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Large volume sample introduction using programmed-temperature injection systems.

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Introduction

State-of-the-art capillary gas chromatography (GC) provides the analyst with a high efficiency as well as a high sensitivity. Unfortunately, however, for true trace level analysis, the sensitivity of capillary GC is all to often insufficient. In these cases, sample enrichment prior to introduction of sample onto the GC column is required. Most of the preconcentration methods rely on time consuming evaporation procedures which are prone to errors due to co-evaporation of volatile sample constituents or degradation of instable components. In recent years, the introduction of large sample volumes has received considerable attention as a means of improving the detection limits in trace analysis.

In trace analysis the introduction of large sample volumes is a simple and efficient way to improve analyte detectability. In capillary GC large volume injection can be carried out either by applying on-column injection using retention gaps, a technique originally developed by Grob [1], or by using temperature-programmable PTV injection. On-column injection is a very accurate and simple injection technique for the introduction of conventional sample volumes (1-3 µl) in capillary GC. In many respects it is superior over any of the other injection techniques that are currently in use for sample introduction in GC. If applied for the introduction of microliters sample sizes, the techniques is easy to use, reliable and rugged. Moreover, this technique is highly accurate as the sample is introduced onto the column without an intermediate evaporation step. The main disadvantage of on-column injection is that it is not really suited for the introduction of dirty samples because in volatile sample constituents easily contaminate the column inlet which results in a poor long term stability of the system. This becomes even true when dealing with large sample volumes. In this respect PTV-based large volume sampling techniques are clearly advantageous because less volatile matrix compounds generally cause less problems here.

The use of temperature programming techniques for sample introduction in capillary GC was proposed by Abel already in 1964 [2]. In 1979 Vogt and co-workers constructed a PTV injector and used it to introduce large sample volumes (up to 250µl) in biomedical and environmental studies [3,4]. Despite the good results obtained by Vogt using the PTV injector in early large volume sampling experiments, there was hardly any interest in the PTV injector for large volume sampling in the decade following that publication. PTV injection has, however, received considerable attention as a means of discrimination free injection and for the injection of thermally unstable molecules.

In this short contribution the various methods for PTV-based large volume sampling will be discussed. The basic principles of the methods are described and guidelines for the selection of the PTV injector and the sampling method that is to be preferred for a given (large volume sampling) application are discussed. Examples of PTV large volume sampling experiments will be given. Special emphasis will be on the optimization of the experimental conditions in PTV- based large volume sampling.

PTV-based methods for large volume sampling.

Large volume injection using PTV injectors is based on selective evaporation of the sample solvent from the liner of the PTV injector while simultaneously trapping the less volatile components in the cold liner. During this stage of the sampling process solvent vapours are discharged via the opened split exit of the injector. When solvent elimination, the split exit is closed and the components are transferred to the column in the splitless mode by rapid temperature-programmed heating of the injector.

In principle three approaches for PTV large volume sampling can be distinguished. The first technique is the so-called multiple injection. With this technique multiple injections of the sample are performed. During the injection procedure the split exit is open and the injector is kept at a temperature some 10 to 50 degrees below the boiling point of the solvent used. The multiple injections are performed at regular time intervals and the solvent is vented in the period between two injections. The interval time used should be sufficiently long to allow all the solvent to evaporate. Too short interval times might result in losses of components due to flooding of the injector and loss of liquid sample via the split exit. If, on the other hand, too long interval times are used, excessive losses of the more volatile sample constituents might occur. Typical interval times range from a few seconds to several tens of seconds and depend on a.o. liner temperature, solvent boiling point, sample volume etc. Even if carefully optimized, losses of volatile sample constituents are difficult to avoid. The volume that can be injected depends on the ability of the liner to retain the liquid sample. For empty liners *(i.e.* liners not packed with a packing material such as *e.g.* glass wool) the maximum volume is typically a few microliters. The multiple injection methods is a very fast and convenient method for the introduction of sample volumes up to approximately 20 µl. It is, however, less suited for a routine environment as it is difficult to automate and does not allow the introduction of really large volumes. For these reasons the 'multiple injection method' is not discussed in more detail here.

