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Short communication

Quantitative injections or calibrations in gas chromatography Anomaly associated with syringe type

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Abstract

When syringes with open needles are used for quantitative injections, e.g. to calibrate gas chromatographic peak areas against the volume of liquid injected, the resultant plot may display an intercept. Current studies on cyclohexane have shown that this effect is so severe that it can make the major contribution to the total signal. Since the magnitude of the anomaly increases with the volatility of the liquid, this means that calibrations of liquid mixtures, or the use of an internal standard, will be unreliable when moderately volatile components are injected with syringes of the above type. The problem is shown to be caused by volatilisation from the needle whilst it is inserted in the heated injection port, and is exacerbated by increasing the temperature of the port or increasing the residence time of the needle. The anomaly is totally avoided if the calibration is performed using a syringe of the type in which the piston passes down the needle. Somewhat similar effects to the ones described can also arise for other reasons, and literature references to such situations are given.

Keywords: Syringe; Injection methods; Calibration; Cyclohexane; Cyclohexanol

1. Introduction

In recent years it has been increasingly recognised that injection technology in capillary gas chromatography can influence the quantitation of the results [1]. For example, the injection of large volumes presents special problems [2–4]; sample evaporation may not be reproducible [5,6]; and special attention needs to be paid to choice of syringe [7]. The speed of depressing the plunger [8] and the cleaning of the outer needle wall [9] have also been noted as factors which can influence the quanititative results obtained. Many other examples of quantitation problems [10,11] and of unrepresentative elution of components associated with syringe characteristics or injection technique could be given [12–16].

The work reported in this paper originated in the course of a study of the enzymic oxidation of cyclohexane to cyclohexanol, in which it was desired to estimate the conversion gas chromatographically. To this end, calibrations were performed using mixtures of these components in defined ratio, but the results obtained were so non-linear and unmeaningful that it was apparent that some anomaly was present with respect to the quantitative assessment of one or both components. The problem was investigated by performing individual calibrations for the components cyclohexane and cyclohexanol (chromatographic peak area versus volume injected). This paper describes how the cause of the anomaly was

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revealed in this way, and shows how the problem can be avoided.

2. Experimental

2.1. Materials

The cyclohexane was supplied by BDH; its boiling point range was 80–88°C, and the purity quoted as 99%. The cyclohexanol was supplied by Fisons; its boiling point range was 158–163°C, and the purity quoted as 95%.

2.2. Apparatus

The GC system was a Carlo-Erba 5300 MEGA series unit. This was equipped with a flame ionisation detector and a data-handling unit.

The GC column was was supplied by Hewlett-Packard: semi-polar DB1 fused-silica megabore, 25 m×0.53 mm I.D. The carrier gas was nitrogen, and the flow conditions kept constant throughout by maintaining the column inlet pressure at 50 kPa. The column was run isothermally at 40°C.

2.3. Syringes

The following syringes were used: (X) A 5-μl open-needle syringe (SGE). (Y) A 1-μl piston-needle syringe (Hamilton No. 7001).

3. Results and discussion

The first cyclohexane calibration was performed using the syringe type X, and the results obtained are shown in Fig. 1. The plot shows acceptable linearity and scatter, but displays a huge intercept, implying that an injection of zero volume has provided a signal equivalent to the injection of about 0.25 μl. It was considered that the most plausible explanation of this was that an additional amount of sample, corresponding to this volume, was being evaporated from the needle whilst this was resident in the heated injector port, and this bonus was being provided whatever the desired volume being injected.

