CHAPTER 3

SAMPLE PREPARATION TECHNIQUES

3.1 INTRODUCTION

Samples, which require analysis, are often too dilute, too complex or incompatible with the chromatographic system. Hence, some form of sample preparation is essential before any instrumental analysis. Ideally, sample preparation should require minimal effort and expense. Moreover, minimal sample preparation will decrease the amount of experimental uncertainty in the results obtained. Due to the nature of our research, we will only discuss those techniques concerned with the pre-concentration of gaseous volatiles from air. The flow diagram below shows a brief summary of the techniques commonly used.

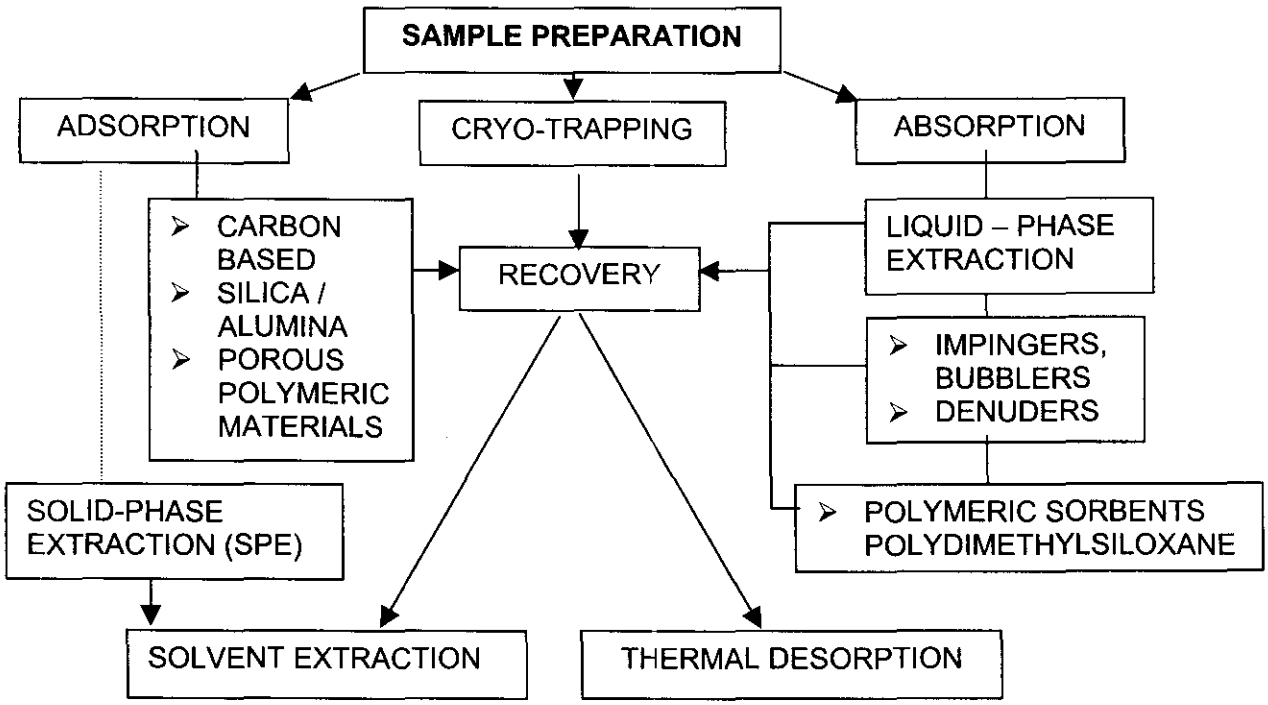


Figure 3.1 Flow diagram of sample preparation techniques for gaseous organic compounds.

3.1.1 DYNAMIC HEADSPACE SAMPLING

This technique is described here as it is used in our study to load our derivatising reagent and for analysing acetaldehyde in beer. Dynamic headspace sampling employs the continuous removal of the headspace vapours from a solid/liquid matrix, followed by collection on a trap such as a cryo-trap, solid phase extraction device or ad/absorbents [67-70].

