especially suitable for nonvolatile samples. A better approach to qualitative and quantitative analysis of volatile samples is through methods based on an on-column interface. However, the retention gap method uses long uncoated pre-columns and only allows modest volumes of HPLC fractions to be transferred. The partially concurrent evaporation method, where only a part of the HPLC eluent is evaporated, works with larger fraction volumes (approx. 200 μL) and with shorter uncoated precolumns.

Future Trends

The development of new, more accurate techniques based on liquid chromatography (especially HPLC, TLC, and new multidimensional or hyphenated techniques) will be increasingly important owing to legislation calling for the reduction of aromatic content in fuels. Therefore, these techniques will continue to play a crucial role in the petroleum industry for the choice of process conditions and the evaluation of fuel quality.

See also: **II/Chromatography:** Liquid Chromatography-Gas Chromatography. **Chromatography: Gas:** High Temperature Gas Chromatography; High-Speed Gas Chromatography. **Chromatography: Liquid:** Detectors: Ultraviolet and Visible Detection; Large-Scale Liquid Chromatography; Mechanisms: Normal Phases; Mechanisms: Size Exclusion Chromatography; Multidimensional Chromatography. **III/Bitumens: Liquid Chromatography. Crude Oil: Liquid Chromatography. Flame Ionization Detection: Thin-Layer (Planar) Chromatography. Flash Chromatography. Geochemical Analysis: Gas Chromatography. Liquid Chromatography-Gas Chromatography. Medium-Pressure Liquid Chromatography. Petroleum Products:** Gas Chromatography.

Further Reading

Adlard ER (ed.) (1995) *Chromatography in the Petroleum Industry*. Amsterdam: Elsevier.

Annual Book of ASTM Standards, vols 05.01 and 05.02. *Petroleum Products, Lubricants and Fossil Fuels*. ASTM, Philadelphia PA 19103-1187, USA (issued annually).

Cagniant D (ed.) (1992) *Complexation Chromatography*. New York: Marcel Dekker.

Cortés HJ (ed.) (1990) *Multidimensional Chromatography*. New York: Marcel Dekker.

Drews AW (ed.) (1989) *Manual on Hydrocarbon Analysis*, 4th edn. Philadelphia: American Society for Testing of Materials.

Herod AA (1994) A review of the uses of planar chromatography in the coal and oil industries. *Journal of Planar Chromatography* 7: 180–196.

Kelly GW and Bartle KD (1994) The use of combined LC–GC for the analysis of fuel products: a review. *Journal of High Resolution Chromatography* 17: 390–397.

Pauls RE (1995) Chromatographic characterization of gasolines. *Advances in Chromatography* 35: 259.

Ranny M (1987) *Thin-layer Chromatography with Flame Ionization Detection*. Dordrecht: D. Reidel.

Standard Methods for Analysis and Testing of Petroleum and Related Products 1992, vol. 2. Institute of Petroleum, London, 1992.

Yau WW, Kirkland JJ and Bly DD (1979) *Modern Size-Exclusion Liquid Chromatography. Practice of Gel Permeation and Gel Filtration Chromatography*. New York: John Wiley.

Thin-Layer (Planar) Chromatography

A. A. Herod and M.-J. Lazaro,
Imperial College, London, UK

Introduction

Thin-layer chromatography (TLC) has strengths not applicable to other chromatographic methods. These can be summarized as cheapness of materials, low volume requirement for solvents, the ability to use any mixture of solvents, and, most important, the intractable materials of a complex sample are retained within the surface area of the chromatographic plate and may be recovered. In addition, chromatographic failures can be disposed of without damaging the budget. Such considerations do not apply to gas chromatography or liquid chromatography, where involatiles (in GC) or insolubles (in LC) are lost on the column or inlet system with the possibility of permanent damage to the column performance. The difference in cost of thin-layer plates and chromatographic columns ranges from a factor of 100 for GC capillary columns, up to a factor of 1000 for preparative HPLC columns. Whereas TLC has most often

been used to examine individual compounds, it has found use (as TLC with flame ionization detection using silica rods) in the examination of crude oils and more recently as a preparative method to separate the high molecular mass fractions of coal and biomass liquids and petroleum vacuum residues for examination free from the smaller, more volatile components.

Uses of Thin-Layer Chromatography

Thin-layer chromatography has been applied to tars and oils in three ways: (1) to identify individual compounds or groups of compounds such as polycyclic aromatic hydrocarbons (PAHs); (2) to measure types of compounds such as oils, maltenes, asphaltenes and preasphaltenes in crudes by the TLC-FID method; and (3) as a fractionation method for examination by other techniques such as NMR or laser ionization mass spectrometry. These uses are considered in more detail below.

Identification of Individual Compounds or Groups of Compounds

Analytical TLC of coal tar has been achieved using silica gel plates and development by a manual method using the series of solvents – tetrahydrofuran, chloroform/methanol (4 : 1 v/v), toluene and pentane. The separated components have been recovered and examined by probe mass spectrometry directly with no prior extraction from the silica. A typical analytical separation is shown in **Figure 1**. The standard compounds on the plate included pyrogallol, perylene and rubrene, with a coal extract produced in a bomb with tetralin solvent. Mass spectra of recovered fractions are shown below.

Measurement of Compound Types

The separation and quantification of compound types – oils, maltenes, asphaltenes and preasphaltenes (terms derived from solvent solubility fractionation)

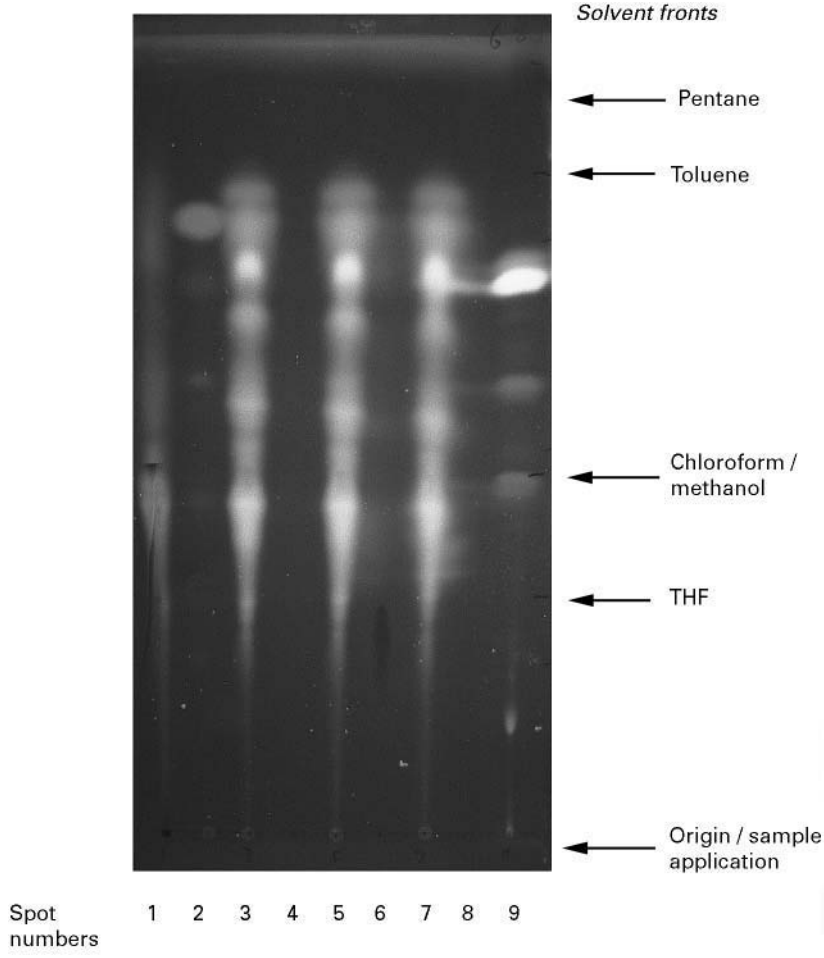


Figure 1 (See Colour Plate 109) Analytical development of coal tar pitch, tetrahydrofuran solubles, on silica developed in tetrahydrofuran, chloroform/methanol (4 : 1 v/v), toluene and pentane. Pitch at lanes 3, 5 and 7; perylene at lane 2; rubrene at lane 9; pyrogallol at lane 6; coal extract at lane 1. Whatman K6 silica; solvent fronts THF 55 mm, chloroform/methanol 83 mm, toluene 153 mm and pentane 178 mm.

