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EFFECT OF INJECTION NEEDLE DIMENSIONS IN GAS CHROMATOGRAPHY

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SUMMARY

The effect of the injection needle characteristics in gas chromatography has been investigated using simple gas laws. In addition to the size of the needle, injector temperature and volatility of the sample, the needle correction has been found to depend on the inlet pressure of the column. Both the needle correction and the slope of the calibration graph are concentration dependent. Representative sampling is unlikely to be achieved with microlitre syringes having an uncalibrated needle volume. It seems necessary to use microlitre syringes with no dead volume in all quantitative methods, including the internal standard method. A simple graphical method is suggested for obtaining the true calibration graph and the true volume injected with microsyringes with a dead volume. This method can be used in physico-chemical studies to extrapolate the basic chromatographic parameters to zero sample size of a given solution of a sample in a volatile solvent.

INTRODUCTION

The measurement of liquid samples is almost universally conducted with some form of syringe. A series of microsyringes (Hamilton, Whitier, CA, U.S.A.) covering the ranges 0–50, O-10 and 0–1 μl is available. With a 1-μl syringe, the sample part of the syringe is the needle and displacement occurs only from the needle, which contains a fine tungsten rod. Total displacement is usually used with such a syringe and no needle correction is needed. Microlitre syringes of higher capacity (10 μl and above) have an uncalibrated needle volume and the displacement of samples is obtained by means of a steel plunger fitted closely to a calibrated glass barrel. During the process of injection with such a microsyringe, a fraction of the needle volume along with the amount as read from the syringe is injected into the column, and this amount is the needle correction. Because of this, the calibration graphs obtained by plotting peak area against the amount injected as read from the syringe do not pass through the origin and the true volume injected cannot be known directly with such microsyringes.

Boček and Novak' applied rigorous statistical methods in absolute calibration and observed systematic constant absolute errors and systematic constant relative errors with such microsyringes. Metwally *et al.*[2] eliminated the needle effect by using

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a high-boiling solvent. However, pure high-boiling solvents are difficult to obtain and are very expensive. Further, there is a risk of overlap of the peaks of low-boiling impurities present in the solvent with the peak of interest, and the clean-up period is long, which makes the total time of analysis unduly long. Hence use of high-boiling solvents has not become popular. The following technique has generally been recommended[2–5]. Once the volume has been adjusted, the plunger is withdrawn until air is visible below the liquid. The volume is read'and the plunger is left in this position. Insertion, injection and removal of the needle are performed quickly. The plunger is then pulled back and the volume is read again. The difference in volume equals the true volume injected. However, the validity of the principle of the method is difficult to elucidate physically here, as it does not take into account the unavoidable losses involved in the procedure. The advantages of the internal standard method of calibration in gas-liquid chromatography have been criticized by Shatkay and co-workers[6–8]. For a system of analyte and standard in the internal standard method it has been concluded that the peak-area ratio depends on both the concentration and the volume injected into the column. However, this conclusion was not considered authoritative by others[9,10].

The above situation led the author to reconsider this subject. Simple gas laws have been applied to realize the effect and for the first time the effect of inlet pressure on needle correction has been demonstrated.

THEORETICAL

Let the volume of the uncalibrated needle be V_0, and P and T the inlet pressure and injector temperature, respectively. When the needle, filled with a given sample, is introduced into the injection port, the temperature of which is sufficiently above the boiling point of the sample, it vaporizes and occupies a volume, V, such that

$$V = V_0 + Z \tag{1}$$

where Z is the needle correction.

Effect of injector temperature

Let us consider two injector temperatures, T_1 and T_2 where $T_2 > T_1$. Then,

$$V_1 = V_0 + Z_1$$

and

$$V_2 = V_0 + Z_2$$

where the subscripts 1 and 2 refer to temperatures T_1 and T_2, respectively. At a constant inlet pressure we can write with simple gas laws

$$(V_0 + Z_1)/T_1 = (V_0 + Z_2)/T_2$$

and, as $T_2 > T_1$,

$$Z_2 > Z_1 \tag{2}$$

Further, let d_1 and d_2 be the densities of the sample at T_1 and T_2 respectively. Therefore,

$$V_0 d_1 + Z_1 d_1 = V_0 d_2 + Z_2 d_2$$

and, as $d_2 < d_1, Z_2 d_2 > Z_1 d_1$. Hence the needle correction (expressed in volume or weight) increases with injector temperature.