The recovery (R) of analyte, from the dynamic headspace onto the trap can be described by the equation [67-70]:

$$R = 1 - \exp [-Ft / KV_L + V_G] \qquad (3.1)$$

Where F is the stripping gas flow rate, t is the stripping time, K the gas-liquid partition coefficient, V_L is the sample volume and V_G the volume of gas passed through the liquid in time t. This equation was derived based on the assumption that the system is in thermodynamic equilibrium, no breakthrough occurs on the trap (i.e. a closed loop), the liquid matrix is involatile and the partition coefficient is independent of concentration [67-70].

3.2 ADSORPTION

Adsorption is a physical process occurring on the surface of adsorbents. As analytes are retained on active surfaces on the sorbent, the amount of adsorption that occurs is related to the available surface area of the sorbent, which in turn is related to the porosity of the material. The rate of adsorption is determined by the structure of both the micropores and the molecules moving into the pores [71]. Table 3.1 in Appendix 1 lists the more common types of sorbents used for pre-concentration, as well as their structure, uses, surface areas and pore diameters, advantages and disadvantages.

Adsorption tubes are prepared by packing the sorbent into glass tubes, of varying sizes depending on the application.

When choosing a sorbent for pre-concentration, it is not only important to see how well compounds are adsorbed that is, their retention, but also how easily they can be recovered.

Carbon-based adsorbents are cheap, all purpose pre-concentration sorbents. However, desorption of the sorbates (particularly polar compounds) may prove difficult and water accumulation is high, making them unsuitable for thermal desorption with cryogenic focusing [19].

Porous polymers are typically used for pre-concentrating high molecular mass and non-volatile compounds such as pesticides. They are popular because they are relatively inert, have large surface areas and are hydrophobic. They also allow for collection of large sample volumes (100L) at high flow rates [73]. However, their general disadvantages include the displacement of VOC's especially by CO_2 [19], and the irreversible adsorption of certain compounds, such as amines [19]. Furthermore oxidation, hydrolysis and polymerisation of the sample may occur [19]. Except for Tenax, these adsorbents are thermally unstable above 250°C, which makes them unsuitable for thermal desorption as this leads to artefact formation [19]. At the same time, sorbents are not reusable after solvent desorption. Careful purification, which usually involves Soxhlet extraction with high purity solvents, of these sorbents is compulsory before they can be used for trace analysis [19]. Finally, porous polymers are more expensive than the charcoals.

All solid sorbents are ideally suited to trapping a particular series of compounds. In an attempt to trap a wider range of compounds, multi-layered traps, which utilise the best features of each adsorbent, have been prepared [74,75]

Sorbents used with solvent extraction are usually silica gel, activated charcoal, Anasorb 747, carboxens (carbonised porous polymers), porous polymers and carbon

molecular sieves. Those used in sampling with thermal desorption include Tenax, Chromosorb 106, Graphitised carbons and carbon molecular sieves [71].

3.2.1 SOLID-PHASE EXTRACTION (SPE)

Solid-Phase Extraction (SPE) is not, traditionally, a technique used for pre-concentrating gaseous compounds. However, it has been included in the discussion because it has been used, predominantly, as a reagent coated sorbent [46,49,54] for derivatisation, and for the extraction of the derivatised products formed during liquid extraction [47].

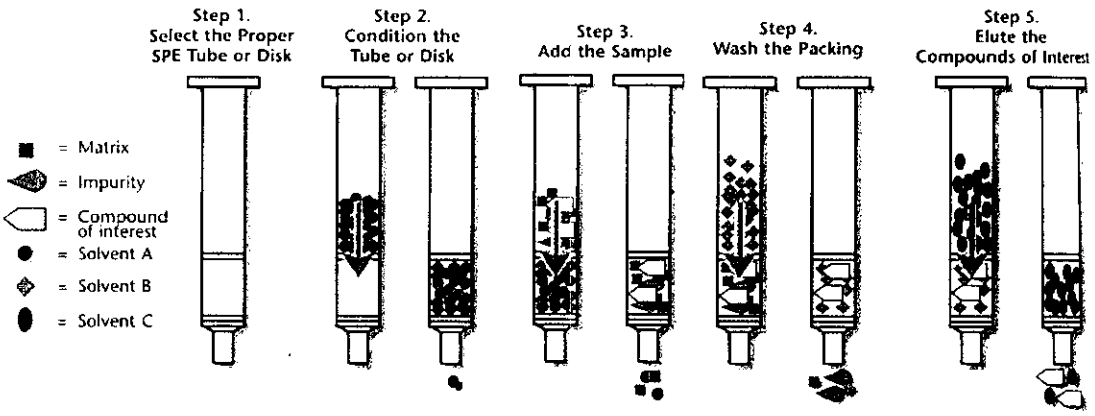


Figure 3.2. Steps involved in the SPE technique [76].