– in oils and coal derived liquids may be achieved using the combination of TLC on rods with flame ionization detection (FID). The normal equipment used for this is the Iatroscan Chromatograph with Chromarods to effect the separation. Rods are cleaned and prepared by passage through a hydrogen/air flame, sample is deposited near one end of the rod and development proceeds as for a plate, using an appropriate solvent sequence. The developed and dried rod is passed through the flame detector to evaluate the separated fractions; calibration of the response factors of the different types of fraction is essential to give quantitative information. The sample is destroyed by the detection method but, as only small quantities of sample are used, several developments can be made at the same time to allow repeatability checks. **Figure 2** shows details of different development sequences to obtain adequate separation of a coal tar pitch. The initial sequence was not satisfactory owing to the aromatic character of the pitch. The final sequence indicated that the hexane development had no effect on the separation of the pitch, since hexane would separate aliphatics, which are absent from the pitch.

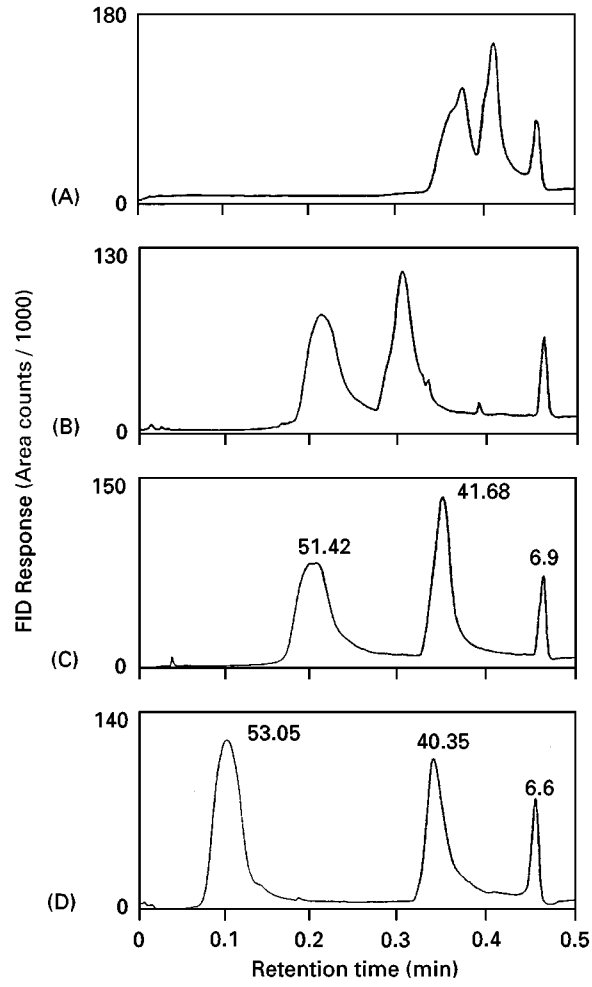


Figure 2 TLC-FID chromatograms corresponding to the following development sequences: (A) 38 min *n*-hexane, 3 min toluene, 30 s dichloromethane (DCM)-MeOH; (B) 38 min *n*-hexane, 20 min toluene, 5 min DCM-MeOH; (C) 45 min *n*-hexane, 20 min toluene, 3 min DCM-MeOH; (D) 35 min toluene, 3 min DCM-MeOH. Numbers correspond to area percentages. (TLC-FID reproduced with permission from Cebolla VL, Vela J, Membrado L and Ferrando AC (1996) *Chromatographia* 42(5/6) March 1996, © Friedr. Vieweg & Sohn Verlagsgesellschaft mbH.)

Separation of Fractions for Examination by Other Techniques

In this mode, TLC has opened a route to the isolation of fractions of coal-derived liquids and tars from biomass as well as petroleum vacuum residues. The essence of the method is the application of sample either in solution or as a suspension or slurry in a volatile solvent. Pyridine has been used for slurrying or dissolving all of these sample types; 1-methyl 2-pyrrolidone (NMP) is capable of dissolving coal and biomass tars, but is involatile (boiling point 202°C) and cannot be removed easily from the plate. After addition to the longer edge of a plate as spots or as a band along the bottom of the plate, usually 10 cm × 20 cm coated with silica gel, the pyridine is allowed to evaporate. **Figure 3** shows a typical preparative development of a synthetic naphthalene mesophase pitch using tetrahydrofuran and toluene, with application of sample in a pyridine slurry. After separation the colours of the bands were black (immobile), brown (mobile in tetrahydrofuran but immobile in toluene) and orange (mobile in both solvents).

Development solvents used include pyridine followed by acetonitrile, pyridine followed by *N*,*N*-dimethylformamide and tetrahydrofuran followed by toluene. In each case, the first solvent used is the more polar of the two and development is not more than half way up the plate, less than 5 cm. After drying, the plate is developed in the second solvent to a distance approximately twice that of the first, but less than 10 cm. In some cases, the development has been achieved using a manual multiple development technique in which the plate is removed from the tank after the solvent front has passed the sample application zone (or the solvent front of the previous solvent), dried and reinserted into the same solvent to continue the development. By this method, the possibility of the mobile material being partially retained by the immobile fraction is reduced.

To avoid contamination of the tar fractions, the plates are usually washed before use with the first

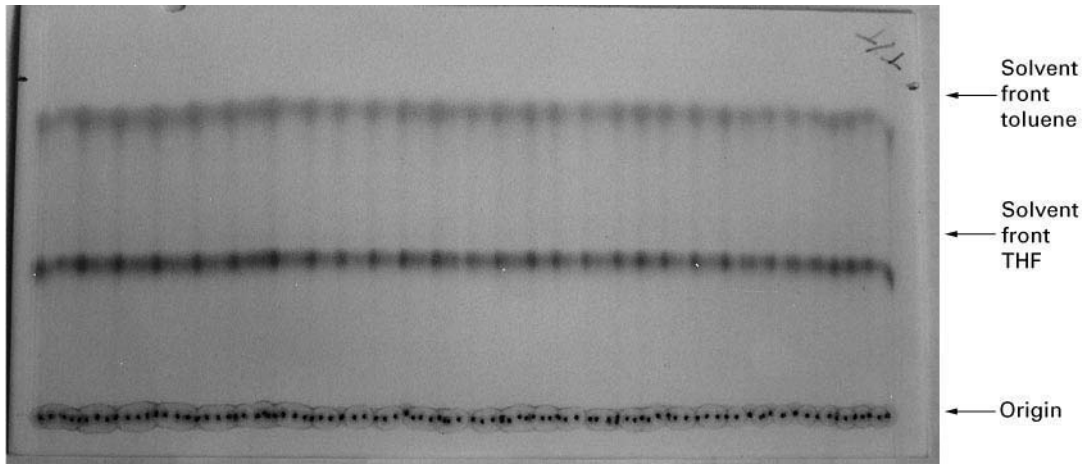


Figure 3 (See Colour Plate 110) Preparative development of a synthetic naphthalene mesophase pitch applied in pyridine slurry and developed in tetrahydrofuran and toluene.

