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Note

Factors affecting sample transfer from microlitre syringes*

J. ROERAADE

Royal Institute of Technology, Department of Analytical Chemistry, S-10044 Stockholm (Sweden)
(Received November 27th, 1987)

Liquid sample injection in gas chromatography (GC) is nearly always carried out with a microlitre syringe. Excellent repeatability can be obtained when automated devices are employed. The volumetric accuracy however is considerably influenced by operating factors such as the speed of injection and the viscosity of the sample, as well as design factors such as the dead volume of the syringe needle and the clearance between the barrel and plunger. In most quantitative analyses, internal standards are employed and therefore deviations in sample volume are not so important, but in calibration work or other quantitative work where internal standards cannot be used, the volumetric accuracy is highly critical.

Microlitre syringes are also essential tools for the handling and transfer of small liquid samples in spectroscopy, life sciences, etc. In this context it is often crucial to minimize sample losses. Quantitative transfer can be accomplished by an abundant rinsing of the syringe, but this will also result in an excessive dilution of the sample.

To diminish these problems, a widely employed procedure is solvent flushing[1]. The syringe is first filled with some microlitres of solvent before the sample is taken up. Usually, the sample and the solvent are interspaced by a small plug of air. This method is generally assumed to result in an effective washout. It has even been stated that quantitative transfer is achieved in this way[2].

However, during practical preparative bench work with micro-samples as well as in work with cold on-column capillary GC, losses and carry-over effects are observed, indicating that the solvent-flush method is not as effective as in commonly believed. Recently, other workers have also expressed doubts after some puzzling observations[3].

This prompted the present investigation, where model experiments using a dye solution were carried out to study the influence of the dispensing speed and other factors on the accuracy of sample transfer.

EXPERIMENTAL

Equipment and materials
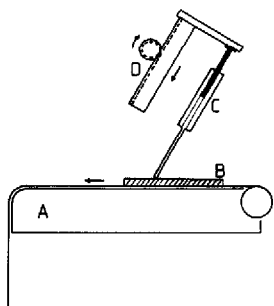   Syringes employed: 10 μl with steel plunger (Hamilton Model 701) and equiv-

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Fig. 1. Schematic picture of the slow dispensing procedure. A = Flat-bed recorder; B = thin-layer plate; C = syringe; D = motor-driven piston.

alent syringes with a PTFE-tipped plunger (Hamilton Model 1701); a 1-μl syringe of the "plunger in needle" type (SGE Model 1B7) was also tested. Motor-driven syringe pump: Sage Model 351. High-performance thin-layer chromatography (HPTLC) plates: Merck, precoated plates, silica gel 60. Scanning densitometer: Zeiss PQM-2. UV–VIS spectrophotometer: Hitachi Model 100-20.

Sample-transfer experiments were carried out with a concentrated solution of methyl violet in ethanol.

Procedure

The efficiency of solvent flushing was investigated at different speeds. For dispensing at slow speed, 1 μl of the dye solution was washed out with 3 μl of ethanol and the plugs were interspaced by 1 μl of air. The sample was taken up on a moving TLC plate in order to study the dilution profile. Linear movement was accomplished by taping the TLC plate on the chart paper of a flat-bed recorder as depicted in Fig. 1. The coloured lines thus obtained were integrated by scanning densitometry at 600 nm.

High-speed dispensing was carried out manually, and the sample remaining in the syringe was washed out with ethanol and quantified by spectroscopy at 580 nm.

RESULTS AND DISCUSSION

In the initial stage, syringes with stainless-steel and PTFE-tipped barrels were evaluated for leak tightness. When tested at a pressure of 2 bar as previously recommended[4] both syringe types showed no sign of leakage. However, when dispensing the dye solution, a clear back-flow of sample between the stainless-steel plunger and the glass barrel was observed. More than ten new syringes of this type were tested, but all showed the same problem, which seems to be inherent to this design. Only the syringe with the PTFE-tipped barrel proved to be leak tight and therefore all further experiments were carried out with this syringe. The results of the manual experiments are shown in Table I.

In capillary GC, an high injection speed is often required in order not to loose chromatographic efficiency or to reduce the risk of discrimination effects caused by sample fractionation in the needle. However, under such conditions, the results in Table I indicate that a significant amount of sample remains in the syringe. Isolating

TABLE I

RESULTS OBTAINED FOR SAMPLE TRANSFER AT HIGH SPEED USING DIFFERENT SOL-
VENT-FLUSH METHODS

The sample/solvent dispositions are shown on the right-hand side of the table.

Dispensing time				
< 100 ms		3 s		
%sample left in the syringe	S.D. (%)	%sample left in the syringe	S.D. (%)	
6.5	8	0.3	14	
7.3	14	0.5	14	
5.2	18	1.2	23	

the sample and the solvent by an air segment is not particularly benefical, in contrast to what is commonly believed.