The SPE device is depicted in Figure 3.2. The SPE cartridge consists of a packed adsorbent column between two fritted plastic/metal disks in a polypropylene open syringe barrel [76].

Unlike LLE, which involves the partitioning of the analyte between two immiscible phases, SPE involves the partitioning of the analyte between a solid and a liquid phase. The analyte is extracted when its affinity for the solid phase is greater than for liquid phase. Later, the analyte is removed by extraction with a solvent for which the analyte has a greater affinity.

The liquid phase is passed through the cartridge by suction or positive pressure (e.g. gravity or gas pressure from a syringe).

Retention is caused by the intermolecular forces experienced between the analyte, the active sites on the sorbent-surface and the liquid phase [77].

Common sorbents used for SPE are based on silica gel with a modified surface. According to the chemical groups bonded to the silica, the phases are classified as non-polar, polar or ion-exchangers.

Octadecyl surface phases (C18) are used for the reverse-phase extraction of non-polar compounds in aqueous solution. The shorter octyl phases (C8), are used to extract medium polarity compounds, while silica gel and alumina oxides are used for extracting polar compounds [78].

SPE is simple, requires less solvent and time than LLE, and it is easily automated. However, it becomes a bit tedious with all the steps required to prepare the sorbent and then extract the analyte, as depicted in figure 3.2. Also, the packing quality varies from cartridge to cartridge [47].

To overcome problems encountered with the SPE cartridges, disk devices have been developed. They are either membranes or sorbents that have been packed into circular disks 0.5mm thick and 4 to 96mm in diameter. The sample processing rates are faster than those of the SPE columns and the small diameter disks are ideal for processing smaller samples [47].

3.2.2 BREAKTHROUGH VOLUME

Breakthrough volume is a measure of the retention of an analyte on a sorbent i.e. retention capability. Tubes that are packed with ad/absorbents can be regarded as chromatographic columns, operating under frontal analysis conditions with a constant concentration of analyte. The analyte will continue to be ad/absorbed in the trap until it reaches its breakthrough volume (V_b). This is usually when 5% of the initial

concentration of the analyte has started to elute from the trap. Therefore, the maximum sampling volume or breakthrough volume (V_b), is described by Raymond and Guiochon [79] as:

$$V_b = V_r \times (1 - (2 / \sqrt{N})) \quad (3.2)$$

Where V_r is the retention volume and N the number of plates of the trapping column. However, for short "columns" which have a low number of plates (N), Lövkvist and Jönsson [80], have suggested a more realistic model for breakthrough volume, which can be described by:

$$V_b = V_r \times (a_0 + (a_1 / N) + (a_2 / N^2))^{-1/2} \quad (3.3)$$

Where a_0, a_1 and a_2 are coefficients for different values of the breakthrough level b described as [80]:

b = total amount of analyte eluted from trap / total amount of analyte sampled

b can vary from 0.1, 1, 2 to 10%, the popular value being 5%.

Baltussen et al [81], have applied this theory for breakthrough volume at 5%, on their silicone packed beds, giving:

$$V_b = V_0 \times (1+k) \times (0.9025 + (5.360 / N) + (4.603 / N^2))^{-1/2} \quad (3.4)$$

Where V_0 is the trap dead volume and k the capacity factor.

3.3 CRYO-TRAPPING

Volatile compounds can be trapped at temperatures lying far below their boiling points. This is usually achieved by collecting whole air samples through a steel tube or capillary, which is cooled by using either liquid nitrogen or carbon dioxide. To increase the condensing surface, the tubes are packed with an inert material possessing a high surface area such as glass wool or beads. The tube is then heated ballistically to a suitable injection temperature and the analytes are transferred onto the column. Unfortunately this set-up is not always sufficiently portable for field work, and extra care must be taken when sampling in humid environments as pre-concentrated water will freeze and block the trap [19].

3.4 ABSORPTION

Absorption is synonymous with dissolution and partitioning. In this process, the analyte will dissolve into a liquid where it is retained until it is thermally desorbed or preferentially extracted into a different solvent for which the analyte has a greater affinity.