solvent to be used in the development, either pyridine or tetrahydrofuran. This is achieved by placing the fresh plate into a development tank containing the solvent and allowing the solvent front to rise almost to the top of the plate. In the subsequent preparative or analytical development, the final solvent front is not allowed to reach the washing-solvent front and, in consequence, the height of the plate used for the separation is probably not more than two-thirds of the available plate height, with allowance for the sample application zone being above the initial level of the solvent when first placed into the development tank.

As indicated earlier, after development three zones are normally visible (black, dark brown and orange or yellow. These zones are recovered by scraping the coated silica into a glass vial and extracting with NMP at room temperature, with ultrasonic agitation if necessary. The extract may be recovered either by decanting the solvent from the silica, using a syringe, or by adding the slurry to a glass syringe equipped with a filter tip (0.6 μm). In this case, the physical pressure necessary to force the solvent through the filter may cause the filter to be blown off the syringe, with loss of sample. The complete removal of black, low mobility material from the silica is difficult to achieve and the residual silica may be dark in appearance; however, the recovered material is unlike the other fractions in molecular mass and spectroscopic behaviour (see below). The solutions derived by solvent extraction may be concentrated by vacuum evaporation or used as recovered. NMP is a difficult solvent to remove completely, but water washing of the almost dried fraction may achieve removal since NMP is very soluble in water.

Instrumentation

For TLC on plates as described here, the equipment needed is minimal: simple development tanks lined with absorbent paper to produce an atmosphere in the closed vessel that is in vapour equilibrium with the solvent pool in the tank, to reduce evaporation from the advancing solvent front on the plate. The plates themselves may be used as commercially supplied, requiring only a solvent wash to remove impurities from the area of the plate to be used for the separation.

More complex automated chromatographic development instruments may be used but the simple separation of tars into three fractions can be achieved without them.

TLC-FID requires specialist equipment since the chromatographic rods require passage through the detector flame by a controlled mechanical technique. The Iatroscan Chromatograph with Chromarod silica rods has been developed for this analysis.

Analyses by Other Methods

Material from analytical spots or bulk fractions recovered from thin layer plates may be analysed by a variety of analytical techniques. In particular, mass spectrometric methods can be applied since they require very little sample. Also, several different mass spectrometric techniques can be applied to either one recovered spot or a bulk fraction. In this section, the analysis of fractions obtained by preparative TLC by probe mass spectrometry, matrix-assisted laser desorption mass spectrometry (MALDI-MS), size exclusion chromatography (SEC) and UV-fluorescence spectroscopy (UV-F) are discussed.

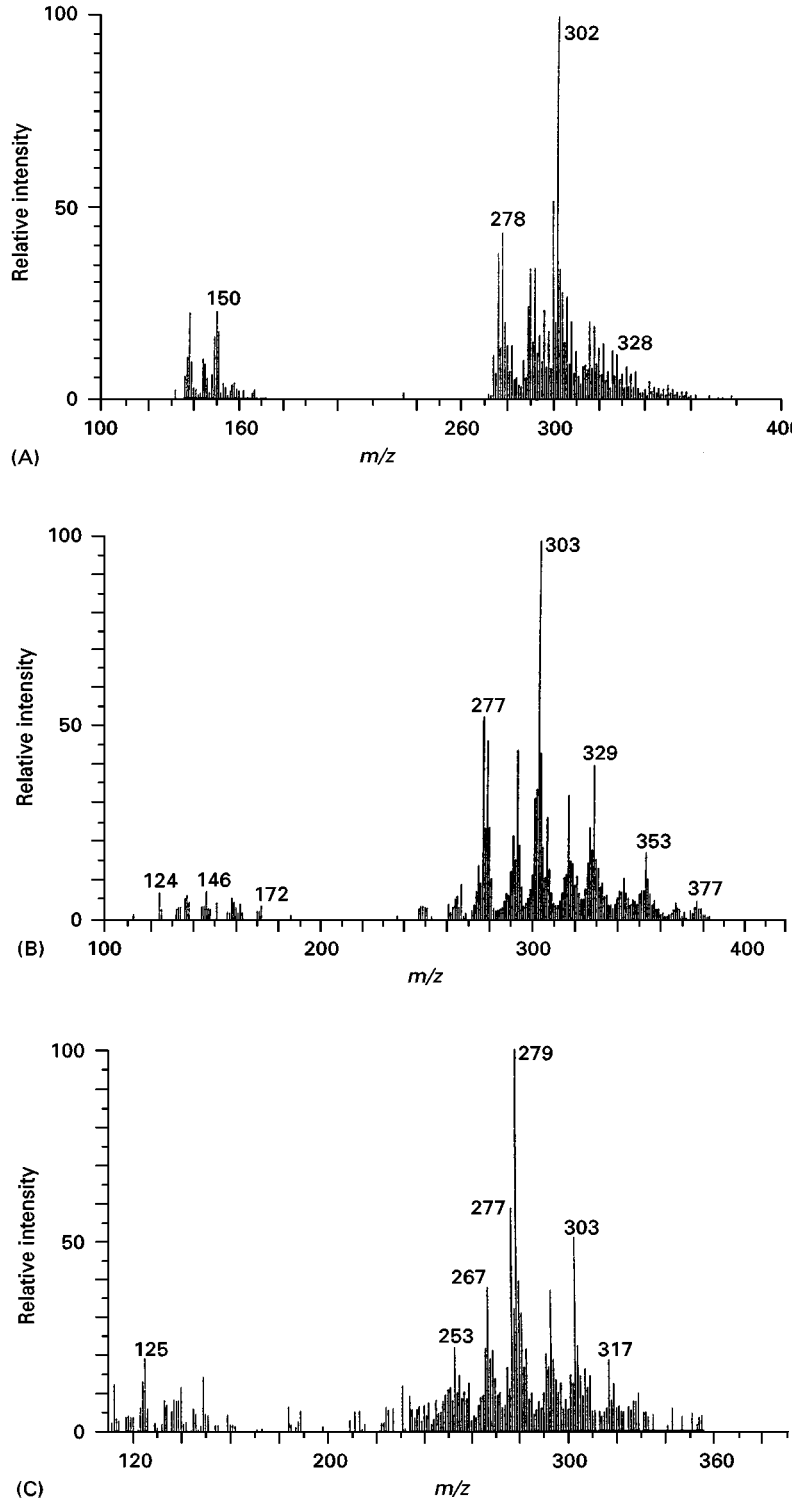


Figure 4 Probe mass spectra of TLC spots from an analytical development of coal tar pitch. Plots are normalized intensity versus mass number (*m*/*z*). (A) Fraction 2, aromatics; (B) fraction 5, neutral nitrogen heterocyclic aromatics; and (C) fraction 9, basic nitrogen heterocyclic aromatics. (Mass spectra reproduced from Herod AA and Kandiyoti R (1995) Fractionation by planar chromatography of a coal tar pitch for characterisation by size-exclusion chromatography, UV fluorescence and direct-probe mass spectrometry. *Journal of Chromatography* A 708: 143–160, © 1995, with kind permission of Elsevier Science NL.

