NOTES ON TECHNIQUES REFERRED TO IN PROCEEDINGS

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A generic name for a series of separation techniques used in analytical chemistry. Discovered by the Russian botanist, Michael Twsett in 1903, it was used initially in the separation of plant pigments, hence the name, but has since been extended to applications in most areas of organic and inorganic chemistry. The extension of this elegant and ingenious technique forms one of the most fascinating studies in the history of science.

Although several forms of the technique exist, they all have the same principle in common, which is illustrated in Fig. 1. A mixture of compounds to be separated is applied in solution to the *stationary phase* and components of the mixture are made to move through the stationary phase by movement of the *mobile phase*. Different substances move at different speeds and are thus separated one from another. The nature of the stationary and mobile phases determines the type of chromatography.

Figure 1. Separation of components on a paper chromatogram. Solvent has soaked upwards, carrying & resolving the components of the mixture.

Column Chromatography, as its name implies, uses a column of adsorbent (generally a powder) down which a solvent is allowed to pass by gravity.

High Performance Liquid Chromatography (HPLC) uses similar principles but employs very much finer powders thus giving superior resolution. These columns require the solvent (mobile phase) to be forced through the powder (stationary phase) at high pressures (about 500-2,000 p.s.i.).

Paper Chromatography makes use of special absorbent paper (rather like blotting paper) as the stationary phase and various liquid solvents as the mobile phase.

Thin Layer Chromatography (TLC) uses a fine powder (generally silica gel) baked upon a glass plate or plastic film as the stationary phase (the plate or film merely acting as an inert support).

Gas Chromatography employs a stationary phase consisting of a liquid of very low volatility, adsorbed onto the surface of fine powder, this being contained in a tube which can be heated in an oven. The mobile phase is a gas, generally nitrogen. Compounds are volatilised by the temperature of the oven and pass as pockets of vapour at varying speeds, to emerge through a special detector.

Thus, all these techniques use the different migrating speeds of substances under the conditions used to discriminate between them.

Ultraviolet absorption spectrophotometry

Many organic compounds absorb ultraviolet light, in a manner analogous to the way coloured compounds absorb visible light. The amount of ultraviolet light absorbed at any wavelength may be used as both an identification and as a quantitative measure of the amount of substance present. Instruments now exist which can scan the absorption of ultraviolet light continuously from short to long wavelength, and plot automatically the amount absorbed. This produces an ultraviolet absorption spectrum (see Fig. 2). Although peak absorptions of compounds are generally recorded to characterise them, the *shape* of the curve (i.e. the ratio of absorptivity at two or more different wavelengths) may be equally valuable in characterisation.

FIG 2 A

Figure 2. Ultraviolet absorption spectra for
A. Caffeine
B. Quinine

Mass Spectrometry

This technique is used extensively in the identification of very small quantities of substances; it is generally, though not always, capable of precise characterisation. The principle is illustrated in Fig. 3. Molecules are introduced into the mass spectrometer in the vapour state and are ionised by bombardment with electrons. Positive ions are thus produced some of which have sufficient excess energy to cause them to fragment into ions of smaller mass. The ions are accelerated through a voltage difference and pass through a magnetic field causing the hitherto linear path to assume a curve. Heavy particles are curved less than light ones and by altering the magnetic field slightly each ion in turn can be

focussed at an appropriate detection device. The relative quantity (abundance) and mass of each fragment is recorded, and constitutes the mass spectrum of that compound. The whole operation must, of course, take place *in vacuo*. The method described here and illustrated (see Fig. 3) is "electron impact mass spectrometry". Other techniques also exist for the formation of charged particles and their analysis, but the principles employed are essentially the same.

Figure 3. Principles employed in the mass spectrometer.

Compounds can be introduced into the mass spectrometer either directly or via a gas chromatograph; the latter technique ensures that the substance introduced is in a highly purified state. Such methods require special separators to remove the carrier gas from the eluate and prevent it entering the mass spectrometer.

The development of chromatography and mass spectrometry probably comprise the most important development in analytical chemistry this century.