Effect of inlet pressure

Let P_1 and P_2 be the two different inlet pressures and $P_2 > P_1$. At a constant injector temperature, we can write

$$P_1(V_0 + Z_3) = P_2(V_0 + Z_4)$$

and, as $P_2 > P_1$,

$$Z_4 < Z_3 \tag{3}$$

Again, we can write

$$V_0 d_3 + Z_3 d_3 = V_0 d_4 + Z_4 d_4$$

where d_3 and d_4 are the densities at P_1 and P_2, respectively, and, as $d_4 > d_3, Z_4 d_4 < Z_3 d_3$. Hence, unlike temperature effect, the needle correction decreases with increase of column inlet pressure.

The behaviour of the vapours in the needle under the experimental conditions may be far from ideality and hence the above derivatives have a qualitative meaning only.

Effect of concentration

At constant P and T and for a given mass of a substance, we have from simple gas laws

$$V \propto 1/M$$

where M is the molecular weight of the substance. As $T_b \propto M$, where T_b is the boiling point of the substance, we can write

$$V \propto 1/T_b$$

Further, we can write for substances A and B

$$V_A T_{bA} = V_B T_{bB} \tag{4}$$

where $T_{bA \text{ and } bB}$ are the boiling points of A and B, respectively. If $T_{bA} > T_{bB}$, we have

$$V_A < V_B$$

and, as $V_A = V_0 + Z_A$

$$Z_A < Z_B \tag{5}$$

Hence the needle correction decreases as the boiling point of the substance increases.

A very dilute solution of the sample in a volatile solvent is injected in gas chromatography, and it is assumed that the volume occupied by the solute present in the needle volume of the solution can hardly exceed the needle volume (V_0) under the experimental condition. Therefore, when the needle filled with a given solution is introduced into the injection port, the amount of solute introduced into the 'column together with the solvent is proportional to the volume (in excess of the needle volume) occupied by the solvent which acts as a carrier. The boiling point of the solvent increases as the solute concentration is increased; if T_{bA} and T_{bB} are the boiling points of the two solutions of a given solute in a volatile solvent having concentrations C_1 and $C_2 (C_1 > C_2)$, respectively, then it follows from eqns. 4 and 5 that the amount of solute introduced from the needle volume of the solution (needle correction) is inversely proportional to the concentration of the solute in the volatile solvent.

Absolute calibration graph

The true amount (X) injected with microsyringes having a needle effect can be expressed by

$$X = z + a \tag{6}$$

where z is the amount injected as read from the syringe and a is the needle correction. In the linear range of detector response an absolute calibration graph is a straight line passing through the origin ***(i.e.,*** no sample, no response), which is expressed by

$$Y = mX \tag{7}$$

where Y is the detector response (usually expressed as peak area in mm^2 or any arbitrary units) and m is the slope. When the calibration graph is established using a microsyringe having a needle effect, the above expression can be written in the form

$$Y = m(z + a) \tag{8}$$

and, when z = 0

$$Y = ma \tag{9}$$

where ma is the response corresponding to the fraction of the needle volume, a, introduced during the process of injection (needle effect). Under given conditions a is fairly constant and the experimental calibration graph obtained by plotting peak area against the amount injected as read from the syringe is a straight line with a positive intercept. As the slopes m in eqns. 7 and 8 are the same, a true calibration graph in the absence of any needle effect is a straight line passing through the origin and parallel to the experimental curve obtained with such micro-syringes. In other words, the true calibration graph is obtained by plotting peak area obtained by subtracting ma from the experimentally found peak area against amount injected as read from the syringe.

