SUMMARY

Chromatography is a powerful and versatile tool for separating closely related chemical species. In addition, it can be employed for the qualitative identification and quantitative determination of separated species. Chromatography is widely used for recognizing the presence or absence of components in mixtures that contain a limited number of species whose identities are known. Chromatography owes its enormous growth in part to its speed, simplicity, relatively low cost, and wide applicability as a tool for separations.

Chromatography is based on selective adsorption of compounds on a solid (or liquid) with high surface area (the *stationary phase*). As the solute mixture passes over the solid, the components are adsorbed and then released from the surface at differing rates. This means that the solutes are continuously partitioned between the adsorbent and the *mobile phase*, either a gas or a solvent mixture. The stronger the interaction of the solute with stationary phase, the slower the solute will progress. The motion of the solute and solvent through the stationary phase in called *elution*. The process is analogous to fractional distillation or extraction, in which different compounds are partitioned between liquid and vapour, or between two immiscible liquids, respectively.

Chromatography can be carried out in many ways. In column chromatography and in thin layer chromatography (TLC) a solution of the mixture flows over a solid adsorbent. Separation occurs as molecules are adsorbed and desorbed while passing over the surface. In paper chromatography applications, the mixture is partitioned between water molecules adsorbed on the paper and a solvent that moves over the paper. In gas **c**hromatography (GC, also called vapour phase chromatography), a mixture of volatile compounds is separated by

passing the vapour over an adsorbent packing in a long, heated tube. Chromatographic methods have high "resolving power", *i.e.* they are capable of sharp separations of closely related compounds, particularly when very small samples are used. Both GC and column chromatography can be carried out with instruments that detect extremely small amounts of compounds in the gas or liquid stream, as it leaves the chromatographic column. In GC, the detector responds to the thermal conductivity of the gas stream or the ionisation of the gas as it passes through a flame. This gas stream can be introduced straight into a mass spectrometer (MS) to give the very important technique of GC-MS which facilitates the separation and identification of samples within a mixture. In liquid (column) chromatography instruments, the detector senses changes in the refractive index or uv-visible absorption of the solution. Signals from the detector corresponding to each component in the mixture and proportional to the amount of the compound are recorded automatically on a chart. These instruments thus provide powerful methods for quantitative analysis.

Chromatographic techniques have become very important in industry for the purification and separation of intermediates in multi-stage syntheses. (Such separations have to be done in batches rather than in continuous flow.) In terms of scientific advances, one of the major innovations in the past five years has been the development of efficient columns capable of separating specific chiral compounds from a mixture. They work by the stereospecific adsorption of one enantiomer onto the surface of the stationary phase. The resin contains only one enantiomer, hence its stereo selectivity towards other chiral molecules. The cost of a column for chiral separations can be roughly three times (or even higher) the cost of a standard analytical column. A similar type of chromatography, affinity chromatography, and works on a similar principle – a substrate is covalently bound to a resin, and only enzymes with vacant sites in the correct orientation can interact with these sites.

Research has also shown that graphite-based HPLC packing materials can separate compounds with low or modest polarities, very efficiently.

Studies of particle and pore sizes have shown that the surface area of graphite-based materials in HPLC columns can be as high as 1000 m^2 g^{-1}. Some classes of compound that have been separated by such columns include:

1. Aromatic hydrocarbons
2. Alkylnapthalenes
3. Methylphenols
4. Polychlorinated biphenyls
5. Steroids; and
6. Some amino acids

By using different solvent systems one area which has been found to have great potential is supercritical fluid chromatography (SFC). A supercritical fluid is one at a temperature and pressure above its critical point. Supercritical carbon dioxide (used to extract caffeine from coffee) in HPLC columns has the solvating properties of a liquid and the transport properties of a gas. Another advantage is that extraction of the solutes from the eluate is easy – the carbon dioxide is simply allowed to evaporate.
However, supercritical carbon dioxide would not be a good solvent for a chromatography column if the eluate were to be analysed by infrared spectroscopy, because of the strong spectral absorption of carbon dioxide which would obscure the absorptions of the eluted compounds. Supercritical xenon has been found to achieve good separation of compounds such as polyaromatic hydrocarbons and if the eluate flows through a microcell no absorption due to xenon is observed. This is a particular advantage if SFC is to be linked with infrared spectroscopy.