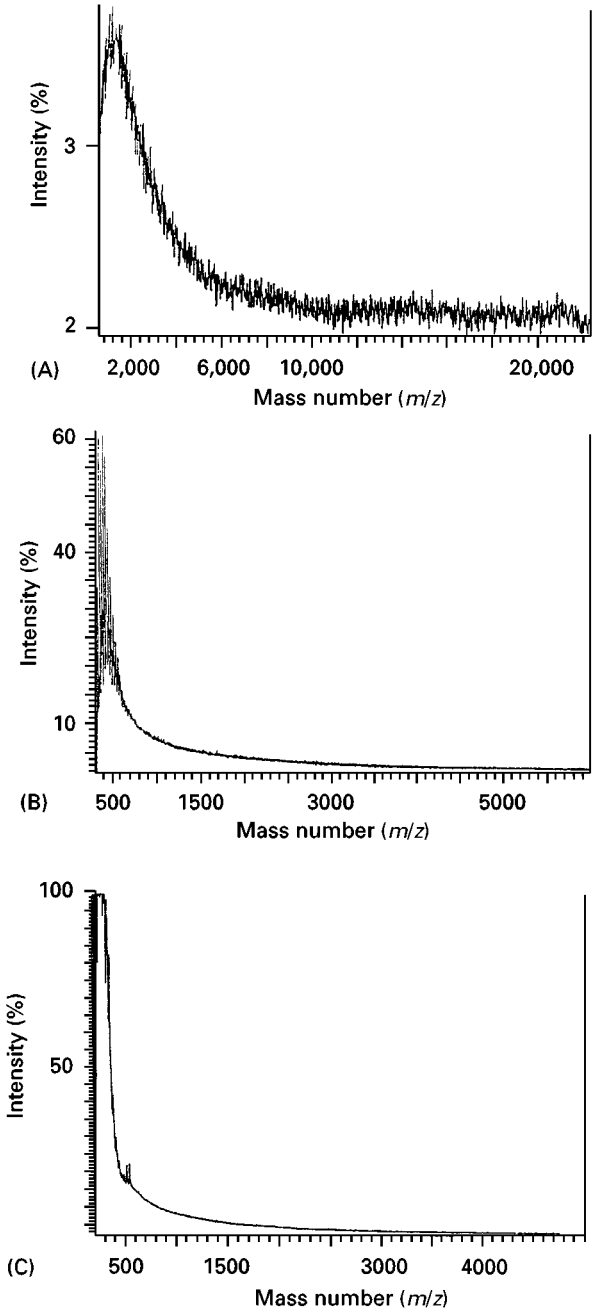


Figure 5 MALDI-mass spectra of coal tar pitch fractions from development in pyridine/acetonitrile. (A) Immobile fraction; (B) fraction mobile in pyridine only; (C) fraction mobile in pyridine and acetonitrile. (Reproduced with permission from Herod AA *et al.* (1996) Matrix-assisted laser desorption/ionization mass spectrometry of pitch fractions separated by planar chromatography. *Rapid Communications in Mass Spectrometry* 10: 171–177, © John Wiley & Sons Ltd.)

Probe Mass Spectrometry (Probe-MS)

This method can be used without extraction of the fraction from the silica. The range of molecular mass achieved depends on the volatility of sample in vacuum and the probe temperature; for fractions of pitch an upper mass of around m/z 600 is possible. The examination of spectra of spots in combination with R_F values of standards can permit the identification of compound types – aromatics, pyridinic and pyrrolic nitrogen heterocyclic aromatics. The absence of signal for the material left at the origin indicates that

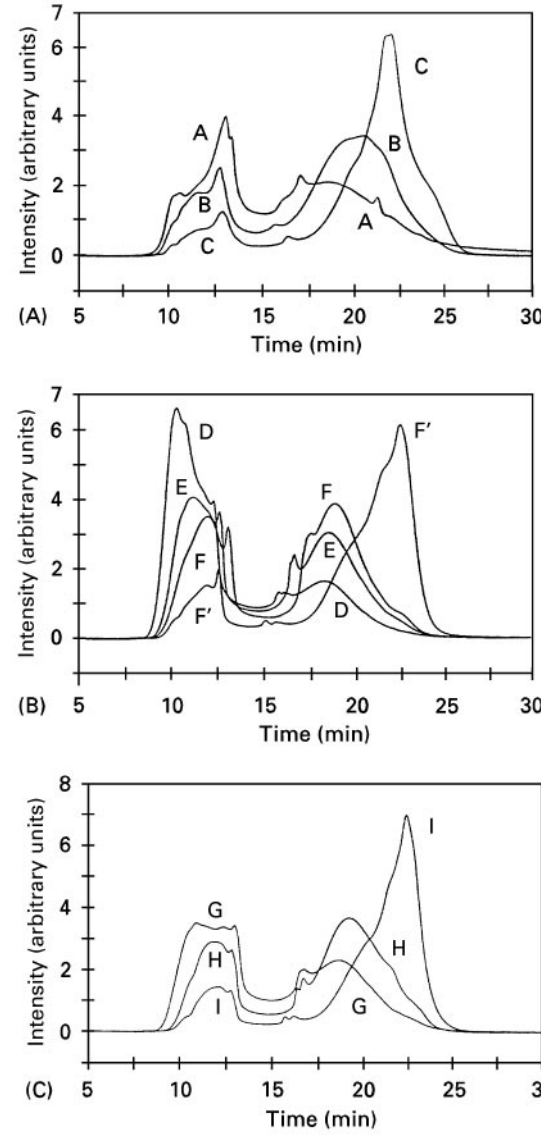


Figure 6 SEC profiles at 350 nm UV absorbance of pitch fractions. (A) Fractions A immobile in pyridine, B mobile in pyridine and C mobile in pyridine and acetonitrile; (B) fractions D immobile in pyridine, E mobile in pyridine, F mobile in pyridine and partly mobile in dimethylformamide and F′ mobile in pyridine and dimethylformamide; (C) fractions G immobile in tetrahydrofuran, H mobile in tetrahydrofuran and I mobile in tetrahydrofuran and toluene. (Reproduced with permission from Herod AA *et al.* (1996) Matrix-assisted laser desorption/ionization mass spectrometry of pitch fractions separated by planar chromatography. *Rapid Communications in Mass Spectrometry* 10: 171–177, © John Wiley & Sons Ltd.)