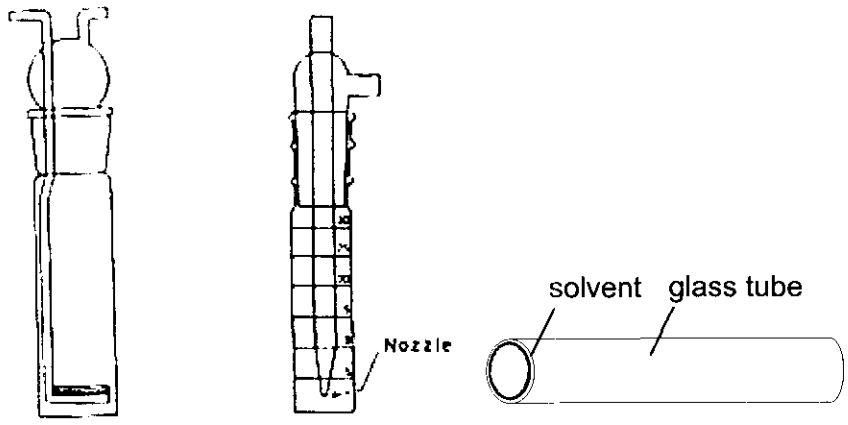


Figure 3.3. Liquid-phase extraction devices[82].

3.4.1 IMPINGERS AND BUBBLERS

Special devices such as impingers and bubblers are used to disperse sampled gas in a solvent, see Figure 3.3. The finely divided gas bubbles rise from the bottom of the vessel, allowing for more contact between the gas bubbles and the solvent as the bubbles move toward the surface. In the case of reactive compounds such as formaldehyde, a derivatising reagent is included with the solvent to improve the extraction efficiency and simultaneously provide a more stable compound [82]. Adjusting the temperature of the solvent may also improve extraction. These devices are often used for sampling of gases from industrial stacks and automobile exhausts. However, large sample volumes are required, which may involve the use of large pumps and the devices themselves are clumsy to wear. Due to the large volumes of solvent used there is also a dilution factor present and an additional concentrating step is required [18,82].

3.4.2 DENUDERS

Denuders are open glass tubes that have been coated on the inside with a thin layer of solvent as in Figure 3.3. Air is sucked through the tube where the analyte gas, present in the air is extracted into the solvent. Unlike impingers and bubblers, higher collection flow rates may be used and the extract is more concentrated because of the smaller volume of solvent used [18]. Impingers and denuders have the advantage that any appropriate solvent can be used to trap a desired compound.

3.4.3 POLYMERIC SORBENTS

Adsorbents, LLE and SPE techniques, are undesirable because they carry contaminants into the final extracted sample, along with the analytes of interest, producing a high background in the analysis. Recently, polydimethylsiloxane (silicone) has emerged as an alternative to adsorbents and organic solvents used for pre-concentration [22,24-27,81,86,87,92-98].

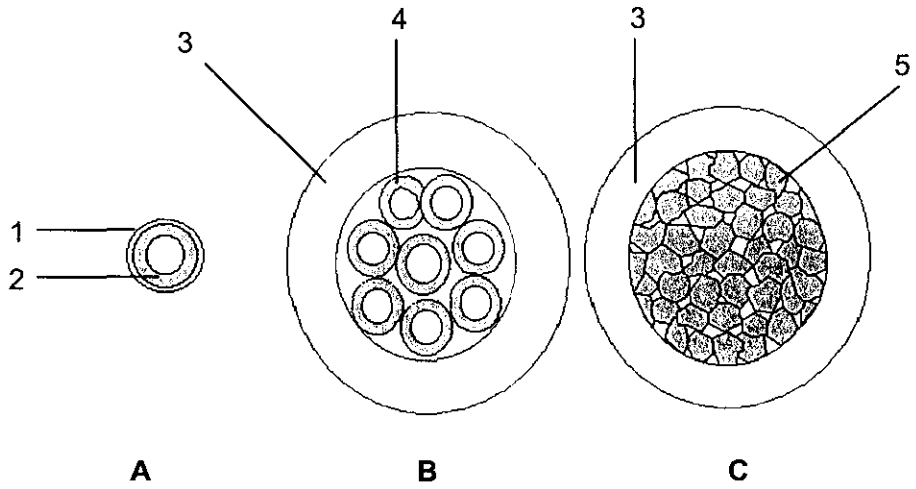
Polydimethylsiloxane is a non-polar, homogeneous liquid stationary phase used in GC capillary columns, generally known as SE-30, DB-1 or HP-1 columns. Just as the sample mixture injected onto a GC column will partition between the mobile and stationary phases leading to a separation of components, so too, will gaseous mixtures in air partition into silicone. The retention of the analytes from air in the trap is determined by their breakthrough volume, as previously discussed. The trapped contents in the silicone can then be extracted using a solvent [83] or by thermal desorption [25,81].