The second method for PTV large volume sampling is the rapid or 'at-once' injection, a method recently developed in our laboratory [5]. In this new and simple method the sample is rapidly injected into a PTV-liner packed with a suitable packing material. During injection the split exit is open and the liner is kept at a temperature below the boiling point of solvent. The liquid sample injected into the liner is retained as a thin film of solvent on the packing material in the liner. Upon evaporation a cold spot is created inside the liner. Volatile sample constituents are retained effectively in this cold spot. Losses of volatile components are therefore greatly reduced in comparison with the situation in the multiple injection mode. The maximum volume that can be injected depends on the ability of the injector to retain the liquid sample. For liners with a large inner diameter (*e.g.* 3.5 mm) the maximum allowable volume of liquid can be up to approximately 150 µl. Optimization of this 'at once' procedure for large volume sampling is relatively easy, as will be shown below. For a more detailed description the interested reader is referred to two recent publications from our group [5,6].

For sample volume in excess of the volume that can be introduced 'at-once', the so-called speed-controlled sampling procedure has to be used. In this third method for large volume sampling the sample is introduced into the liner of the injector at a controlled rate. Speed-controlled sampling is necessary because the volume of liquid that the packed liner can retain is exceeded. For optimum performance the speed of sample introduction should equal the rate of solvent elimination. If the sampling flow rate exceeds the evaporation rate, the excess liquid will accumulate in the liner which will eventually result in flooding of the liner and, hence, in losses of both volatile and non-volatile components. Oppositely, too low sample introduction rates will result in a lengthy sampling procedure and in severe losses of the more volatile sample constituents, unless liners packed with an adsorbent are used. In contrast to the situation in both the multiple injection mode and the 'at-once' injection the sample volume in the speed controlled mode is not restricted to a maximum. Speed controlled PTV sampling has received considerable attention in chromatographic literature in the last four to five years [7-9]. For more details the reader is referred to these articles and reference cited therein. In Table 1 a brief comparison of the tree PTV-based methods for large volume sampling is given. Below a more detailed description of the 'at-once' methods is given.

Table 1. Comparison of the three PTV modes for large volume sampling.

	Liner type	Maximum volume
Multiple injection	Open	≈ 20 ul (10*2µl)
Rapid 'at-once' injection	Densely packed	150 µl*
Speed controlled injection	Packed	infinite

* can be increased by performing multiple 'at-once' injections.

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PTV large volume sampling using the rapid ‘at-once’ injection method.

Principles and instrumentation

The rapid ‘at-once’ injection method for PTV large volume sampling consist of three subsequent steps. First the sample is rapidly injected into a densely packed PTV liner. Next, the solvent is evaporated by flushing the liner with helium at a low temperature for some time. Solvent vapours formed are discharged via the split exit. Finally, the split exit is closed and the components are transferred to the column in the splitless mode

In its basic principles the ‘at-once’ method for PTV large volume sampling is an ‘in injector’ evaporation technique. The evaporating liquid creates a cold spot that efficiently retains the analytes. Figure 1 shows the results of temperature measurements performed in the liner of a PTV injector.
A time *t* is zero, 100 µl of hexane was rapidly injected into the liner. The figure clearly shows the creation of a cold spot in the injector. When solvent evaporation has reached completion the temperature inside the liner rapidly returns to the original set-value.