EXPERIMENTAL

The experiments were performed on a CIC (Chromatography and Instruments, Baroda, India) gas chromatograph equipped with dual flame-ionization detectors and an Omniscribe (Digital Electronics, Bombay, India) Series 5000 strip-chart recorder complete with integrator. A copper column (1 m × 3 mm I.D.) packed with 20% Apiezon L on 80–100-mesh Chromosorb W and a stainless-steel column (2 m × 2 mm I.D.) packed with 20% OV-17 on 80–100-mesh Chromosorb W were used. Nitrogen was used as the carrier gas. The flow-rates of nitrogen, hydrogen and air were maintained at 30, 20 and 350 ml/min, respectively. A dilute solution of authentic pure solute in volatile solvents was injected with both 10- and 1-μl Hamilton syringes. Recommended chromatographic practice was adopted in order to reduce errors[11]. The temperature of the injection port and the depth of needle penetration were kept constant. Insertion, injection and removal of the needle were performed in a quick rythmic cycle. The peak area in counts was calculated from the integrator trace. The needle volume of the 10-μl Hamilton syringe used in the experiment was found to be 1.0 μl. In the figures the amount of solute introduced into the column was derived from the respective volume of the solution (in microlitres) of the solute in a volatile solvent injected.

RESULTS AND DISCUSSION

Effect of injection port temperature

It is known that the absolute calibration graphs with microsyringes having a needle effect do not pass through the origin[1,2,12]. This effect is shown[12] in Fig. 1. All the graphs except 3 in Fig. 1 were drawn through points which were averages of five determinations at each level. A maximum relative error of ± 5% was observed for the areas measured in all instances except for curve 3. The calibration graphs obtained by plotting peak area against the amount injected as read from the syringe have positive intercepts which increase as the injector temperature is increased. The needle correction is thus sensitive to injector temperature, in accordance with eqn. 2.

Further, the true amount injected cannot be derived from the readings on the syringe taken before and after the injection, as mentioned in the Introduction. The graph obtained by plotting peak area against the volume injected as reed from the syringe before and after the injection has a negative intercept (curve 3, Fig. 1). This graph is the best (regression line) drawn through individual data points. In this instance, the relative error for measured peak areas was higher than in other instances. A maximum relative error of 20% was observed. In the injected position the vapours in the needle are at a very high temperature and at the carrier gas pressure. As soon as the needle is removed from the hot injection port to take the reading of the remaining volume, some vapour flashes out of the needle to attain equilibrium at ambient temperature and pressure. The negative intercept, highly scattered points and the difference in slope between this line and the others are probably due to this loss of sample and unequal amounts of adhering liquids retained in the capillary of the needle. Further, the negative intercept was found to be sensitive to injector temperature.

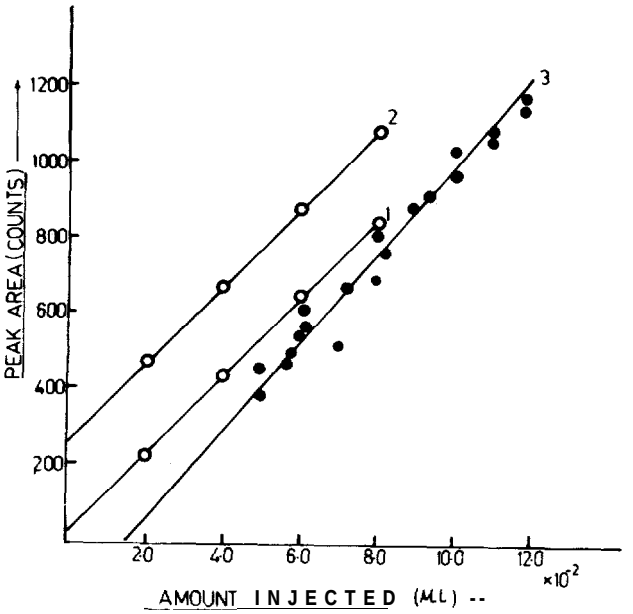

Fig. 1. Calibration graphs with a 10% (v/v) solution of toluene in benzene on an OV-17 column at 80°C. 1, Peak area *versus* amount of toluene injected as read from the syringe at 120°C injector temperature; 2, as 1, except at 220°C injector temperature; 3, as 2, except the amount injected was read from the syringe before and after the injection.