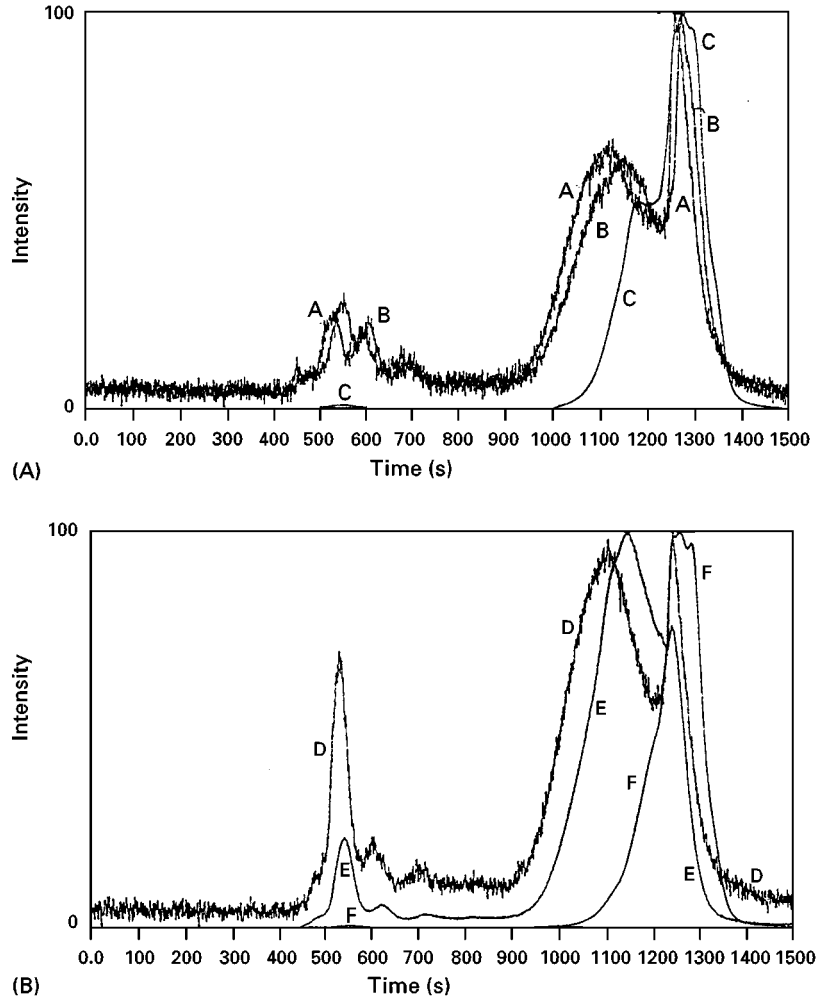


Figure 7 SEC profiles of pitch in NMP solvent of (A) fractions A, B and C from TLC in pyridine and dimethylformamide and (B) fractions D, E and F from TLC in tetrahydrofuran and toluene; detection by UV fluorescence with excitation at 320 nm and emission at 380 nm (fractions C and F) or 480 nm (fractions A, B, D and E). Fractions A and D are immobile; fractions B and E are partly mobile; fractions C and F are very mobile. (Reproduced with permission from Herod AA and Kandiyoti R (1996) Fractionation of coal tar pitch by planar chromatography for the characterisation of large molecular mass materials. *Journal of Planar Chromatography* 9: 16–24, © Research Institute for Medicinal Plants, H-2011, Budakalasz, Hungary.)

it contains large, involatile molecules rather than aggregates of small polar molecules.

Figure 4 shows mass spectra for some spots recovered from an analytical separation of a coal tar, following a separation similar to that shown in Figure 1. Fractions 1 (mobile in pentane) and 2–4 (mobile in toluene close to toluene front) gave molecular ions for polynuclear aromatic hydrocarbons ranging from fluorene (m/z 166) to m/z 482, corresponding to a dimethyl tetrabenzobinaphthyl type. Fractions 5, 6 and 7 correspond to the range of mobility between aromatics in the toluene zone and the chloroform/methanol solvent front and show evidence of the presence of nitrogen-containing heterocyclics. Fractions 8–11 were taken from material mobile in chloroform/methanol but not mobile in toluene and correspond to basic nitrogen heterocyclic aromatics. The probe mass spectra of thin-layer fractions have allowed the identification of isomer classes rather than individual isomers but have extended the mass range of identified nitrogen PAH to nearly m/z 500. The identification of neutral and basic nitrogen components can be achieved during one rapid, simple and inexpensive separation with the use of standards to define the separation. Also, interference from the ^{13}C isotope peak of the more abundant polycyclic aromatics which have molecular masses one unit less than the nitrogen heterocyclics, is avoided, as opposed to the situation in GC-MS where both types elute together.

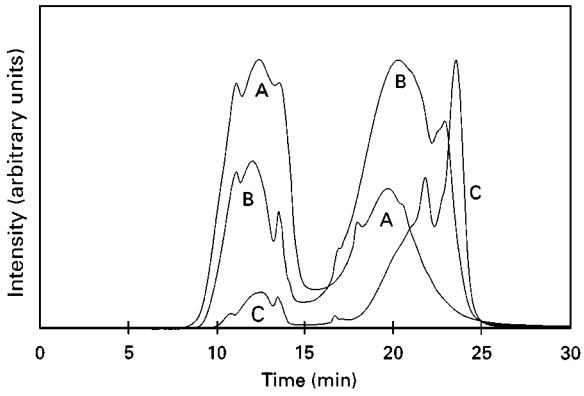


Figure 8 SEC profiles in NMP of TLC fractions of Point of Ayr liquefaction pilot plant coal digest. Fractions A immobile in pyridine, B mobile in pyridine, C mobile in pyridine and acetonitrile; UV absorbance detection at 350 nm. (Reproduced with permission from Herod AJ *et al.* (1996) Planar chromatography as a method of fractionation of a coal liquefaction extract for Mössbauer spectroscopy. *Journal of Planar Chromatography* 9: 361–367, © Research Institute for Medicinal Plants, H-2011, Budakalasz, Hungary.)

Matrix Assisted Laser Desorption Mass Spectrometry (MALDI-MS)

The application of MALDI-MS to coal-derived fractions is at an early stage. Much of the published work corresponds to laser ablation MS, where the fractions are examined with no added matrix but with the small molecules of the sample itself acting as the matrix. In the absence of small molecules (indicated by SEC) to form an effective matrix for kerogen extracts, no significant mass spectrum can be generated for the large molecules; addition of suitable matrix materials allows the generation of mass spectra, however. The upper limits observed for coal-derived materials by MALDI are in excess of 100 000 u but so far, it has not proved possible to generate mass spectra from the TLC-immobile fractions containing the largest molecules. Similarly, the techniques and matrix materials used to generate spectra for coal-derived fractions have not proved successful with immobile fractions from either biomass tars or petroleum vacuum residues.

One essential requirement for producing good mass spectra is the reduction of polydispersity (ratio of mass average to number average molecular mass) of fractions. **Figure 5** shows the MALDI-mass spectra of coal tar pitch fractions from development in pyridine/acetonitrile: (A) immobile fraction; (B) fraction mobile in pyridine only; and (C) fraction mobile in pyridine and acetonitrile. Increasing the mobility leads to shifts of molecular masses to smaller values and to changes in the shapes of spectra; the spectra become narrower and sharper with increasing mobility. Comparing relative intensity scales, intensities of immobile fraction spectra were only 1–4%, whereas the spectra of mobile fractions gave signal at full scale (100%). Smaller-mass molecules appear to be preferentially ionized and desorbed, thus skewing the molecular ion distribution in favour of the smaller molecules.

