spectrophotometric analysis a few milliliters, the solutions may first have been extracted from several liters of sample.

The precautions necessary to avoid either contamination or loss of material at these low concentrations, during the subsequent analysis, are often not appreciated.

Precautions

A few typical precautions should indicate the caution that is necessary:

1. The analysis should be performed in a laboratory as free as possible from the analyte. Remember that many of these trace contaminants are solvents frequently found in analytical laboratories.
2. Any stock solvents should be safeguarded, minimising exposure to the atmosphere and avoiding sample withdrawal with potentially contaminated pipettes or syringes.
3. Samples and working standards should be placed well away from more concentrated solutions or stock solvents.
4. As traces of pesticides are commonly found in laboratory solvents, pesticide free grade solvents should be used for these analyses.
5. Glassware should be scrupulously cleaned or new, if at all possible.

Such is the problem of contamination that the practical lower limits of detection can often be limited by the background concentrations of the analyte (or of interfering components) in the reagents or laboratory atmosphere.

Extraction techniques for chromatographic analysis

Extraction of the compound of interest from the aqueous sample into an organic solvent is commonplace before any chromatographic analysis. The major reasons for this are as follows:

1. To separate unwanted components present in large excess.
2. To separate minor components which have overlapping peaks with the components of interest.
3. To concentrate the components of interest.

For some samples, the extraction may be the only pre-treatment necessary before injection into the chromatograph while for more complex samples it may be just one stage of a multi-stage process. Most of the techniques described may be integrated with the chromatographic stage and subsequent data handling. There is no one method of choice. The best method will be dependent on the following:

1. The chemical and physical properties of the compounds being determined and potential interferences.
2. The choice of gas or liquid chromatography as the separative technique.
3. Whether solvent-free methods are preferred. Such methods remove the concern of possible contamination of the laboratory and its atmosphere (health effects and cross-contamination of other samples), contamination of aqueous waste and the cost of disposal of the waste solvent.
4. The number of samples to be analysed. If you have a large number of samples and are working in a well-equipped laboratory, the techniques which are fully integrated with the chromatograph may be preferable. In a smaller laboratory, dedicated instruments may not be justifiable and simpler methods may be preferred.
5. Whether you would wish to perform the field extractions.

The extraction methods are common in many areas of chemical analysis. Try thinking of methods you have already come across before studying the following sections. These methods are summarised in Fig. 16.18.

Table 16.4. Steps in a solid-phase extraction process.

Condition column with methanol
Load sample
Wash column with water
Pass air through the column to remove as much water as possible (an option if the elution solvent is immiscible with water)
Elute with a suitable organic solvent

Manifolds are available which allow processing of a number of samples simultaneously. In addition, SPE set-ups can be directly coupled to HPLC systems.

Advantages of solid-phase extraction

Advantages of solid-phase extraction over liquid-liquid extraction are:

1. It is a very rapid process and can easily be automated.
2. High concentration factors can easily be achieved.
3. Solvent consumption can be much lower.

There are, however, instances where liquid-liquid extraction may still be the method of choice. These are usually when there are solids present or there is a high loading of organic material in the sample (e.g. humic acid) which could block or overload the column.

A further development is the use of extraction discs where the adsorbent material is held within the fiber structure of a polytetrafluoroethylene (PTFE) filter disc. After pre-washing the disc with a portion of the final eluting solvent and conditioning with methanol, the extraction procedure is simply to pass the sample, by suction, through the filter. The extracted components are then eluted by using a suitable solvent. The advantages of discs over columns include a higher sample throughput (several hundred milliliters of sample may need to be passed through the filter if a high concentration factor is needed) and the lower likelihood of the filter clogging with particles. Some standard methods now include liquid-liquid extraction and solid-phase extraction as alternative procedures.

Head-space analysis

In this technique, the water sample is placed in a container with a septum seal in the lid and an air space above the sample. The most simple procedure is then, after allowing for the air to equilibrate with the water, to inject an air sample (containing volatile organic components) into the gas chromatograph. This technique overcomes problems found in liquid-liquid extraction resulting from solvent interference. The sensitivity towards a particular component will, however, be dependent on its volatility, favouring low-molecular-mass, neutral components. The overall sensitivity of the technique may be increased by heating the sample. Be aware, however, that you are also increasing the vapour pressure of the water and care should be taken to check the water compatibility of the chromatographic column.

Purge and trap techniques

These techniques extract the volatile organic content from the sample by using a purge gas stream. In many instruments, the organics are collected in a short tube of adsorbent material such as activated charcoal or a porous polymer (e.g. 'Tenax'). After the collection period, the tube is flash-heated to release the organics into the gas chromatograph. Other instruments collect the volatile components into a secondary liquid nitrogen cold trap. Rapid heating of this trap then releases the organics into the chromatograph.

Solid-phase microextraction

This technique could be seen as using both the principles of solid-phase extraction and head-space sampling. A fiber which is originally contained within a syringe needle (Fig. 16.20) is exposed either to the stirred sample or to the head-space above the sample. The fiber typically consists of fused silica with a coating of polydimethylsiloxane, or alternatively polyacrylate, with the phase being chosen according to the compound being determined. The dissolved components partition between the sample and fiber. After equilibration is complete (2–15 minutes for liquid samples), the fiber is withdrawn into the syringe needle for storage prior to analysis.

