The availability of relatively inexpensive and safe isotopically labelled substrates and precursors will benefit tracer studies and allow improved precision in quantitative measurements of a wide variety of analytes. GC–SIRMS will facilitate dynamic studies of metabolism and migration. The capacity to distinguish exogenous from endogenous materials is bound to be a significant and expanding area of application that will find great use in the identification of abuse, adulteration and authenticity.

## Acknowledgements

The following are acknowledged: Nick Ordsmith and Keith Hall from Hall Analytical Laboratories Ltd. Andy Phillips from Micromass Ltd. Nick Bukowski from ThermoQuest.

*See also:* **II/Chromatography: Gas:** Detectors: Mass Spectrometry. **III/Drugs of Abuse: Solid-Phase Extraction.**

## Further Reading

Chapman JR (1993) *Practical Organic Mass Spectrometry. A Guide for Chemical and Biochemical Analysis*, 2nd edn. Chichester: John Wiley.

Johnstone AW and Rose ME (1996) *Mass Spectrometry for Chemists and Biochemists*. 2nd edn. Cambridge: Cambridge University Press.

Krumbiegel P (1991) *Stable Isotope Pharmaceuticals for Clinical Research and Diagnosis*. (*Drug Development and Evaluation*), vol.18. Jena: Fischer.

McLafferty FW and Turecek F (1993) *Interpretation of Mass Spectra*. 4th edn. Sausalito: University Science Books.

# Thin-Layer (Planar) Chromatography

*See* **III / CLINICAL CHEMISTRY: THIN LAYER (PLANAR) CHROMATOGRAPHY**

# BITUMENS: LIQUID CHROMATOGRAPHY

**K. Dunn, G. V. Chilingarian and T. F. Yen**,
University of Southern California, Los Angeles,
CA, USA

## Introduction

Great confusion exists in the literature on the definition of the term *bitumen*. It occurs in the earth's crust in various forms: firstly, in a dispersed state, in trace quantities; and secondly, as accumulations, where bitumen either impregnates the rock or occurs in a pure or nearly pure form. In the first half of the 20th century, the term bitumen was applied to crude oil and its natural derivatives (maltha, asphalt, ozokerite). In this original meaning, bitumens are called naphthides. The term bitumen is also used for asphalts and asphalt-like manufactured substances utilized in road construction, including products from the processing of coals and/or oil shales. In crude oils, in distillate cuts beginning with kerosene, and in distillation residues, there is a group of high molecular weight heteroorganic compounds that are lumped under the name of resins and asphaltenes. Their content may be as large as 40% in heavy crude oils. Carbon and hydrogen constitute 80–95% of the resin and asphaltene molecules, with oxygen always present. Sulfur, nitrogen and metals are also usually present. The content of resins in crude oil (2–40%) exceeds by far (from 3 to 40 times) the content of asphaltenes (trace to 6%; usually $<1\%$). Carboids and carbenes, which resemble asphaltenes, differ from them by having a higher oxygen content. However, they are absent in crude oils and distillate cuts. In small quantities, they are found in residues from vacuum distillation, in cracked tars, and in native and petroleum asphalts.

Bitumens and the related heat-treated asphalt are a very complex mixture of compounds with a wide range of molecular weights, which are difficult to isolate into classes of pure compounds. Resins are semi-liquid (sometimes almost solid) dark brown to black substances which have a specific gravity around 1 and a molecular weight of 500–2000 (usually 600–1000). Asphaltenes, on the other hand, are dark, amorphous powders and have a specific gravity greater than 1 and molecular weights of 1000–10 000 (average 5000). Using various methods, the observed molecular weights suggest that asphaltenes form molecular aggregates or colloids, even in dilute

**Table 1** Unit molecular weight of asphaltenes

| *Method* | *Particle weight* | *Researcher* |
|---|---|---|
| Viscosity | 900–4000 | Fischer and Schrem (1959) |
| High angle X-ray | 805–3360 | Yen *et al.* (1961) |
| Light absorption | 1000–4000 | Markhasin *et al.* (1966) |
| Electron microscopy | 3440–5430 | Dickie *et al.* (1969) |
| VPO | 2950–8130 | Dickie and Yen (1967) |
| VPO | 2000–4600 | Dickie and Yen (1967) |
| VPO | 1220–2160 | Speight *et al.* (1985) |
| Cryoscopic, naphthalene | 1700 | Hilman and Barnett (1937) |
| Cryoscopic, phenathrene | 2500 | Boyd and Montgomery (1962) |
| Cryoscopic, benzene | 5000–6000 | Katz (1934) |

solutions. The results are influenced by solvent polarity, asphaltene concentration and the temperature used. Different molecular weights of asphaltenes can be obtained by different instruments. For example, the data obtained from solution viscosity and cryoscopic method are low, whereas those obtained from the ultracentrifuge and electron microscope are high. Vapour pressure osmometry (VPO) is considered to be the most accurate method at present when a good solvent is used to disperse the asphaltenes. Because the solvent affects the degree of asphaltene aggregation, the true molecular weights of asphaltenes are generally much lower than those measured. Some of these results are listed in **Table 1**.

The asphaltene content is a variable value depending on the relative amounts and characteristics of the source material and on the procedure adopted for separation. The physical and chemical characteristics are of considerable importance: the higher boiling point components of crude oil can precipitate in certain solvents, whereas the lower boiling point components (cyclic and aromatic compounds) are soluble in solvents. Most of the studies on bitumens and asphalts are concentrated on groups of compounds with particular physical and chemical properties. Depending on the solubility in certain solvents, asphalt or bitumen is generally fractionated into four main fractions: saturates, aromatics, resins and asphaltenes. The polarity of these fractions increases in the following order: saturates < aromatics < resins < asphaltenes. A detailed scheme for bitumen fractionation is shown in **Figure 1** and explained as follows:

1. Gas oil is propane-soluble and *n*-pentane-insoluble.
2. Resins are *n*-pentane-soluble but propane-insoluble.
3. Asphaltenes are toluene-soluble but *n*-pentane insoluble.
4. Preasphaltenes are insoluble in both *n*-pentane and toluene.