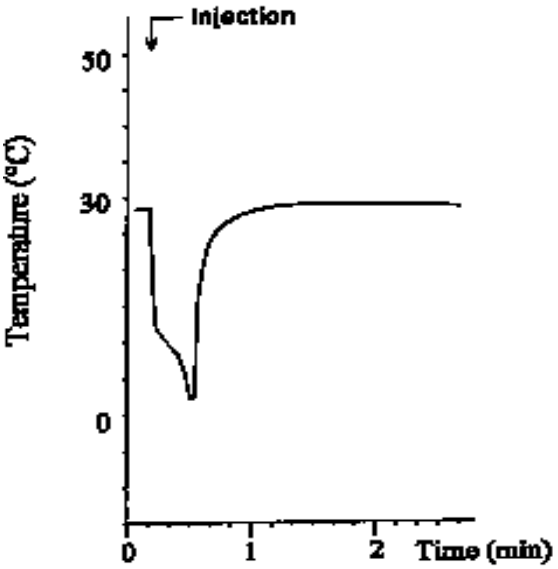

Figure 1. Cooling inside the liner caused by evaporation of the solvent.

In the ‘at-once’ injection method the nature of the packing material is extremely important. First of all the packing should be capable of retaining a large volume of the liquid sample. In addition to this, the material should be inert and thermo stable. Furthermore it should not retain the high-boiling analytes too strongly in order to minimize thermal stress applied to the molecules upon splitless transfer to the column. A wide range of packing materials has been used in PTV ‘at-once’ large volume sampling. These include silanized glass wool, glass beads, Tenax TA, PTFE (Teflon) wool, polyimide wool as well as different stationary phases for packed column GC [6].

With regard to the instrumentation required, the ‘at-once’ large volume sampling mode is truly straightforward. What is required is a PTV injector with a packed liner. Although in principle each PTV injector is suited, it is advantageous to use a PTV injector equipped with a large inner diameter liner. This is because the volume of the liner, and hence also the volume of the packed bed in the liner, is proportional to the square of the liner diameter. As the volume of liquid that can be retained in the liner is proportional to the volume of the packed bed this implies that the maximum sample volume that can be accommodated in the liner is proportional to the square of the liner I.D. As a rule of thumb: If a packing material with a sufficiently large surface area and favourable wetting characteristics is used, the maximum sample volume that can be injected onto a 4 mm I.D. packed liner is approximately 150 µl For smaller I.D. liners the maximum allowable volume decreases proportional to the square of the liner inner diameter

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Optimization

Optimization of the 'at-once' method for large volume sampling requires optimization of each of the three steps of the procedure, *i.e.* injection, solvent elimination and splitless transfer. It is generally recommendable to start the optimization procedure with the optimization of the last step of the experimental procedure, *i.e.* the splitless transfer. For this purpose the liner is packed with the selected packing material. Next, 1 µl of a test sample containing the components of interest is injected and the minimum splitless time required to obtain quantitative transfer of the components to the column is determined at the selected final temperature of the injector. If quantitative recoveries can not be obtained this can be due to either adsorption of the components on the packing or to degradation of the solutes. In the first case higher PTV final temperatures can be tried. Care should be taken, however, not to exceed the maximum temperature of the packing material. If thermal degradation of the solutes occurs, either lower PTV final temperatures should be used or, eventually another packing material should be selected.

Once the adsorption materials has been selected and the conditions required for rapid and quantitative splitless transfer of the components have been established, the maximum sample volume that can be rapidly injected without flooding the injector has to be determined. A first rough indication of the maximum injection volume can be obtained by visual observation. Despite the rough nature of this experiment, the value obtained this way will very often prove to be sufficiently accurate. For these experiments the liner is packed with the packing material under investigation and placed in the injector which is maintained at a low temperature, *e.g.* 30º C at a gas flow of about 200 ml/min. No column is installed in the injector. When too large volumes are injected the liquid can not be retained in the liner and liquid droplets are seen to occur at the bottom end of the injector. The maximum volumes determined in this way can later be confirmed by injection of increasing sample volumes and checking the plot of experimental peak area *vs.* injection volume for linearity.

