strength will cause a decrease of pK_a values for humic acid [11], resulting in a lower sorption of PAC. The effect of a decreasing pK_a will be the same as that of an increasing pH (see the following discussion).

It is obvious that these three factors cause different effects on the degree of sorption and that one should not expect a clear pattern. The higher variation in retention between N-PAC and PAH at the lowest electrolyte concentrations can be explained by the polarity of the N-PAC molecules compared to PAH.

A possible explanation for the striking decreases, especially for N-PAC, in sorption when using a mobile phase without electrolyte might be that the humic acid is uncurled, leaving no voids for sorption. The same experiments carried out at pH 7.9 showed the same pattern (not shown in this paper), but generally the sorption was lower than at pH 5.9, in agreement with the results presented here.

In Figure 3, one can see that the sorption of quinoline and isoquinoline is highest between pH 4 and 6, whereas the sorption of PAH continues to increase when pH decreases. A decrease in pH causes a change of the overall charge of humic acid toward neutral; PAH will therefore be more strongly attached to humic acid, as this phase gets more energetically favorable for neutral, nonpolar compounds compared to the negatively charged humic acid.

The same consideration cannot be used totally on N-PAC, as they are polar molecules with an electron lone pair on the nitrogen atom. Because of this, they are also bases with pK_a values of approximately 5 (quinoline 4.90 and isoquinoline 5.42 at 20°C [12]). The stronger sorption of the two quinolines compared to naphthalene in the pH region from 3.2 to 6 is therefore probably a result of ionic binding between positively charged quinoline molecules and negatively charged carboxylic groups in humic acid [4].

The sorption of PAC (Fig. 4) increases as the inverse temperature changes from 3.3 to $3.45 \times 10^{-3} K^{-1}$. Isoquinoline, 4-azafluorene, 5,6- and 7,8-benzoquinoline, phenanthridine, and phenanthrene exhibit a similar pattern. This is not surprising, as the sorption process is a thermodynamic process of which the equilibrium constant, according to the van't Hoff equation, is inversely related to the temperature [13]. From the regression lines in Figure 4, it is possible to estimate differences in ΔS (from the intercept) and ΔH (from the slope) for various compounds. For the fluorenes (Fig. 4C), the ΔH seems similar, whereas the differences are in ΔS. For the PAH (Fig. 4B), 1,2-benzanthracene is the one that is different. For the N-PAC (Fig. 4A), ΔH is similar within this group, with ΔS causing the difference. Very few and scattered results are available for the enthalpy and entropy values for the sorption of PAC to humic acid. More measurements are needed in order to be able to determine the correct thermodynamic values [14].

CONCLUSION

The results show that this method is suitable for determination of log K_{oc} for unsubstituted PAC with up to four aromatic rings. Experiments have also demonstrated that the method can be used to investigate effects of environmental parameters on the degree of PAC sorption to soil organic material. Preliminary measurements indicate that the method also can be applied to determine ΔS and ΔH. The advantage of the method is that it is much less time and cost consuming compared to batch experiments.

When comparing the different PAC, it was observed that the sorption was increasing with the size of the molecule and decreasing for basic N-PAC compared to PAH at pH 7. Variation of pH showed an increase in sorption with decreasing pH for PAH and an optimum for sorption of N-PAC between pH 4.5 and 6. The sorption decreases with increasing temperature from 17 to 30°C, and finally a more distinct variation of sorption was observed as a result of changes in ionic strength for N-PAC than PAH, which might due to changes in pK_a values for the carboxylic acid groups in the humic acid.

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