Apart from being inert, the silicone "fluid" is thermally stable (150-250°C) under oxygen-free conditions [78]. The advantage of thermally desorbing the silicone lies in its immediate reusability. In addition, all the silicone degradation peaks reveal repeatable retention times as well as easily identifiable Electron Impact (EI) mass spectral fragments. The main volatile silicone degradation products are methylcyclosiloxanes, with the most abundant of these being hexamethylcyclotrisiloxane (D3) followed by gradually decreasing amounts of the higher molecular mass cyclic siloxanes (D4, D5, D6...)[85].

Unfortunately, as we know that "like-dissolves-like", polar compounds will be virtually unretained on a non-polar phase. Hence modified polymers e.g. polymethylacrylates etc. [86-88] have been developed in an attempt to increase the polarity of the stationary phase. However, these polymers no longer exhibit a dissolution process,

but rather an adsorptive process with all associated disadvantages and particularly high backgrounds during thermal desorption [86].

Due to the remarkable properties of silicone, it has been widely used, leading to several possible configurations as described below, and depicted in figure 3.4 and 3.5.



A - Ultra Thick Film Open Tubular Trap
B - Multichannel Silicone Rubber Trap
C – Silicone Packed Bed Trap

1 – Wide bore capillary column.
2 – Silicone rubber tube d_f 145 μm.
3 – Glass tube (60mm o.d. 40mm i.d. length~16cm).
4 – Multiple silicone rubber tubes (0.63mm o.d. 0.3 mm i.d.) arranged in parallel.
5 – Pulverised Silicone rubber particles.

Figure 3.4. Cross-sections of various trap configurations using 100% polydimethylsiloxane.

3.4.3.1 OPEN TUBULAR TRAPS (OTT)

Grob and Habich [89] introduced the use of OTTs to overcome the problems experienced due to incomplete transfer of desorbed analytes from packed column traps onto GC capillary columns. The difference in flow rates, obtained when moving from a packed column to a capillary column, was eliminated by using the OTT, which

has similar dimensions to a capillary column. Various coatings, ranging from activated charcoal to SE30, were used inside the OTTs for the pre-concentration of various compounds [83,89-92]. This also led to the development of ultra thick film OTTs, by Blomberg and Roeraade [93,94], and Burger et al [92,95]. Blomberg and Roeraade used dynamic coating techniques requiring special instrumentation, whereas Burger's technique is easier to prepare. A single 1m long silicone rubber tube is inserted into a fused silica capillary, to provide a film thickness of 145µm. The silicone tube needs to be first stretched and immersed into liquid nitrogen. In this way it is sufficiently manageable to be inserted into the capillary, figure 3.4A. The capillary then fits into a modified GC where it can be thermally desorbed onto another GC column for analysis. However, the OTTs show limited sampling capacity and can only operate under low sampling flow rates (10ml/min).

3.4.3.2 THE MULTICHANNEL SILICONE RUBBER TRAP (MCT)

Ortner and Rohwer developed the multichannel silicone rubber trap [72]. It is based on the same principle as the open tubular traps developed by Burger et al [95]. However, instead of one long silicone rubber tube inside a fused silica capillary, the trap is made more compact by having several shorter lengths of silicone rubber tubes arranged in parallel inside a glass tube, depicted in figure 3.4B. This makes the trap suitable for desorption in a conventional desorption unit. The trap exhibits a low pressure drop, allowing for collection at high flow rates, particularly of non-volatile compounds. However, to improve the extraction of semi-volatile analytes into the silicone [24,26] it is operated under low sampling flow rates (15ml/min) to increase the number of plates (N). The MCT has also been applied to the analysis of aqueous samples [25,27].