Asphaltenes are multipolymer systems containing a great variety of building blocks. The statistically average molecule contains a flat sheet of condensed aromatic systems that may be interconnected by sulfide, ether, aliphatic chains or naphthenic ring linkages. Gaps and holes appear as defect centres in the aromatic systems most likely involving free radicals. Heterocyclic atoms may be coordinated to transition

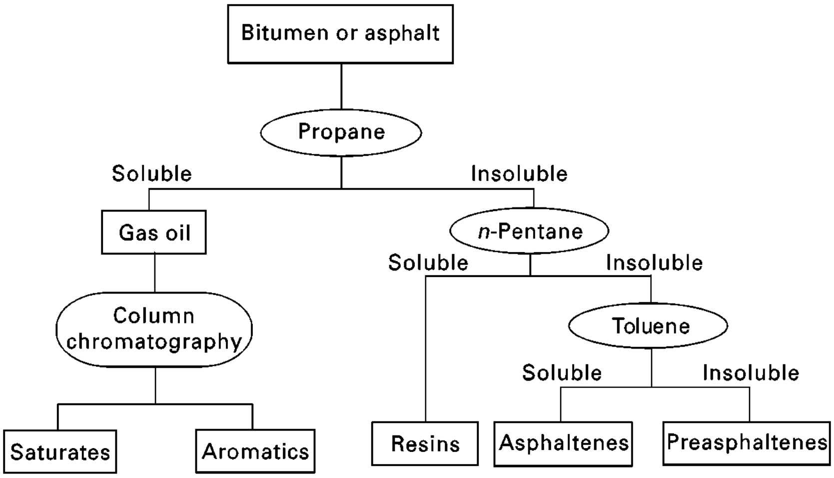

**Figure 1** Fractionation scheme of bitumen or asphalt.

metals such as vanadium, nickel and iron. Approximately five of these sheets are associated by $\pi$–$\pi$ interaction. They are stacked one above the other: the distance between the sheets ranges from 0.36 to 0.38 nm, giving an overall height for a stack of 1.6–2.0 nm; the average sheet diameter appears to be 0.85–1.5 nm. The hypothetical structure of an asphaltene is shown in **Figure 2**.

Resins are considered smaller analogues of asphaltenes with a much lower molecular weight. The resins contain aromatic compounds substituted with longer alkyl chains and a greater number of side chains attached to the rings than asphaltenes. The combination of the saturated and aromatic characteristics of resins stabilizes the colloidal nature of asphaltenes present in the oil.

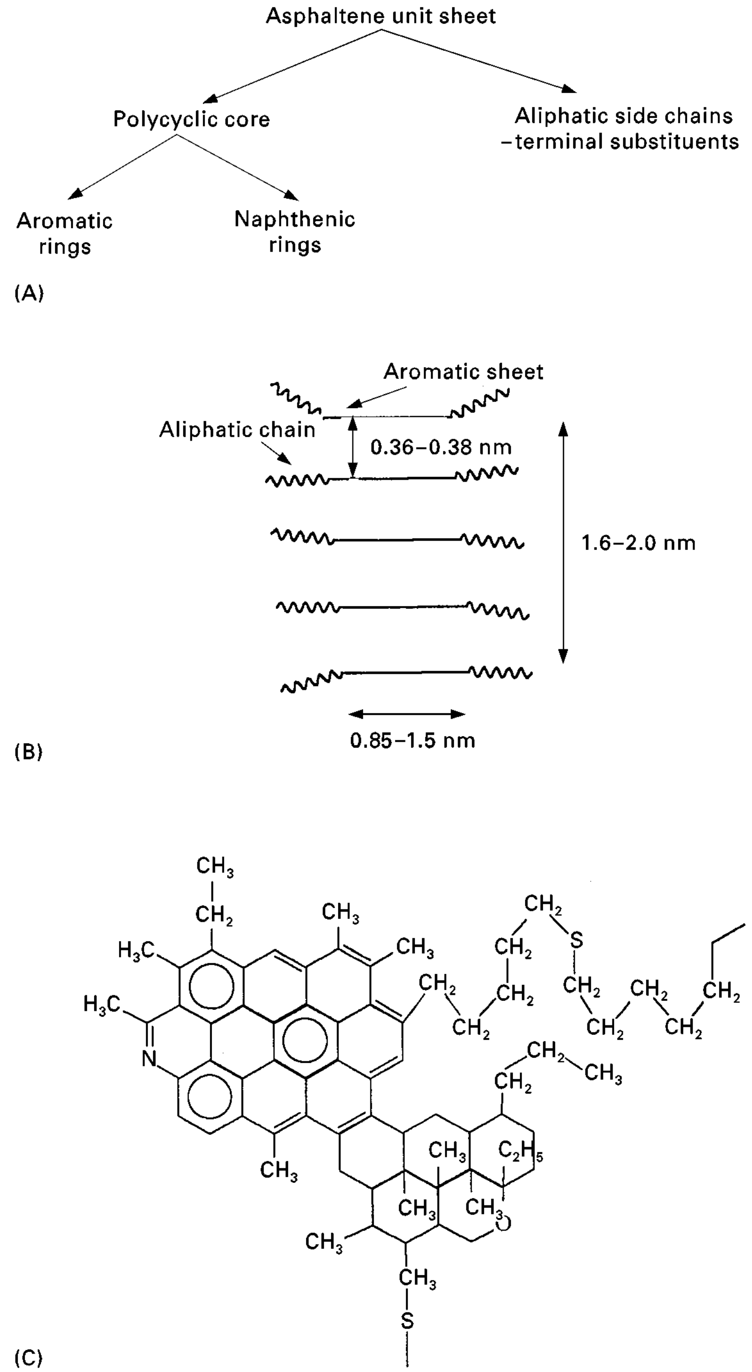

**Figure 2** (A) The classification of asphaltene structure. (B) The structure of asphaltene stack. (C) The structrure of one sheet of asphaltene stack.

The gas oils constitute the lowest molecular fraction of the asphalt and serve as the dispersion medium for the peptized asphaltenes. Structurally, gas oils consist mostly of naphthenic–aromatic nuclei with a greater proportion of side chains than the resins. Alkyl naphthenes predominate and straight chain alkanes are rarely present. The naphthenic content is 15–50%, with naphthenics containing two to five nuclei per molecule.

The fractionation techniques for crude oil involve solvent fractionation and precipitation, column chromatography, ion exchange and complexation methods. No matter what method is used, the important step is the isolation of asphaltenes. Of course, all these separation processes are carried out on the heavy fractions of petroleum only; usually the volatile fractions have been removed by distillation.

The most popular method of column chromatography is the fractionation into saturates, aromatics, resins and asphaltenes (SARA). Solvent fractionations have been used in the industry since 1930, becoming widely accepted between 1950 and 1970. Recently, centrifugal thin-layer chromatography (TLC) has been used to separate crude oil successfully. This method reduces the separation time and cost, as well as preserving each fraction for further study. TLC with flame ionization detector (TLC-FID) can achieve quantitative analysis quickly and simply. The combination of solvent fractionation by silica gel and ion exchange chromatography can achieve a more detailed separation showing the complexity of petroleum. The polar fraction of the crude oil is separated by ion exchange. For chromatographic methods for bitumen or asphalt separation (SARA method, centrifugal TLC, TLC-FID and the combination of silica gel chromatography and ion exchange) are described in detail in the following sections.