Size Exclusion Chromatography (SEC)

Size exclusion chromatography has been used extensively for the examination of oils and tars from coal, biomass and petroleum. Until recently, tetrahydrofuran (THF) was the solvent most used, for example for asphalts for road tars. In work with coal tar, we have shown that the use of THF gives erroneous results since the high-mass portion of the tar is lost to the guard column, which gradually blocks. It can be

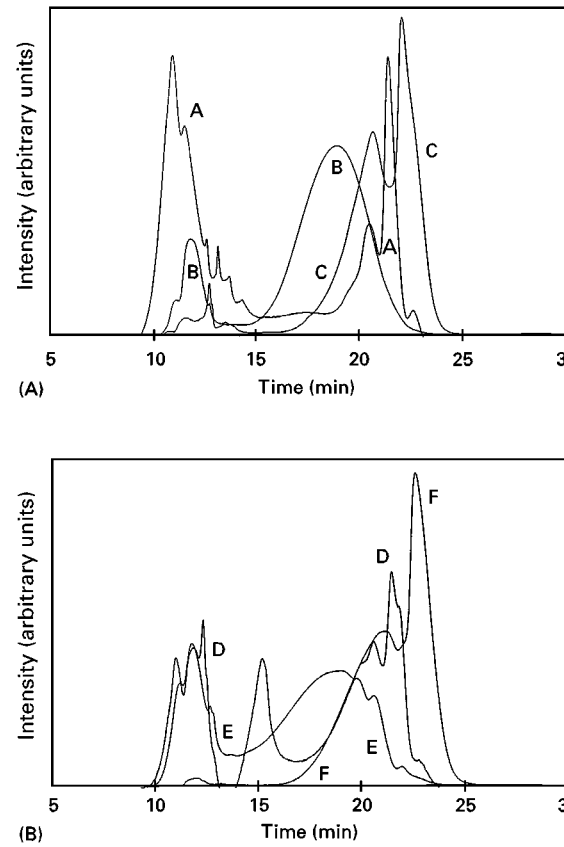


Figure 9 SEC profiles of Stockholm tar (a commercial pinewood tar) fractions. (A) A immobile in pyridine, B mobile in pyridine and C mobile in pyridine and acetonitrile; (B) D immobile in tetrahydrofuran, E mobile in tetrahydrofuran and F mobile in tetrahydrofuran and toluene. UV absorbance at 300 nm. (Reproduced from the work of Lazaro MJ, Domin M, Herod AA and Kandiyoti R (1999) Fractionation of a wood for pitch by planar chromatography for the characterisation of large molecular mass materials. *Journal of Chromatography A* 840: 107–115; not previously shown in this form.)

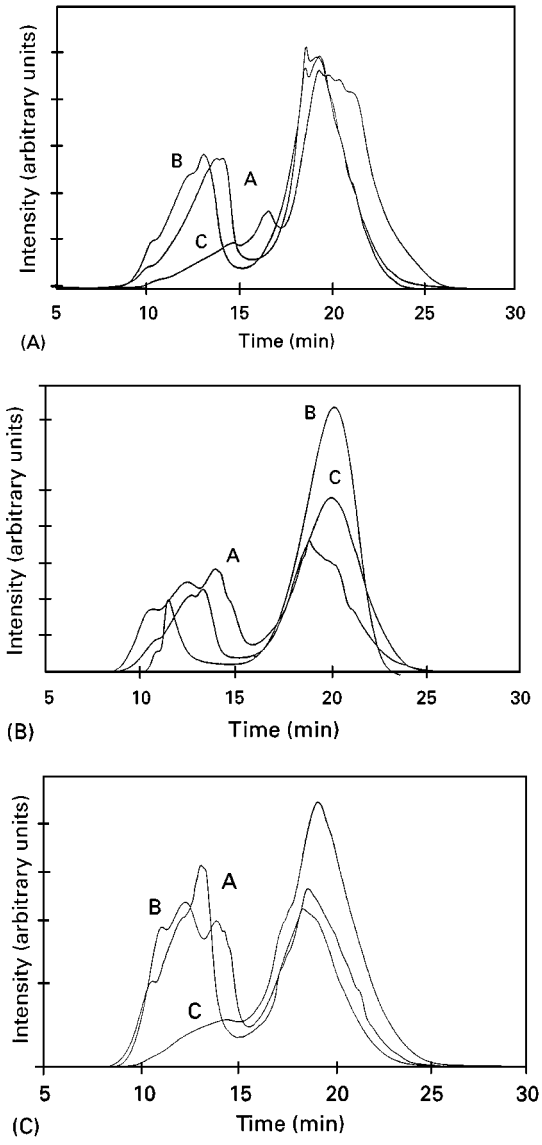


Figure 10 SEC profiles of petroleum vacuum residues. Fractions from TLC of (A) residue 1 in pyridine and acetonitrile, (B) residue 2 in pyridine and acetonitrile, (C) residue 1 in THF and toluene; curves are A immobile, B mobile in first solvent and C mobile in both solvents. (Reproduced from Deelchand J-P Naqvi Z, Dubau C, Shearman J, Lazaro MJ, Herod AA, Read H and Kandiyoti R (1999) Planar chromatographic separation of petroleum residues and coal-derived liquids. *Journal of Chromatography A* 830: 397–414; Copyright Elsevier Science.

cleaned using 1-methyl-2-pyrrolidinone (NMP) and restored to a usable state. In addition, THF does not completely dissolve the coal-derived materials. In the work described here, NMP has been used to dissolve tars and as an eluent for SEC. Although NMP dissolves coal tars and biomass liquids completely, it is a poor solvent for alkanes and therefore petroleum-derived materials may be only partially soluble. However, the use of TLC fractionation allows the removal of alkanes from the aromatics by applying a final development of pentane. If UV absorbance is the method of detection for SEC, then alkanes are not observed.

SEC chromatograms of coal tars, biomass tars and petroleum vacuum residues are shown in **Figures 6–10**. All of the chromatograms show at least two major peaks, the first near the exclusion limit of the column and the second corresponding to material resolved by the column. The signal for the excluded material is thought to correspond to material the column is unable to resolve. If resolved, this material will appear as a long trailing distribution of apparently larger-mass material. More important though is the increasing proportion of excluded material in the TLC fractions with increasing immobility. This indicates that the TLC separation is on the basis of molecular size and not just polarity. Indeed, the definition of polarity for the fractions of tars described as oils, maltenes, asphaltenes and preasphaltenes on the basis of solubility or insolubility in particular solvents may apply equally well as a measure of increasing molecular

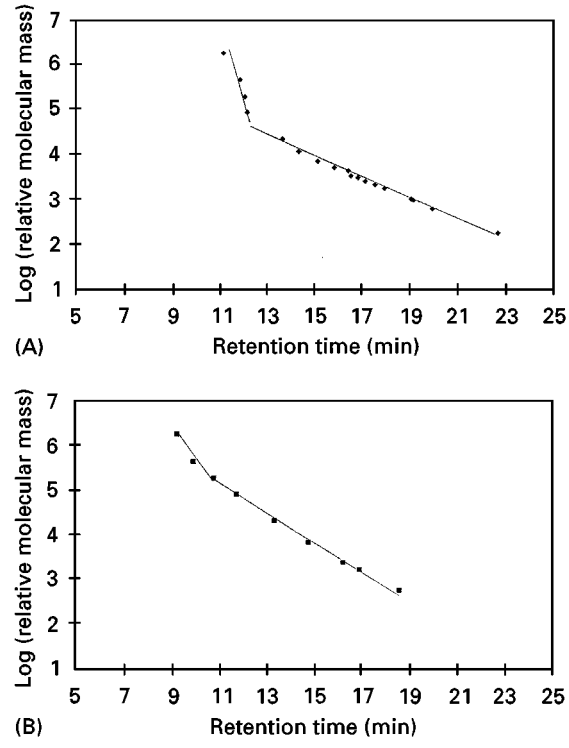


Figure 11 Calibration graphs for two SEC columns using polystyrene standards; $\log_{10}$ molecular mass versus elution time. (A) Mixed E column and (B) Mixed D column from Polymer Laboratories Ltd, Church Stretton, UK.

mass or size. In SEC, the sequence of solvent-derived fractions shows a trend towards increasing molecular size from oil to preasphaltene. The combination of evidence from SEC, TLC, solvent solubility and MALDI-MS indicates that the immobile fractions do contain the largest molecular masses present in the tars from different sources. The SEC profile of the whole pitch shows a relatively smaller peak of excluded material compared with the immobile fractions. This points to the masking effect of the greater concentration of smaller masses and more mobile material, and emphasizes the utility of the planar chromatographic separation.