3.4.3.3 PACKED SILICONE BEDS

Baltussen et al [81,86,96-98] packed a glass tube with equally sized particles of pulverised 100% polydimethylsiloxane, shown in figure 3.4.C. As this method of packing allows for a low-pressure drop over the trap along with turbulent flow, high sampling flow rates (500ml/min) can be used. These packed beds have successfully been applied to the analysis of organic acids, PAHs and nitro-PAHs from air [96], for characterisation of natural gas [81], monitoring nicotine in air [98], and amines, pesticides and PAHs in aqueous samples [86,97]. An added benefit of these traps is that breakthrough volumes can be calculated and predicted based on the retention of analytes on an SE-30 column [81].

3.4.3.4 SOLID PHASE MICROEXTRACTION (SPME)

The SPME technique is in principle a solventless liquid-extraction, developed by Pawliszyn [87]. The SPME device resembles a syringe. A 1cm long thin polymeric fibre, normally silicone, is attached to the tip of the syringe plunger, which can be retracted into the syringe barrel, as depicted in figure 3.5[99]. This device is practical for piercing septa and exposing only the fibre to a hot GC inlet, vial etc.
Unlike the other pre-concentration techniques, which are typically dynamic because they involve a flowing stream of gas passing over the sorbent, SPME is a static sampling technique.
The fibre is exposed either to the headspace of a sample or immersed in a liquid sample in a sealed vial for a precise period of time. The analytes will partition into the liquid phase until a distribution-equilibrium has been reached. This process usually takes between 2-30min. Equilibrium can be attained more rapidly in headspace SPME than in immersion SPME, as the analytes can diffuse more rapidly towards the

fibre. This extraction step is equivalent to one theoretical plate (N). From the equation below [87,99], it can be seen that the amount extracted (n), is directly proportional to the concentration of the analyte in the sample (C_o).

$$n = \frac{K_{fs}\ V_f\ V_s\ C_o}{K_{fs}\ V_f + V_s} \qquad (3.5)$$

Where K_{fs} is the distribution coefficient between the fibre and sample. V_f is the volume of the fibre, V_s is the sample volume and C_o the initial concentration of the analyte in the sample [87,99]. Consequently, trace analysis of analytes having a small partition coefficient (K_{fs}) will require sensitive instrumentation.

As for solvent extraction, the extraction efficiency can be improved by adjusting the pH, temperature, fibre ("solvent") polarity, fibre thickness, salt content and agitation. Various SPME fibre coatings, of differing thickness, have been developed by forming copolymers with the silicone (e.g. PDMS/DVB for non-polars), adding adsorbent material to the coating (e.g. Carbowax/PDMS), or by using a different polymer (e.g. polyacrylate for polar compounds). However, these variations do not exhibit dissolution properties as described for the liquid silicone polymer above [99].

In addition, when the analyte is too volatile or unstable derivatisation techniques can be used. This is done by coating the fibre with derivatising reagent followed by reaction with the analyte (*in-situ* derivatisation) [22,43]. SPME is suitable for the analysis of large sample volumes, as shown by equation (3.6), taken from (3.5) where $V_s >> K_{fs}V_f$ [87,99],

$$n = K_{fs}\ V_f\ C_o \qquad (3.6)$$

As the amount extracted by the fibre is independent of the sample volume, the thickness of the fibre plays a larger role. Compounds with a low K_{fs}, are efficiently extracted by using a thicker fibre and vice versa.

After extraction, the fibre is conveniently thermally desorbed in a hot GC inlet during the splitless mode.

For precision and to save time, reproducible fibre exposure time, desorption time, vial size, sample volume and other sampling parameters are much more important than obtaining full equilibration between fibre and analyte.

This sample preparation technique has become popular because it is simple, rapid, solventless and has demonstrated low detection limits. However, the fibre has proven fragile and is easily destroyed if not handled with care. Also, depending on the sample complexity and desorption conditions, the fibre may not be reusable due to memory effects. Under ideal conditions, the fibre assembly can provide 50-100 extractions [99].