The last parameter that has to be optimized experimentally is solvent elimination time. It is evident that the time needed for elimination of the solvent is determined by the evaporation rate of the solvent. This parameter in turn is determined by various operational parameters including the initial liner temperature, the pressure inside the liner and the split flow rate. Typical initial liner temperature are again some 10 or
50º C below the boiling point of the solvent used. For fast evaporation of the solvent it is generally recommendable to use high split flow rates (> 100 ml/min). Optimization of the solvent elimination time required is easy when the sample contains only non-volatile components. For these components the moment of closing the split valve is not critical provided that it is not closed too early. Closing the split valve too late will hardly cause losses as the vapour pressure of the compounds is very low. In case of too early a closure, however, an excessive amount of solvent will be transferred to the column which can, in turn, result in peak distortion of components of intermediate volatility. This situation is illustrated in figure 2. The easiest method to determine the solvent evaporation time is to ignite the vapour leaving the exit. The yellow flame (hexane, pentane) or weak blue flame (ethyl acetate) indicates the presence of high concentrations of solvent vapour in the split gas. The flame extinguishes once all solvent is evaporated. More sophisticated approaches for determining the solvent evaporation rate are based on the use of a thermal conductivity detector as a monitor detector or on calculation of the evaporation time from the peak width of the solvent peak observed in a large volume experiment [5].

The quantitative introduction of large volumes of a sample that contains low boiling components is slightly more complicated. When using the 'at-once' injection volatile solutes are retained in the liner by two mechanisms: cold trapping and solvation. Upon evaporation of the solvent a cold is created in the injector. This cold spot provides strong retention for the solutes. In addition to this, the liquid solvent present in the cold spot also helps to prevent the volatiles from being lost via the split exit. When the last fraction of the solvent evaporates, both the cold trapping effect and solvation rapidly become less efficient and severe losses of volatiles can occur in a short time. It is therefore crucial to close the split exit of the injector slightly before solvent evaporation has reached completion. Methods to determine the solvent evaporation time are discussed above. Next, the solvent vent time is successively reduced until peak distortion as shown in figure 2 starts to occur. Optimum conditions for solvent trapping exist slightly before this point.

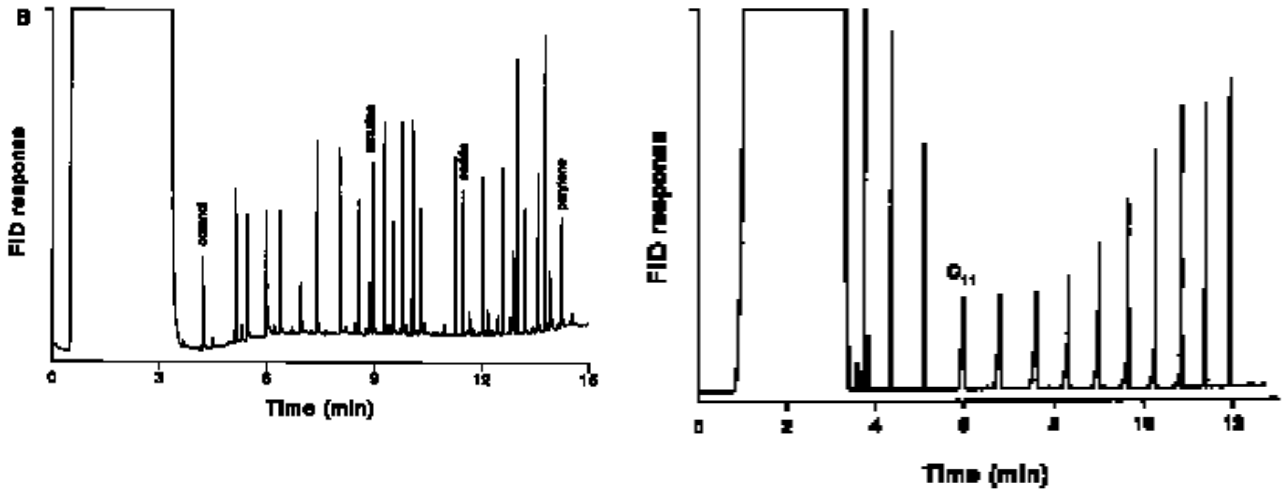

Figure 2. Peak distortion due to excessive recondensation of solvent in the column.