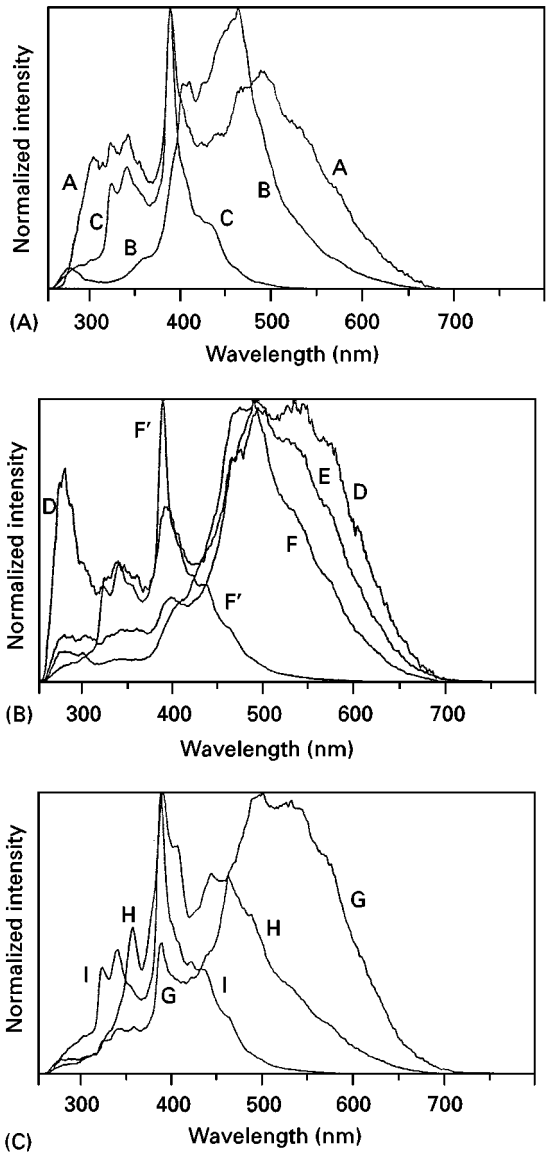


Figure 12 UV-fluorescence synchronous spectra of coal tar pitch fractions. (A) A immobile in pyridine, B mobile in pyridine, C mobile in pyridine and acetonitrile; (B) D immobile in pyridine, E mobile in pyridine, immobile in dimethylformamide, F partly mobile in dimethylformamide and F′ mobile in dimethylformamide; (C) G immobile in tetrahydrofuran, H mobile in tetrahydrofuran, immobile in toluene and I, mobile in toluene. (Reproduced with permission from Herod AA *et al.* (1996) Matrix-assisted laser desorption/ionization mass spectrometry of pitch fractions separated by planar chromatography. *Rapid Communications in Mass Spectrometry* 10: 171–177, © John Wiley & Sons Ltd.

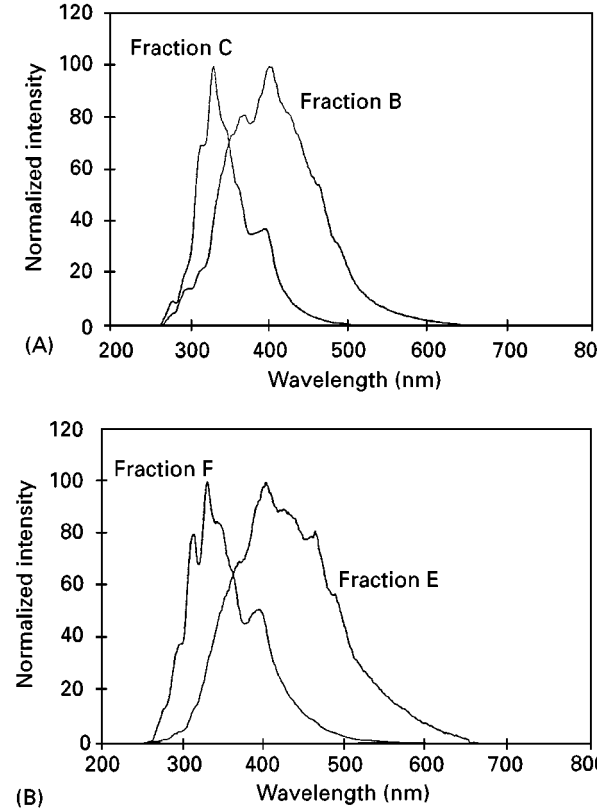


Figure 13 UV-fluorescence synchronous spectra of Stockholm tar fractions. (A) B mobile in pyridine and C mobile in pyridine and acetonitrile, the immobile fraction A did not show fluorescence; (B) E mobile in THF and F mobile in THF and toluene, the immobile fraction D did not show fluorescence. (Reproduced from the work of Lazaro MJ, Domin M, Herod AA and Kandiyoti R (1999) Fractionation of a wood for pitch by planar chromatography for the characterisation of large molecular mass material. *Journal of Chromatography A* 840: 107–115; not previously shown in this form.)

Calibration of the column separation is normally achieved using polymer standards; in this work polystyrene standards have been used. The calibration then appears to apply to polycyclic aromatic hydrocarbons and their N, S and O derivatives up to masses of approximately 1000 u. At higher masses, two problems apply: (1) there are no standard PAH available; and (2) the structures of the tar molecules are totally unknown. Calibration curves based on polystyrenes up to relative mass 1.84 million are shown in **Figure 11** for two columns. The linear regions from low mass (A) 20 000 u or (B) 200 00 u correspond to

the working region in which solute molecules penetrate the pores of the column packing and separate by size. The linear relation for larger polystyrenes at shorter elution times may correspond to separation in the space between the particles of the packing and molecules eluting in this region are described as excluded from the porosity; the discontinuity is described as the exclusion limit of the column.

UV-Fluorescence Spectroscopy (UV-F)

UV-fluorescence spectroscopy does not measure molecular size directly, but shifts of fluorescence maximum intensity to longer wavelengths indicate an increasing aromatic cluster size since the fluorescence originates from the largest aromatic system within a molecule, fed by energy absorbed by the smaller pendant aromatic groups. Such shifts to longer wavelengths point to increasing molecular size. Decreasing fluorescence quantum yields are a consequence of increased molecular size and complexity since there are more pathways for the electronic excitation to progress to vibrational and thermal energy rather than being lost as fluorescence.

UV-F spectra of coal- and biomass-derived liquids and petroleum residues are shown in **Figures 12–14**. In coal-derived tars, the fluorescence intensity of the materials showing reduced mobility in TLC (and being largely excluded from the SEC porosity) decreases and the maximum shifts towards red wavelengths, indicating that these fractions contain large molecules. With biomass tars, immobile fractions do not show any fluorescence at all, indicating the presence of very high molecular mass material.