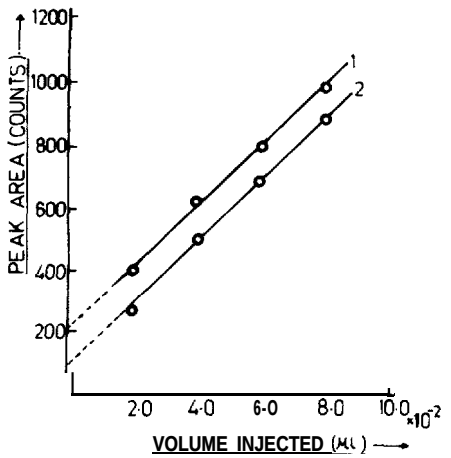

Fig. 2. Effect of inlet pressure on needle correction. 1, Peak area versus amount of o-xylene injected [IO% (v/v) solution in cyclohexane] as read from the syringe at 120°C column temperature and 200°C injector temperature on Apiezon L at an inlet pressure of 1.50 atm; 2, as 1, except the measurement was made on an OV-17 column at an inlet pressure of 3.00 atm.

Effect of inlet pressure

The needle correction is known to depend on the size of the needle, injection temperature and volatility of the sample. The effect of inlet pressure on the needle correction has not been studied earlier. This effect is shown in Fig. 2. Both of the columns were thoroughly aged at elevated temperature before the experiments were performed. The background signal at the column temperature was negligible. The experiments were completed in a day and the column outlet pressure (atmospheric) was virtually the same. Thus the observed effect cannot be ascribed to the role of background detector response[13] and/or to the effect of pressure on the changes in ionization efficiency[14] of the flame-ionization detector. Similar results were obtained with an n-undecanecyclohexane sample system. Thus, unlike the effect of injector temperature, the needle correction is inversely proportional to the column inlet pressure and this is in accordance with eqn. 3.

Effect of sample concentration

The effect of the concentration of the solute in the volatile solvent on the needle effect is shown in Fig. 3. The data obtained experimentally were compared at the same sensitivity and concentration (10% solution). Similar results were observed by Metwally *et al.*[2]. Although the change in slope with o-xylene is not significant, that with n-undecane is appreciable. Thus, both the slope and the needle correction are concentration dependent. The needle correction increases as the concentration of the solute is decreased. This is in agreement with the present derivation.

The difficulty of representative sampling of alkane samples with a wide boiling

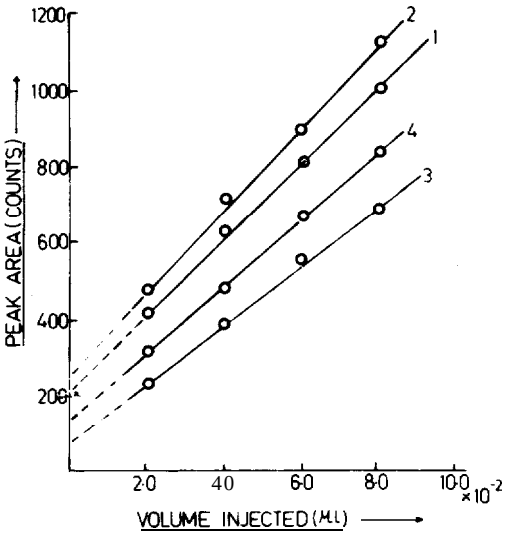

Fig. 3. Effect of concentration of solute on needle correction. 1, Peak area versus amount of o-xylene injected [10% (v/v) solution in cyclohexane] as read from the syringe at 120°C column temperature and 200°C injector temperature on Apiezon L; 2, as 1, except the measurement was made with a 1% solution of o-xylene; 3, as 1, except the measurement was made with 10% n-undecane at 150°C column temperature and 205°C injector temperature; 4. as 3, except the measurement was made with a 1% solution of *n*-undecane.