## The SARA Method

### Background

The SARA method enables the separation of bitumen into groups. The method is an extension of column chromatography in order to permit more standardized separation. Two packed columns are employed: Firstly, a SARA column (ion exchange resins) and secondly, an activated silica gel column. The resin fractions are irreversibly retained in the SARA column, whereas the gas oil (saturates and aromatics) fraction remains in the solvent after elution of this column. The gas oil fraction can be further separated into saturates and aromatics by the activated silica gel column. A flow diagram of the SARA method is given in **Figure 3**. Although the SARA method has been considered as a standard separation technique for asphaltenes, there are some drawbacks to prevent it from becoming popular: it is time-consuming (it takes at least 1 week) and there is incomplete recovery (resin fraction is difficult to recover).

### Separation Procedure

**Separation of asphaltenes** The asphaltene fraction is separated by a precipitation process. The bitumen and asphalt samples can be dissolved first in toluene to form dispersions for precipitation by another

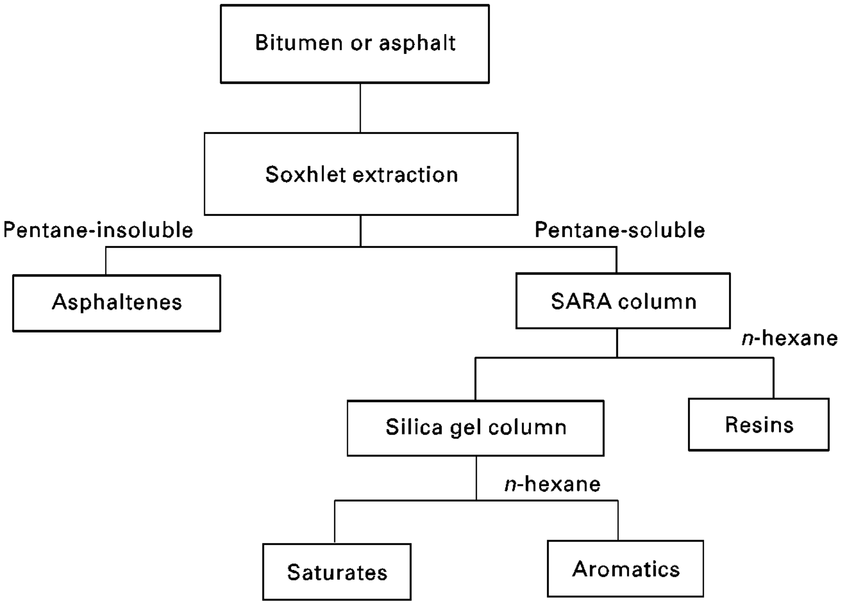

**Figure 3** Flow chart of SARA separation method.

liquid. Asphaltene precipitation occurs when an excess of *n*-pentane is added to the toluene dispersion. A toluene–*n*-pentane volumetric ratio of 1 to 50 is required to ensure the precipitation of asphaltenes. After the precipitaion process, the toluene dispersion can be separated into two fractions: pentane-insoluble (asphaltenes) and pentane-soluble (maltenes). To purify asphaltenes, the precipitation process is followed by Soxhlet extraction. *n*-Pentane is used during Soxhlet extraction until it becomes clear. This requires over 48 h. The maltene fraction can be further separated by column chromatography.

**Separation of saturates and resins** The SARA column is dry-packed under vibration with various packings, purged with nitrogen and equilibrated with *n*-pentane. The column contains four discretely packed zones with a sequence of $H^+$ cation exchange resin, $OH^-$ ion exchange resin, clay-coated with ferric chloride, and $OH^-$ ion exchange resin, as illustrated in **Figure 4**. The maltenes present in the sample are dissolved by the *n*-pentane, placed in the top reservoir and carried into the column. The leaching process is continued by recycling until the eluate is colourless. Liquid must always be present in the top reservoir. All operations are carried out under nitrogen. The result of the separation is that the gas oils (saturates and aromatics) collect in the bottom flask, whereas the resins remain in the column. The gas oils can be further fractionated into saturates and aromatics on a silica gel column using *n*-hexane as the eluent, with the saturates being eluted first. The cut-off point of elution between saturates and aromatics can be distinguished by UV examination. Under the UV light, the saturates are colourless, whereas the aromatics exhibit a yellow-green colour.

**Separation of resins** In the analytical SARA separation, the resin content is obtained from the mass balance, by deducting the weights of asphaltenes and gas oils from the original weight of the sample. This method is easy and rapid to quantify the resin content in the bitumen or asphalt sample; however, the fact that resins cannot be collected for further study is a drawback. The resin fraction retained in the SARA column may be semiquantitatively recovered (approximately 90%) by exhaustive elution of the packing with chloroform. For complete recovery of