Selection of the packing material

Apart from optimization of the solvent vent time and the splitless transfer process also the packing material used in the 'at-once' large volume sampling experiments requires careful optimization. Especially the activity of the material is critical. The packing material selected should have a high degree of inertness to the components of interest. Glass wool is an ideal material for the analysis of stabile components such as for example PCB's. An example of an 'at-once' large volume injection in PCB analysis is shown in figure 3.

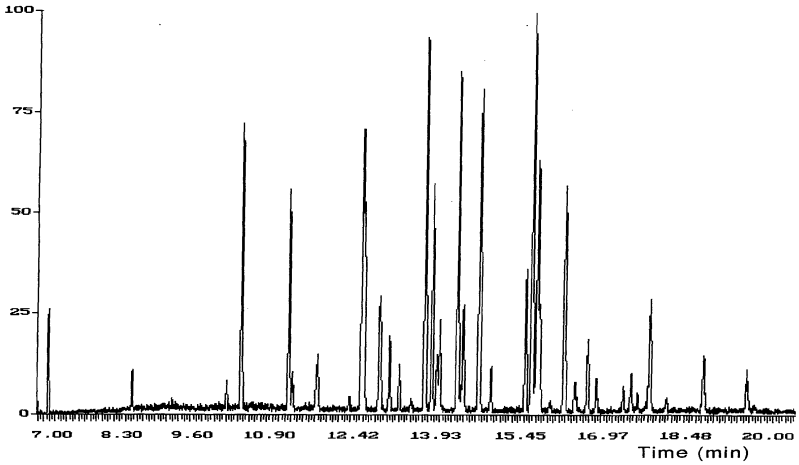

Figure 3. Analysis of a 100 µl sample of PBC's hexane. Total PBC concentration 20 ppb. PTV conditions: 50º C to 350º C at 3ºC/s. Liner packed with glass wool. Solvent time 30 s, split flow 260 ml/min. GC: column: 25 m x 0.25 mm. i.d. 0.25 µm CP-Sil-5, 50º C to 150º C at 30º C/min. than to 250º C at 5º C/min. Detector: MS.

Unfortunately, glass wool is difficult to deactivate in a way that thermal degradation of polar analytes is prevented. This is illustrated in figures 4A and B. Figure 4A shows a chromatogram obtained for a test sample containing a wide variety of environmentally relevant components in a large volume sampling experiment using a liner packed with deactivated glass wool. Serious degradation is observed for a number of components including p-dinitrobenzene, cyanazine, vamidothion, endrin and DDT. Figure 4B shows the same analysis but now using a liner packed with Teflon wool. With this material much better results were obtained for the particular set of components under investigation. For samples containing only relatively volatile analytes, liners packed with Tenax were found to give good results.

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Figure 4. GC-FID chromatograms for large volume injection using the 'at-once' PTV injection mode. A. 100 µl, 50-100 ng/ml, glass wool packed liner. B. 60 µl 8-16 ng/ml, Teflon packed liner. Components in elution order. Octanol, Naphtalene, Benzothiazole, Indole, Nicotine, p-Dinitrobenzene, Pentadecane, Diethylphtalate, Trifluralin, Dimethoate, Atrazine, Diazinone, Caffeine, Parathion-methyl, Fenitrothion, Cyanazine, Vamidothion, Dieldrin, Endrin, p,p'-DDT, Methoxychlor, Azinfos-methyl, Mirex, Azinfos-ethyl, Coumaphos, Octacosane, Perylene.

In conclusion, large volume sampling is an attractive means for improving the detection limits in capillary gas chromatography. The technique has been applied to a wide variety of real-world samples. In these experiments different solvents, chromatographic columns as well as a wide range of selective gas chromatographic detectors has been used. PTV large volume sampling has proven to be a very reliable, rugged and easy to optimize technique in our laboratory in the last five years. This is in particular true for the rapid 'at-once' injection methods.

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