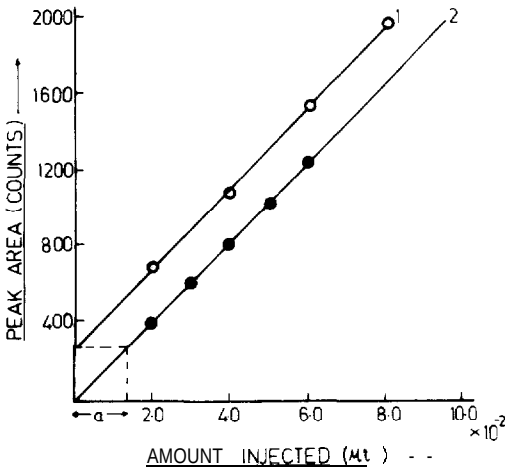

Fig. 4. Calibration graphs with a 10% (v/v) solution of toluene in benzene on Apiezon L at 80°C. 1, As 2 in Fig. 1; 2, true calibration graph passing through the origin and parallel to curve 1 with data points (●) obtained with a 1-μl syringe under the same conditions.

range with such microsyringes has been studied by Grob and Neukom[15]. The sample components usually do not leave in the same proportion. High-boiling substances are more likely than volatile substances to remain inside the needle and they are therefore discriminated in the chromatogram. They found that the losses inside the syringe depend strongly on the temperature gradient in the upper part of the injector and could not avoid the losses completely even when the entire needle was heated to a high temperature. As already discussed (Fig. 1, curve 3) these unrecognized losses can be ascribed to the loss of the samples due to equilibration at ambient temperature and pressure when the needle is removed from the injector port. The net conclusion is that representative sampling is unlikely to be achieved with a microsyringe having a needle dead volume. The result of Grob and Neukom[15] and the concentration effect discussed above seem to indicate that the "solute effect" and the "volume effect" observed by Shatkay and co-workers+* represent errors of the experimental procedure but not of the method. They used a 10-μl syringe in their experiments. Thus, it seems necessary to use microsyringes having no needle dead volume in all methods of quantitative analysis including the internal standard method by gas chromatography.

However, it is possible to construct a true calibration graph for a given sample with such microsyringes [12], and this is shown in Fig. 4. The true graph passes through the origin and parallel to the experimental graph. It is expected that the data points obtained experimentally under the same conditions with microsyringes having no needle effect should fit this true graph. An excellent fit of data points to this line obtained with 1-μl Hamilton syringe is shown. The needle correction is known from the experimental and true graphs. A straight line parallel to the abscissa is drawn from the intercept till it meets the true graph and the volume corresponding to this point is read from the abscissa, which gives the appropriate needle correction. The exact amount injected is derived from the amount injected as read from the syringe and the needle correction (eqn. 6). This method can be used in physico-chemical

studies to extrapolate the basic chromatographic parameters to zero sample size of a given solution of a sample in a volatile solvent.

REFERENCES

1 P. Boček and J. Novak, *J. Chromarogr., 51 (1970) 375.*
2 M. M. E. Metwally, C. H. Amundson and T. Richardson. *Anal.* Chem., 39 (1967) 551.
3 J. W. Amy and W. E. Baitinger, in H. A. Szymanski (Editor), *Lectures on Gas Chromatography 1962,* Plenum Press. New York, 1963, p. 19.
4 S. Dal Nogare and R. S. Juvet, Jr., *Gas Liquid Chromatography,* Interscience, New York, 1966, p. 177.
5 0. E. Schupp, III. in E. S. Perry and A. Weissberger (Editors), Technique **of Organic** *Chemistry,* Vol. XIII, Interscience, New York, 1968, p. 99.
6 A. Shatkay and S. Flavian, *Anal. Chem., 49 (1977) 2222.*
7 A. Shatkay. *Anal.* Chem., 50 (1978) 1423.
8 A. Shatkay, *J. Chromatogr.,* 198 (1980) 7.
9 G. C. Royston, *Anal. Chem., 50 (1978) 1005.*
10 M. Ravey, *Anal.* Chem., 50 (1978) 1006.
11 D. W. Grant and A. Clarke, *J. Chromatogr., 97 (1974)* 115.
12 0. K. Guha and K. P. Mishra. *Indian J. Technol., 20 (1982) 280.*
13 J. Novak, J. B. Růžičková, S. Wičar and J. Janák, *Anal. Chem., 43 (1971) 1996.*
14 P. Boček, J. Novak and J. Janák, *J. Chromatogr., 43 (1969) 431.*
15 K. Grob, Jr. and H. P. Neukom, *J. Chromatogr., 198 (1980) 64.*