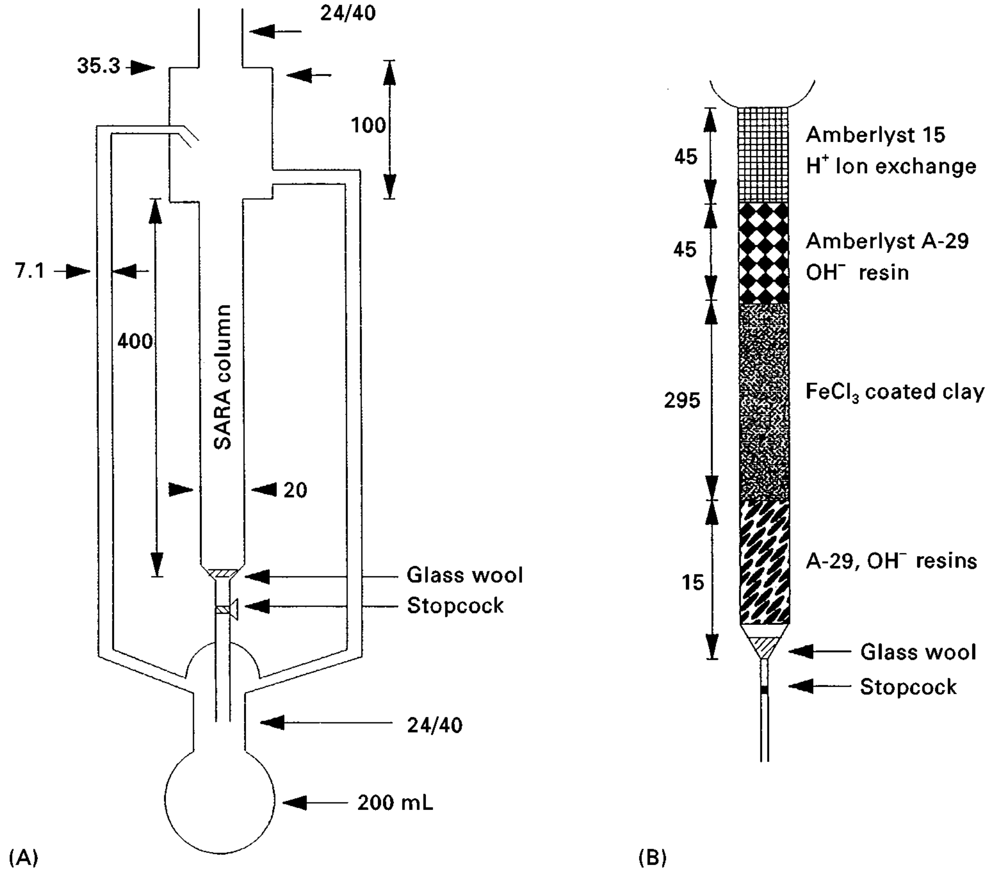

**Figure 4** Preparative SARA column. (A) Recycle column for separating resins and oils; (B) SARA column packing sequence. All dimensions are in millimetres. (Modified after Atgelt *et al.*, 1979.)

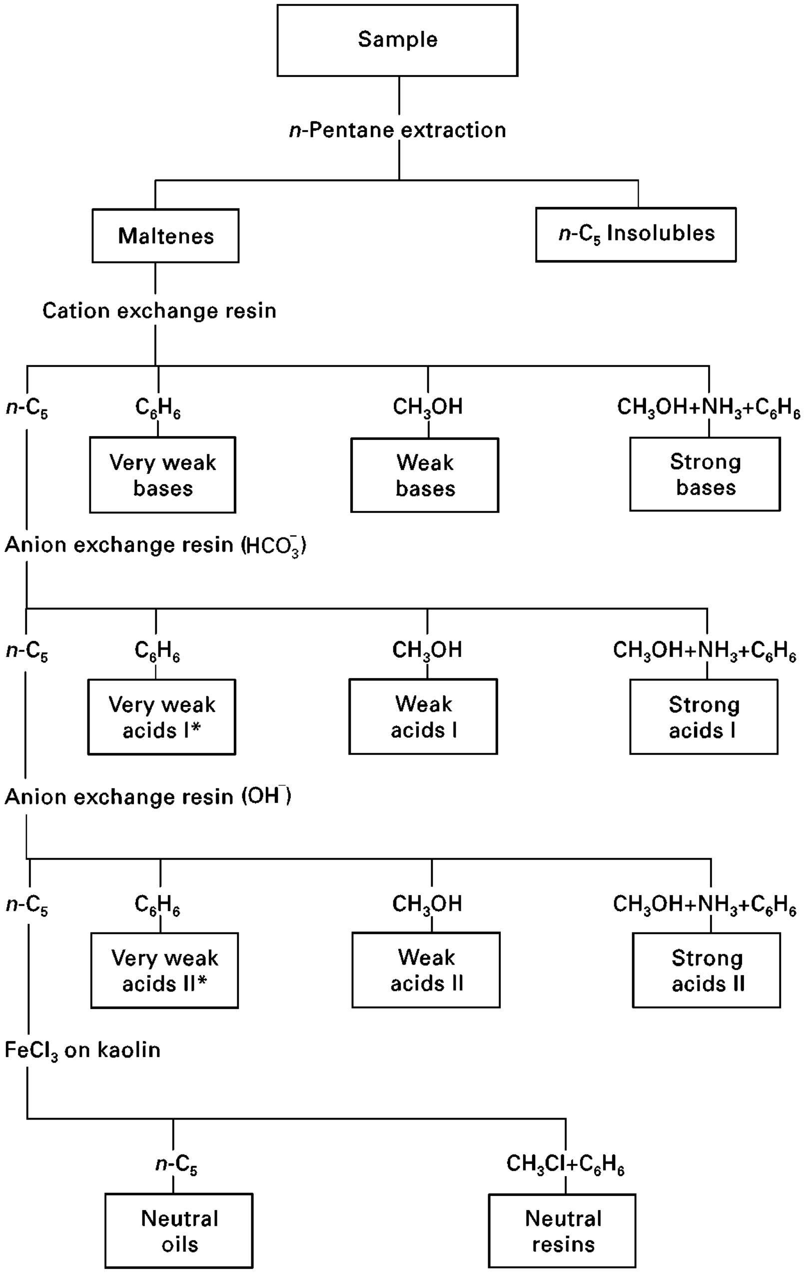

**Figure 5** Stepwise separation of resins. *Acids I are stronger than the corresponding acids II. (Reproduced with modification from Jewell, 1979.)

resins, individual cation/anion columns for stepwise separation are used. This separation process, shown in **Figure 5**, includes cation exchange, $HCO_3^-$ anion exchange, $OH^-$ anion exchange, and clay-$FeCl_3$ complexation. This scheme provides 10 groups of resins, and this demonstrates the great complexity of petroleum composition.

Separation using the SARA method is a time-consuming process. The extraction time varies with the amount and nature of the sample and especially with the content of asphaltenes and other insolubles present. Petroleum residues are commonly extracted overnight, whereas samples of tar sands, shale oils and coal liquids may require 3–4 days.

## Centrifugal Preparative Thin-layer Chromatography

### Background

Hydrocarbon-type fractions from bitumen, heavy crude and distillation residues can be separated by

TLC. This is a rapid, effective and inexpensive method which has the potential to become the major tool for semi-quantitative analysis of composition of the heavy hydrocarbon mixtures. The application of TLC to the separation of petroleum products has been successfully employed by many investigators (e.g., Lian *et al.*, 1992). A conventional TLC method can differentiate (and characterize) among heavy and residue fractions from petroleum, coal liquids, tar sands, bitumens and asphalts. A preparative, centrifugally accelerated, radial, thin-layer chromatograph (the Chromatotron) is suitable not only for quantitative determination of bitumens and asphalts, but also for the collection of various fractions. It is very important to select the appropriate solvents or solvent mixtures for separation into four fractions. **Figure 6** shows the solubility parameters of common solvents in relation to those required to dissolve the various asphalt fractions.