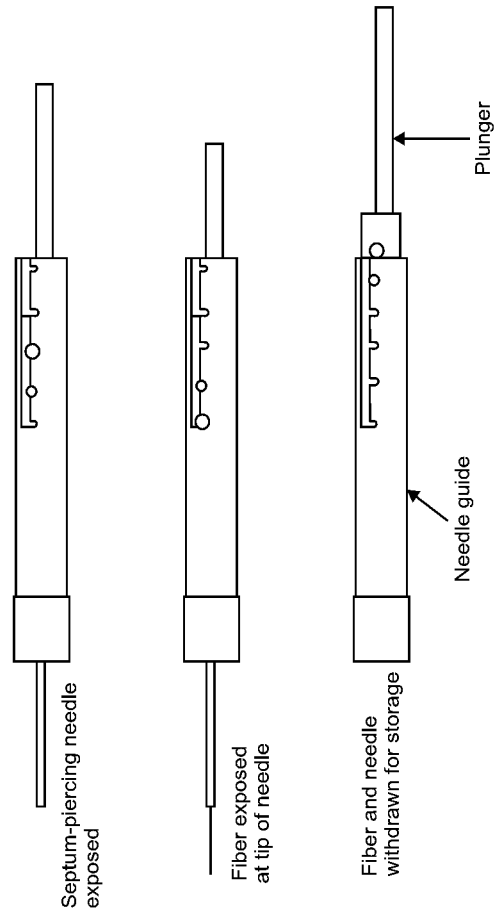

Fig. 16.20. Schematic of the solid-phase microextraction process.

The method cannot only be used for volatile organics but also for semi-volatile pollutants, such as chlorinated pesticides. Different fibers are used. A smaller depth coating (7 μm) is suitable for the semi-volatile compounds and a thicker (100 μm) coating for volatiles. Fibers are also available for the extraction of polar organic compounds (e.g. phenols) which are often very difficult to extract by other techniques. In the case of phenols, sample modification (e.g. lowering the pH and adding sodium chloride) increases the extraction efficiency.

Subsequent analysis can be carried out by either gas or liquid chromatography. With gas chromatography (GC), the fiber is directly introduced into the GC injector inside the syringe and re-exposed once the needle has pierced the injection septum. Most GC systems can be used without modification. Desorption of the organics takes place into the carrier gas, although this can take 20–30 seconds. In order to overcome this problem, a technique known as *cryo-focusing* is used. The sample is condensed on to the top of the column held at a low temperature, typically 40°C. Rapid heating of the column then releases the sample. If the subsequent chromatographic method is high performance liquid chromatography (HPLC), then the compounds can be desorbed by immersion of the fiber into a suitable solvent. The solution is then injected into the chromatograph. Injection systems are also available which permit the introduction of the fiber directly into the mobile phase, where the latter flows along the length of the fiber on to the head of the analytical column.

The fibers can be re-used as many as 50–100 times. The advantages of the technique include its simplicity and low cost of apparatus. As the complete extract is introduced into the chromatograph, this can lead to 100–700× lower detection limits than liquid-liquid extraction. No solvent is injected and short narrowbore columns can be used with gas chromatography. These columns would become flooded with solvent if used after liquid-liquid extraction. The fibers can cope with high levels of contamination and so they can be used for dirty samples such as waste water. One disadvantage of the method is that it is an equilibration technique. Extraction of each compound will be different and so calibration is necessary for each of these. In addition, changes in composition of the water samples could alter the extraction equilibria and hence the extraction efficiency.

Gas chromatography

Chromatographic separation of a mixture occurs by the differential partition of the components between a stationary phase and a mobile phase. In gas-liquid chromatography, the mobile phase is a gas and the stationary phase is a liquid adsorbed on, or chemically bonded to a solid. The main components of a gas chromatograph are shown in Fig. 16.21.

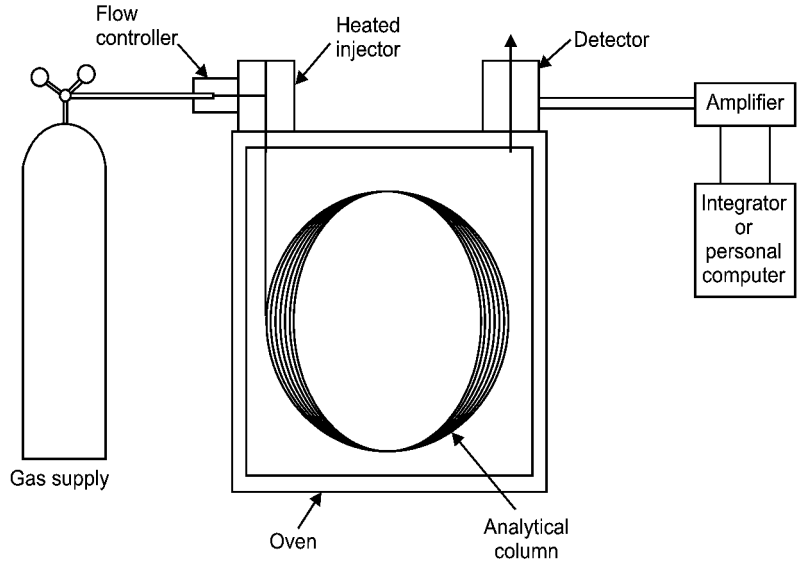

Fig. 16.21. Major components of a gas chromatograph.

Contents at a Glance