The fractions mobile in both solvents showed relatively strong fluorescence intensities, the position of the peaks at lower wavelengths suggesting the presence of relatively smaller polynuclear aromatic ring systems and probably also the presence of lower molecular mass material. Similarly fractions mobile in one solvent gave less intense fluorescence than fractions mobile in both solvents. TLC fractions of petroleum vacuum residues show no similar shift to red wavelengths with increasing immobility, or markedly reduced quantum yield but tend to cover the same range of wavelengths with shifts of intensity of peaks within that range. However, SEC of the immobile fractions indicates that the lack of mobility results from molecular size.

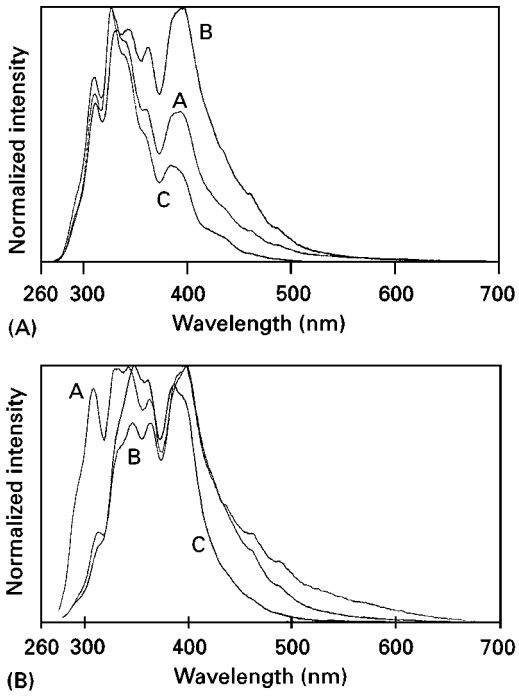


Figure 14 UV-fluorescence synchronous spectra of petroleum residues. (A) Sample 1 and (B) sample 2 (A immobile in pyridine, B immobile in acetonitrile and C mobile in pyridine and acetonitrile). (Reproduced from Deelchand J-P, Naqvi Z, Dubau C, Shearman J, Lazaro MJ, Herod AA, Read H and Kandiyoti R (1999) Planar chromatographic separation of petroleum residues and coal-derived liquids. *Journal of Chromatography A* 830: 397–414; Copyright Elsevier Science.

Conclusions

Several examples of the fractionation by TLC of coal- and biomass-derived liquids and petroleum residues have been shown. TLC improves the isolation and characterization of large molecular mass fractions in oils and tars for examination by other techniques such as probe mass spectrometry, MALDI-MS, SEC and UV-F. The separation is relatively rapid and inexpensive and requires only small volumes of solvents. The fractionation has led to structural information not readily available by direct characterization of the original mixture. Molecular-mass distributions, determined by SEC and MALDI, increase with decreasing mobility of the fractions in thin layer chromatography. UV-F spectroscopy has distinguished structural features by showing the presence of large polycyclic aromatic systems that increase in proportion to decrease in mobility of fractions. Detailed structures of the largest molecules remain unknown. Probe mass spectra have allowed the identification of isomer class and extended the mass range of identified nitrogen PAH to nearly m/z 500, allowing the identification of neutral and basic nitrogen types as well as the major components through one rapid, simple and inexpensive separation.

See Colour Plates 109, 110.

See also: **II/Chromatography: Liquid:** Mechanisms: Size Exclusion Chromatography. **III/Bitumens: Liquid Chromatography. Crude Oil: Liquid Chromatography. Flame Ionization Detection: Thin-Layer (Planar) Chromatography. Geochemical Analysis: Gas Chromatography. Polycyclic Aromatic Hydrocarbons:** Gas Chromatography; Solid-Phase Extraction; Supercritical Fluid Chromatography; Thin-Layer (Planar) Chromatography.

Further Reading

Davison RR, Glover CJ, Burr BL and Bullin JA (1995) SEC of asphalts. In: Chi-san Wu (ed.) *Handbook of Size Exclusion Chromatography*, ch. 8, pp. 211–247. New York: Marcel Dekker.

Herod AA (1994) A review of the uses of planar chromatography in the coal and oil industries. *Journal of Planar Chromatography* 7(3): 180–196.

Herod AA (1998) Azaarenes and thiaarenes. In: Neilson AH (ed.) *The Handbook of Environmental Chemistry*, vol 3. I: *PAHS and Related Compounds*, *Chemistry*, ch. 7, p. 271. Berlin: Springer-Verlag.

Jork H, Funk W, Fischer W and Wimmer H (1990) *Thin Layer Chromatography*: *Reagents and Detection methods*, vol. 1a. Weinheim, Germany: VCH Verlagsgesellschaft.

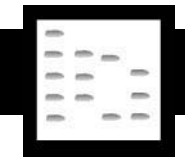
Lakowicz JR (1983) *Principles of Fluorescence Spectroscopy*. New York: Plenum Press.

Somsen GW, Morden W and Wilson ID (1995) Planar chromatography coupled with spectroscopic techniques, a review. *Journal of Chromatography* A 703: 613–665.

Touchstone JC (1992) *Practice of Thin Layer Chromatography*, 3rd edn. New York: John Wiley & Sons.

Wolfbeis OS (1993) *Fluorescence Spectroscopy – New Methods and Applications*. Berlin: Springer-Verlag.

PHARMACEUTICALS



Basic Drugs: Liquid Chromatography

B. Law, AstraZeneca Pharmaceuticals, Mereside, Alderley Park, Macclesfield, UK

Introduction

High performance liquid chromatography (HPLC) is the most important technique for the separation, analysis and quantification of a wide range of drug types. Although there are a variety of approaches available for the chromatography of basic drugs, analysis of these compounds is still one of the main challenges for the practising chromatographer in the pharmaceutical industry. The general approaches have remained the same since the early days of HPLC, but there have been many refinements and developments since the late 1960s. In the main these have involved modification and improvements to the stationary phase, which are still continuing today.

This article focusses on the main methods of separation and analysis of basic drugs that are currently in use. Consideration is given to the relative pros and cons of the different approaches, as well as the development and evolution of the techniques.

Liquid–Solid Chromatography

Liquid–solid, or normal-phase chromatography (LSC) was one of the first approaches employed for the separation of bases in modern LC. Its use, however, has decreased dramatically since the 1970s and it is now rarely employed for the routine separation of basic drug molecules.

LSC was originally carried out using native silica or alumina, with the former being preferred for the separation of bases. Recently, there has been a gradual shift towards the use of polar bonded phases such as cyanopropyl, amino or diol, the last two showing preferential retention of bases compared with cyanopropyl. These bonded materials overcome some of the problems associated with silica phases such as deactivation by water and long equilibration times. The problem of deactivation is particularly acute in the area of bioanalysis, where it can be difficult to obtain extracts that are totally dry. To a certain degree this problem can be overcome by the inclusion in the eluent of a small amount (1% v/v) of water or a short-chain alcohol.

Eluents for LSC typically consist of mixtures of a nonpolar hydrocarbon, such as hexane or isooctane, and a polar modifier, e.g. dichloromethane, 2-propanol, methyl *t*-butyl ether or ethyl acetate. Frequently, the addition of an amine modifier such as triethylamine may be necessary to give satisfactory peak shapes.