## Separation Procedure

One type of centrifugal TLC equipment for analysis of the constituents of hydrocarbon mixtures and expecially for determination of the composition of bitumen is illustrated in **Figure 7**. The preliminary preparation (such as rotor coating, sample preparation and application to the rotor) is important in order to achieve good results. Proper preparation of the sample and the appropriate selection of solvent to dissolve the sample is very important. Details are discussed in the following sections.

**Rotor preparation** The glass rotor should meet certain requirements: chemical resistance to solvents and developing reagents, ability to withstand temperatures needed for reaction in the rotor, mechanical strength, thickness uniformity and low cost. Silica gel 60 HF254 + 366 (with a binder) can be used as an adsorbent coated on the rotor as a thin layer. The thickness of the adsorbent layer determines the proper flow rate of mobile phase and solvent volume. **Table 2** shows the interrelationships of adsorbent layer thickness, solvent volume and flow rate.

**Sample preparation and application** The process of adding the sample to the Chromatotron rotor is one of the most important steps to ensure the success of the separation. The solvent used must be highly volatile and nonpolar because solvents with a high boiling point or high polarity are difficult to remove from the adsorbent during sample introduction. Sample injection is performed by means of a pump operated at a low flow rate in order to add a large volume of sample to the rotor and to achieve vaporization of the solvent. Sample introduction as a narrow initial band can enhance the resolution greatly. It is necessary to wait for the solvent to vaporize completely before starting the elution process. If a small

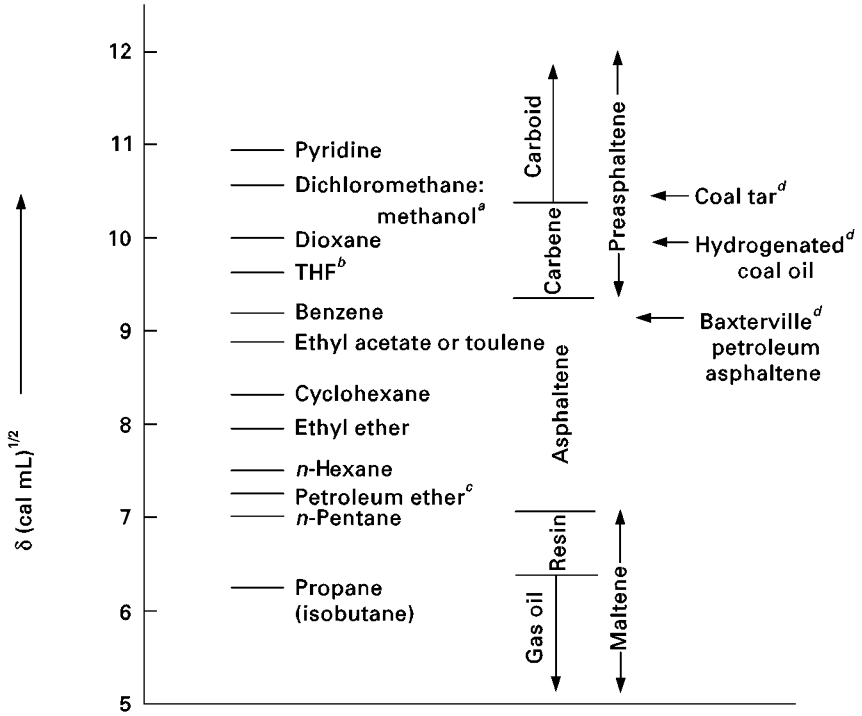

**Figure 6** Solubility parameters of common solvents in relation to the values required for dissolution of asphalt fractions. $^a$95 : 5 (v/v); $^b$tetrahydrofuran; $^c$estimated, mixture; $^d$calculated value.

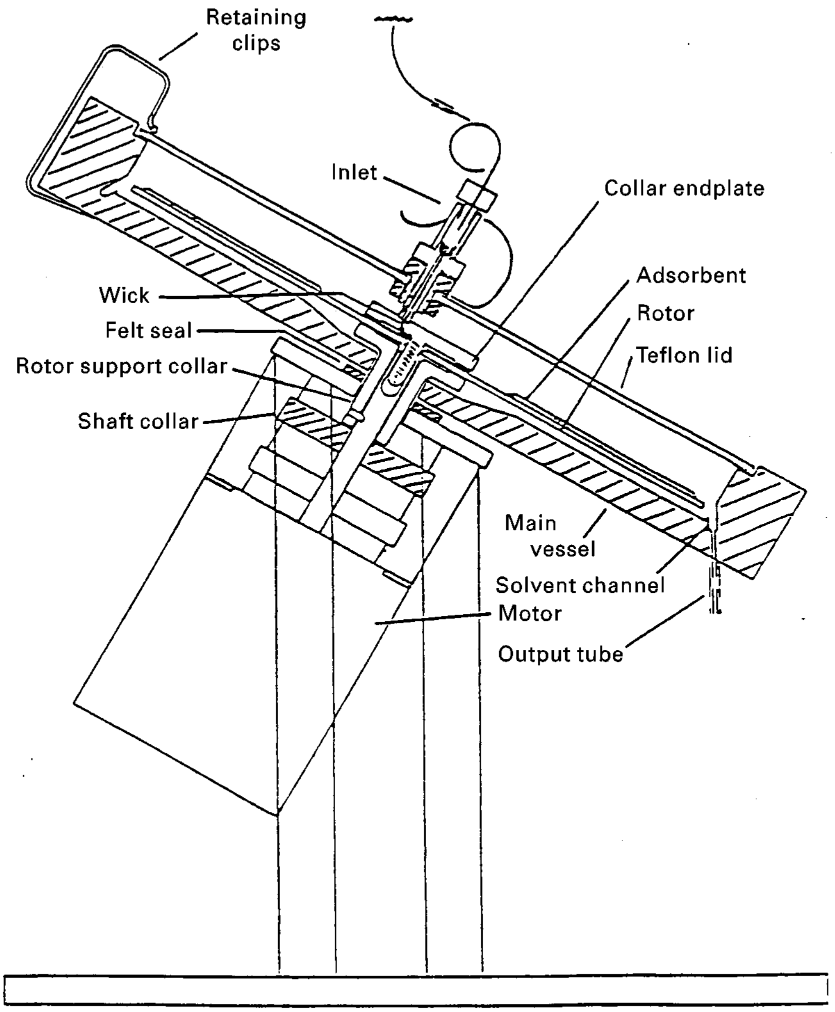

**Figure 7** Schematic diagram of the Chromatotron. (Courtesy of Harrison Research.)

amount of solvent is retained in the adsorbent after introduction of the sample, it will adversely affect the separation process due to spreading of the sample in the rotor.