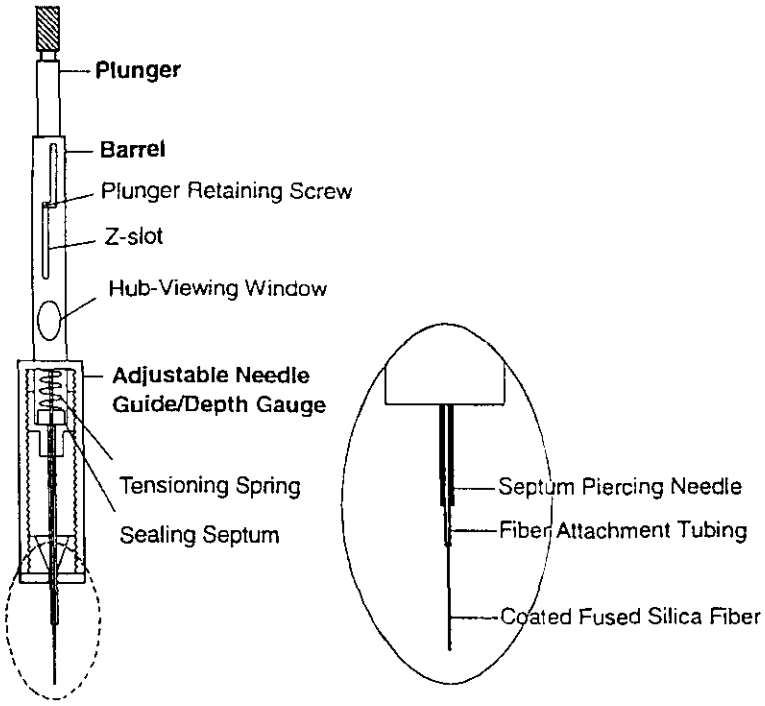


Figure 3.5. A commercial SPME device from Supelco [87].

A similar approach called Headspace Sorptive Extraction (HSSE) [100] uses a small glass rod coated with a large amount of polydimethylsiloxane (50mg). This is used similarly to the SPME fibre by exposure to headspace samples, except it is thermally desorbed in an automated thermal desorber. In addition, HSSE shows increased sensitivity over SPME as the volume of absorbent (V_f) is much larger.

3.5 RECOVERY

3.5.1 SOLVENT EXTRACTION

This technique is otherwise known as liquid-liquid extraction [72,82]. A solvent can be used to isolate analytes from a liquid sample or from a solid, in our case the sorbents. The technique relies on a distribution of the analyte between two immiscible phases. For acids and bases the distribution coefficient ($K = C_{solvent} / C_{sample}$) is easily affected by the pH of the solution and in this way the extraction can be made more selective. In general, the principle that "like-dissolves-like" is applied. Polar analytes will dissolve into polar solvents and non-polar analytes into non-polar solvents. The sample solution is shaken up with an equal amount of solvent in a separation funnel. When the 2 phases separate, the desired fraction is collected. The extraction efficiency increases with the number of extractions. Because the final fraction still contains a large amount of solvent, an extra step is required to concentrate the extract before it can be analysed. Analytes with small K's or large sample volumes require continuous extraction or counter current extraction to achieve a complete separation [72,82]. Overall, this is a simple but time-consuming technique and the general trend is to move away from these methods. In addition, the large volumes of high purity solvents required for such extractions are toxic and expensive.

Recently, these disadvantages were minimised with the introduction of liquid-liquid micro-extractions. Typically, 1ml of solvent is added to 10ml of sample in a vial and the extract can be injected without further pre-concentration.

3.5.2 THERMAL DESORPTION

Thermal desorption is the process through which the analytes on a sorbent are removed by heat energy. During this process, the analytes are transferred onto the chromatographic column. However, it is common to have a refocusing step before transfer onto the column. Usually, a second trap is cooled, using either liquid nitrogen or CO_2 gas, to sub-ambient temperatures ranging from 0°C to –100°C. This second trap is heated ballistically after desorption, in order to transfer the analytes in a narrow plug onto the column. A description of the instrument used for thermal desorption is given in chapter 5.

Thermal desorption has several advantages over solvent extraction. The main one being the removal of the dilution effect. With solvent extraction only a small fraction of the entire extract is injected for analysis. In addition, thermal desorption requires no expensive high purity solvents or labour to perform the liquid extractions as automated thermal desorption units allow for the desorption of several traps overnight. Disadvantages include the occasional blocking of the cryogenic trap. Although this can be prevented by avoiding the use of hydrophilic sorbents. In addition, instrumentation and use of large quantities of liquid nitrogen becomes expensive [18,19].