The presence of an air segment proved to be more efficient in slow sample transfer. However a complete transfer without excessive dilution of the sample seems possible only if the dispensing speed is kept very low. As seen from the results in Fig. 2, at least 3 μl of washing solvent were necessary to transfer the whole sample in 1 min. Further, to reduce sample dispersion in the washing fluid, the transfer speed has to be much lower (Fig. 2b–d).

The following explanation for the observed results is suggested. During the slow transfer, a film of sample is formed in the air segment section. The thickness of this film (d_f) is dependent on the transfer speed, v, the sample viscosity, η, the surface tension, γ, and the inner diameter, d, of the syringe, as expressed by Concus[5]:

$$d_f = Cd \left(\frac{v\eta}{\gamma} \right)^{2/3} \tag{1}$$

The sample film is taken up by the solvent plug and dispersed backwards, and exponentially diluted in accordance with the well known Taylor parabolic profile. This is evident from the results shown in Fig. 2.

For rapid sample transfer, eqn. 1 is not applicable. During the impulse-like movement, the sample is largely torn apart and spread out in the form of a wave-like film, which is completely mixed with the flushing liquid. Hence, the isolation of solvent and sample by an air segment is not effective.

Plunger-in-needle syringes are in principe superior since no dead volume is present. However, I did not obtain very much better results with these syringes. An appreciable part of the liquid enters the clearance between the needle and the plunger, especially during rapid injections. This is not surprising, since very high pressures can arise, particularly when dealing with viscous fluids.

What improvements can be thought of? When considering a slow sample transfer, the air-segmented solvent-flush method is recommended. The film thickness in the air segment should be kept to a minimum and, therefore, solvents with a low
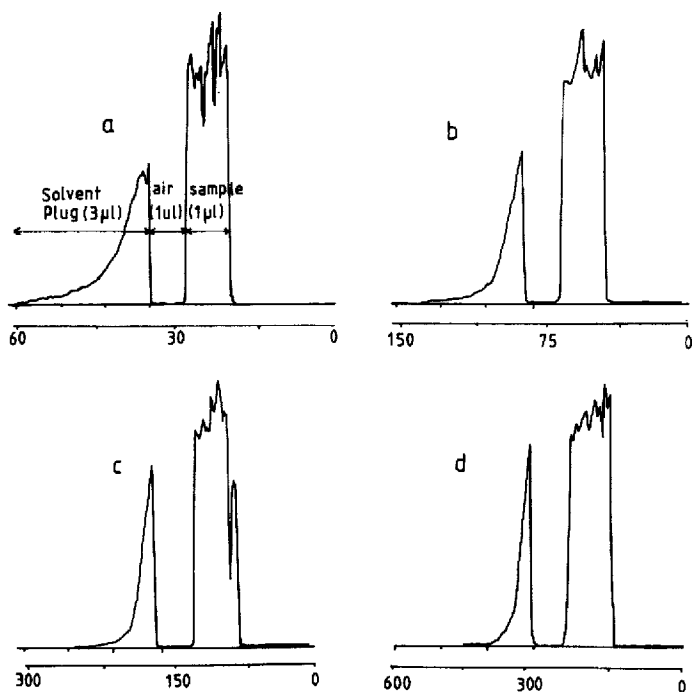
Fig. 2. Scanning densitograms of four different samples (a)–(d) dispensed at different speeds on a moving TLC plate. The transfer times (s) are indicated on the horizontal scales.

viscosity and an high surface tension are preferable. Additionally, the surface tension of the inner wall of the syringe should be as low as possible. Silylation is clearly beneficial. If the contact angle between the liquid and the surface is $>90°$, no film should be formed in the air segment and a total sample transfer with a minimum or no flushing liquid should be possible. This was confirmed in preliminary tests, where PTFE syringes ($\gamma = 11$ dyn/cm) and polar solvents were used. Unfortunately, in many practical situations the applicability of PTFE is limited due to its adsorptive properties.

For rapid sample transfer, these concepts are not very useful. Although the conditions may be such that no film is formed, the sample plug breaks up due to excessive shear forces and spreads as small droplets which are mixed with the solvent plug. Thus, reducing the dead volume of the sampling device remains the only way of improvement.

It is obvious that similar effects to those described are expected to occur in valves, sample loops, etc. Thus, when designing new such devices, it seems worthwhile to aim for a very smooth flowpath to reduce the contact-angel hysteresis (very smooth channels can e.g. be obtained by etching in monocrystalline silicon), and for a chemical modification, e.g., covering the surface with CF_3 groups, which have the lowest known surface tension (6 dyn/cm). This could lead to significant improvements in transfer techniques for liquid micro-samples, provided that the transfer speed is kept at a level where the integrity of the sample plug is maintained.

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