**Chromatotron** The Chromatotron is a glass rotor coated with a thin layer of adsorbent. The mobile phase and solutions of compounds are introduced to the adsorbent through an inlet, which delivers them close to the centre of the rotor. A motor drives the rotor at a constant speed, which establishes radial elution of solvent and sample, forming concentric bands of separated substances that move to the edge of the rotor and are channelled by an output tube for collection. The four steps used in the procedure are:

1. Petroleum ether elution of saturated and mono- and diaromatic hydrocarbons.
2. Petroleum ether–ether mixture (80/20, v/v) elution of polyaromatic compounds and resin-1 fraction. This solvent is most suitable for ensuring that the aromatic hydrocarbons form a separate concentric band.
3. Ethyl acetate elution of resin-2 fraction.
4. Tetrahydrofuran elution of asphaltenes.

The method can be used for both qualitative and quantitative purposes; the complete process is summarized in **Figure 8**. It is very important to determine the cutoff points for the separation process for collecting four high purity fractions. There are two

**Table 2** Interrelationships among the thickness of the adsorbent layer, solvent volume and flow rate using the Chromatotron

| *Thickness of adsorbent layer (mm)* | *Flow rate (mL min$^{-1}$)* | *Solvent volume (mL)* |
|---|---|---|
| 1 | 2–4 | 5 |
| 2 | 6–8 | 10 |
| 4 | 8–10 | 20 |
| 6 | >10 | 30 |

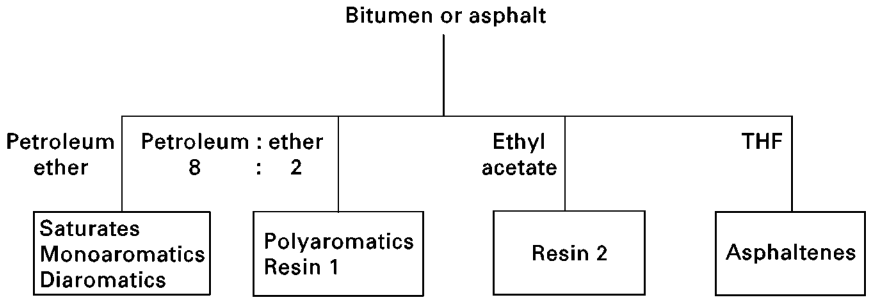

**Figure 8** Characterization of bitumen or asphalt by preparative method using the Chromatotron.

methods which can be used to identify the cutoff points:

1. UV examination: this method is useful for estimating the cutoff point between the saturates and aromatics. To identify this point, the rotor can be examined by illumination with UV light at 254 nm. The aromatics will exhibit a yellow-green colour, whereas the saturates are colourless.
2. Spot test: a spot test can be used to identify the cutoff points of saturates, aromatics and resins. Spots obtained using a freshly prepared sulfuric acid–formaldehde solution (98 + 2, v/v) have the following colours: saturated compounds, colourless; monocyclic aromatics, reddish brown; dicyclic aromatics and polycyclic aromatics, dark green.

## Thin-layer Chromatography with Flame Ionization Detection

### Background

The fingerprinting of asphalt and its components can be made by TLC-FID much more rapidly and simply than by classical column chromatography and preparative TLC. TLC-FID involves separation of solvent-extractable organics on silica-coated quartz rods called Chromarods into saturates, aromatics and polar component classes combined with semiquantitative detection by automated FID.

### Separation

The working principle of a commercially available instrument, the Iatroscan TH-10, designed to scan the adsorbent-coated Chromarods, is illustrated in **Figure 9**. A set of up to 10 coated rods is mounted on rod holders used for both chromatography and subsequent scanning. The samples to be analysed are dissolved in a solvent and applied near one end of the coated rods, which are then developed using suitable solvents, as in conventional TLC. Thereafter the developing solvent is removed by evaporation, and finally the coated rods are scanned in the FID device. The fractions resolved on each of the Chromarods are thereby succesively vaporized/pyrolysed and the ionizable carbon is detected at the collector electrode. The FID signals from each fraction are amplified and recorded as separate peaks.

In as much as the bitumens comprise a complex mixture of various substances which are impossible to isolate, a compositional analysis technique is generally used whereby a sample is separated into four fractions by performing multistage development with eluting solvents of varying polarities. The solvent elution sequence for asphalt separation is firstly, hexane for separating saturates; secondly, toluene for separating aromatics; and thirdly, dichloromethane–methanol (95/5, v/v) for separating resins. The order of eluted components from the top of the rods is: saturates, aromatics, resins and asphaltenes.

### Analysis

TLC-FID is a fast, multiple-analysis method which can be performed simultaneously on 10 samples. The total elapsed time for the analysis is less than 2 h (compared to about 2 days per sample with the SARA procedure). It is, however, destructive and does not yield fractions for further examination.

Linearity of response versus concentration is important in obtaining quantitative results. The sample size, the flow rate of hydrogen fed to the FID and the speed of scanning all have an effect on the linearity of response versus concentration, baseline stability of the FID signal and reproducibility of response values.

## Silica Gel and Ion Exchange Chromatography – Separation of Polar Fractions

### Background

The separation and study of polar compounds in crude oil and bitumens is of considerable importance in the fields of petroleum recovery and processing. Numerous methods for separation of polar compounds

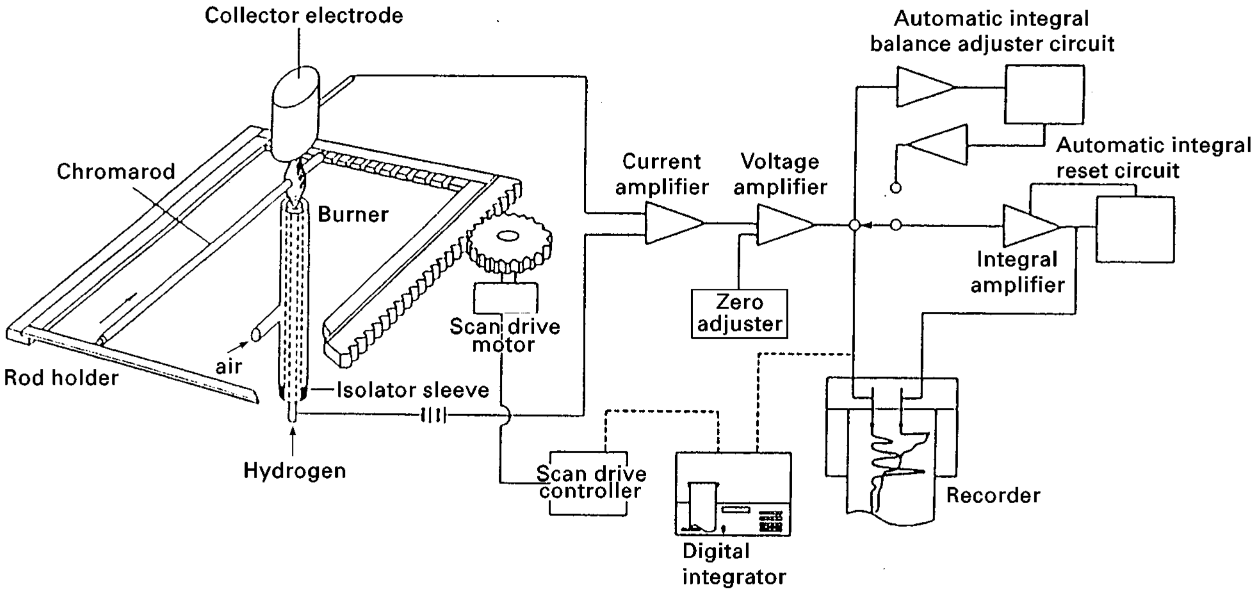

**Figure 9** Construction of a coated-rod TLC scanner, Iatroscan TH-10 Mk III. (Modified from Mukherjee, 1991.)

from crude oil and bitumens have been developed. Among these, the most widely used is the method developed by the Bureau of Mines and American Petroleum Institute. The acid and base fractions of heavy petroleum distillates or residues are isolated by ion exchange chromatography, the neutral nitrogen compounds by complexation chromatography on ferric chloride and saturates, and mono-, di- and polyaromatics by adsorption chromatography on activated alumina or on a combined alumina–silica gel column. The definition of acids or bases by ion exchange methods is based on the hydrogen donating or accepting tendency of the molecules. In as much as many polar compounds are amphoteric, their definition as acids or bases depends on the analytical sequence employed. Although this method is rather complex and tedious, it is used to separate the polar fractions from crude oil and bitumens.

### Separation Procedure

There are two stages in the separation: firstly, silica gel chromatography and secondly, ion exchange chromatography. The first stage separates the crude oil or bitumen into four fractions (I–IV: **Figure 10**). The polar fraction obtained from the first stage is further split into subfractions by the second-stage chromatography.

**Silica gel chromatography** Fractionation of crude oil or bitumen is carried out by solvents with increasing polarity through a column using Baker reagent grade, 60–200 mesh silica gel. The silica gel is thermally activated before use. The sample (10–20 g) is dissolved in 200 mL tetrahydrofuran (THF) and then filtered. The volumetric ratio of the sample to the adsorbent is about 1:35. The columns are eluted with *n*-hexane, toluene, 4:1 (v/v) toluene–methanol and 2:1 (v/v) toluene–methanol, to obtain fractions I, II, III and IV, respectively. Figure 10 shows the overall separation scheme. All samples must be evaporated to a constant weight by (rotary) evaporation and using a vacuum oven. Recovery of fraction IV is generally lower than 5% and, therefore, this fraction has not been considered for further study. The most polar

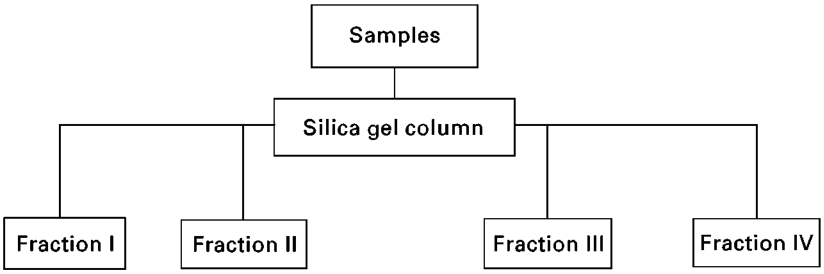

**Figure 10** Solvent fractionation scheme using a silica gel column for crude oil and shale oil.

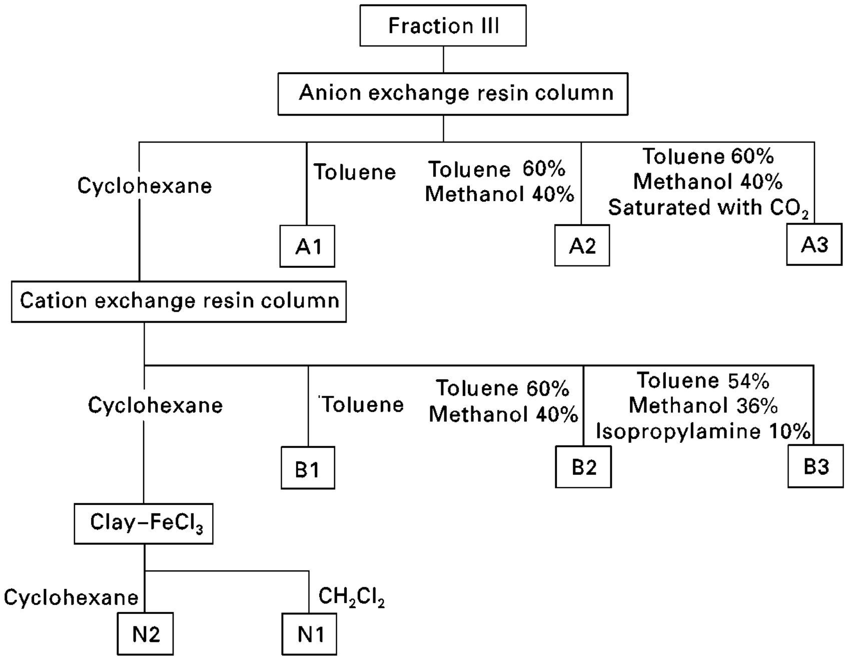

**Figure 11** Separation of fraction III (see Figure 10) to subfractions by ion exchange chromatography: A, acid; B, base; and N, neutral.

groups are present in fraction III because of the high interfacial tension compared with those for fractions I and II. Fraction III is further fractionated by ion exchange chromatography.

**Ion exchange chromatography** The polar material (fraction III) obtained using the silica gel column is mixed with cyclohexane. There are three further stages of separation: anion exchange, cation exchange and clay–$FeCl_3$ complexation. In order to obtain acidic fractions from the polar material, a strongly basic anion exchange resin is used and an eluting scheme is employed, based on increasing polarity of the solvent. This scheme includes four elution steps: cyclohexane; toluene; a 3:2 mixture of toluene–methanol; and a 3:2 mixture of toluene–methanol saturated with $CO_2$. The first fraction eluted by cyclohexane from the anion exchange resin is further fractionated by passing it through a strongly acidic cation exchange resin. The same elution scheme as in the first elution step is used, with a slight modification for the cation exchange elution in that the fourth step uses a 5.4: 3.6:1.0 mixture of toluene–methanol–isopropylamine. Three basic fractions result from these fractionations.

A column packed with clay–$FeCl_3$ is employed to separate further the material obtained from the cyclohexane elution in the second stage. The solvents used to obtain the neutral fractions are cyclohexane and dichloromethane. The volumetric ratio of sample to adsorbent is around 1:10 for the three adsorbents used. Two neutral fractions can be obtained at this last stage of separation process. A detailed diagram of the scheme is given in **Figure 11**.

## Conclusion

The SARA separation method is at present the most comprehensive and most widely used method for the study of heavy oil fractions. Despite its shortcomings (tedious, time-consuming and possible losses), it can be viewed as a standard by which other methods should be evaluated. Preparative, centrifugal, TLC with the Chromatotron is capable of isolating crude oil and bitumen in the amounts required for further identification by other instrumental techniques.

TLC-FID has a great potential of becoming a standard method for quantitative evaluation of crude oil and bitumen. It is rapid, accurate, requires only small samples and does not involve tedious procedures in comparison to the SARA method. Solvent fractionation using silica gel rods simply separates crude oil and bitumen into four fractions. With automatic instrumentation, the method can be used routinely for industrial purposes. The combination, of fractionation by solvents and ion exchange followed by complexation, is a good method for studying the composition of crude oil and bitumen. Ion exchange processes rely on the great selectivity for acidic and basic components in the crude oil. The complexation process enables the neutral nitrogen compounds to be separated. This scheme provides 10 resin fractions, which demonstrates the great complexity of crude oil composition.

The choice of a procedure primarily depends on the information required. Combining different separation steps and/or short-cuts to achieve a specific purpose is possible.

*See also:* **II/Chromatography: Liquid:** Mechanisms: Ion Chromatography. **Chromatography: Thin-layer (Planar):** Modes of Development: Conventional. **III/Crude Oil: Liquid Chromatography. Flame Ionization Detection: Thin-Layer (Planar) Chromatography. Flash Chromatography. Metal Complexes. Petroleum Products:** Liquid Chromatography.

## Further Reading

Atgelt KH, Jewell DM, Latham DR and Selucky ML (1979) *Chromatography in Petroleum Analysis*, pp. 186–214. New York: Marcel Dekker.

Boyd ML and Montgomery DS (1962) Structural-group analysis of the asphaltene and resin components of Athabasca bitumen. *Fuel* 41: 335–350.

Dickie JP and Yen TF (1967) Microstructures of the asphaltic fractions by various instrumental methods. *Analytical Chemistry* 39: 1847–1852.

Dickie JP, Haller MN and Yen TF (1969) Electron microscopic investigation on the nature of petroleum asphaltics. *Journal of Colloid Interface Science* 29: 375–484.

Eremenko NA (1990) *Petroleum Geology Handbook*. Los Angeles, CA: OSI.

Hilman ES and Barnett B (1937) The composition of cracked and uncracked asphalts. *Proc. Am. Soc. Testing Materials*, 37: 558.

Hirsch DE, Hopkins RC, Coleman HJ *et al.* (1972) Separation of high-boiling petroleum distillates using gradient elution through dual-packed (silica gel–alumina gel) adsorption columns. *Analytical Chemistry* 44: 915.

Jewell DM (1979) *Chromatography in Petroleum Analysis,* pp. 284–285. New York: Marcel Dekker.

Jewell DM, Weber JH, Bunger JW *et al.* (1972) Ion-exchange, coordination and adsorption chromatographic separation of heavy-end petroleum distillates. *Analytical Chemistry* 44: 1391.

Katz M (1934) Alberta bitumen. *Canadian Journal of Research* 10: 435–451.

Lian H, Lee CZH, Wang YY and Yen TF (1992) Characterization of asphalt with the preparative Chromatotron. *Journal of Planar Chromatography* 5: 263–266.

Mukherjee KD (1991) *Handbook of Thin-layer Chromatography,* pp. 339–350. New York: Marcel Dekker.

Sadeghi KM, Sadeghi MA, Wu WH and Yen TF (1989) Fractionation of various heavy oils and bitumen for characterization based on polarity. *Fuel* 68: 782–787.

Shue FF and Yen TF (1981) Concentration and selective identification of nitrogen and oxygen-containing compounds in shale oil. *Analytical Chemistry* 53: 2081–2084.

Suatoni JC and Swab RE (1976) Preparative hydrocarbon compound type analysis by high performance liquid chromatography. *Journal of Chromatographic Science* 14: 535.

Speight JG, Wernick DL, Gould KA *et al.* (1985) Molecular weight and association of asphaltene, a critical review. *Rev. Inst. Fr. Pét.* 40: 51–62.

Wang YY and Yen TF (1990) Rapid separation and characterization of fossil fuels by thin-layer chromatography. *Journal of Planar Chromatography* 3: 376–380.

Weinberg VA, White JL and Yen TF (1983) Solvent fractionation of petroleum pitch for mesophase formation. *Fuel* 62: 1503–1510.

Yen TF (1990) Asphaltenic materials. In: *Encyclopedia of Polymer Science and Engineering*, 2nd edn, pp. 1–10. New York: John Wiley.

Yen TF and Chilingarian GV (1994) Asphaltenes and asphalts 1. In: *Developments in Petroleum Science* 40A. New York: Elsevier Science.

Yen TF, Erdman JG and Pollack SS (1961) Investigation of the sturcture of petroleum asphaltenes by X-ray diffraction. *Analytical Chemistry* 33: 1587–1594.

Yen TF, Shue FF, Wu WH and Tzeng D (1983) Ferric chloride-clay complexation method. Removal of nitrogen-containing components from shale oil and related fossil fuels. *ACS Symposium Series* 230: 457–466.

---

# CARBAMATE INSECTICIDES IN FOODSTUFFS: CHROMATOGRAPHY AND IMMUNOASSAY

**G. S. Nunes**, Federal University of Maranhão/UFMA, São Luís-Ma, Brazil
**D. Barceló**, CID/CSIC, Barcelona, Spain

## Introduction

Pesticides have received special attention over the years, due mainly to the problems of environmental and food contamination. Analytical methods for determining pesticide residues have their main application in the control of food for human consumption,