In order to test this proposal, a series of blank

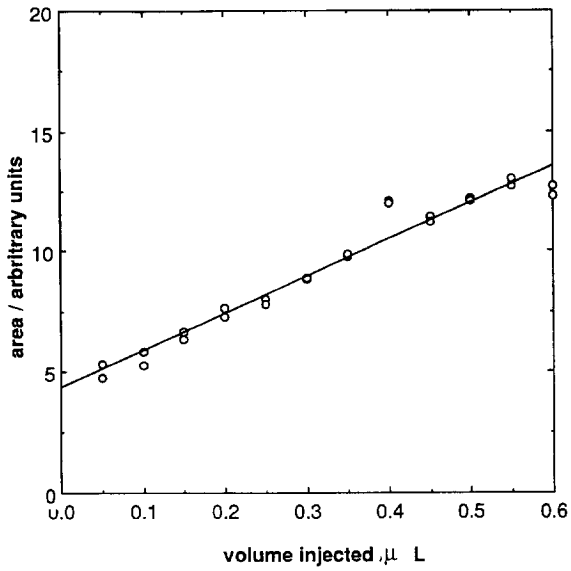

Fig. 1. Cyclohexane calibration performed using syringe X. The gas chromatographic conditions were as described in Section 2. The large intercept represents the anomaly referred to in the text.

injections were performed, in which the syringe X was loaded (according to the scale on the barrel) with volumes of 0, 1, 2, 3, and 4 μl of cyclohexane. Since it was clearly desirable to standardise on the residence time of the needle in the injection port, it was decided to insert the syringe for a duration of 5 s for each of the blank injections, after which the syringe was removed without plunging down the piston.

The results obtained are shown in Fig. 2, which also shows the calibration curve on the same axes. The almost horizontal line is in accord with the explanation proposed for the intercept on the calibration graph, but displays a larger intercept. The operator was then timed as he made some specimen injections, and it was found that the average time in which the needle was present in the heated port was ca. 2 s. A further series of blank injections were performed, using the same procedure as above, except that the nominal volumes of cyclohexane in the syringe were 0.2, 0.4, 0.6 and 0.8 μl, and the residence time of the needle in the heated port was 2 s. The results obtained are shown in Fig. 3, and confirm the idea that a bonus of about 0.25 μl is being provided in all cases by evaporation from the needle.

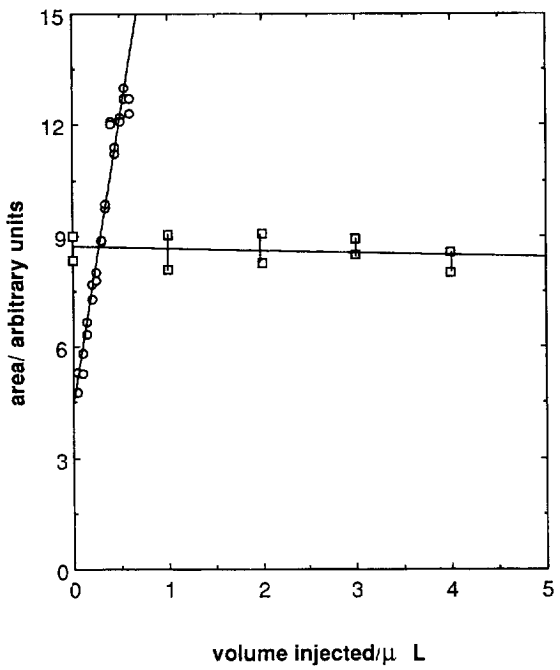

Fig. 2. Observed peak areas when syringe X, nominally loaded to the extents shown on the abcissae, was allowed to remain in the injector port at 180°C for 5 s, and then removed without pushing the plunger. The calibration from Fig. 1 is shown on the same axes for comparison.

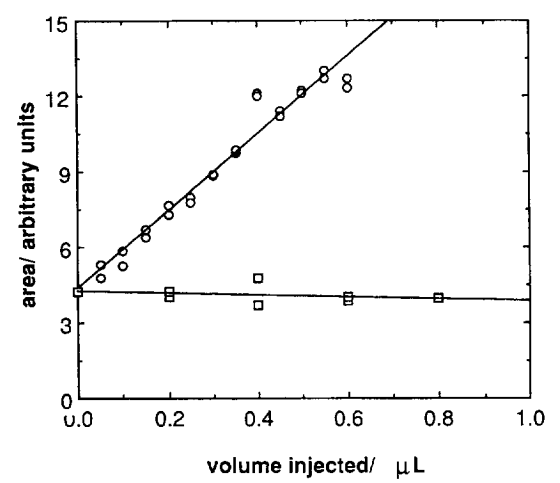

Fig. 3. Repeat of the procedure used in Fig. 2, except that the residence time of the needle in the heated port was 2 s in each case.

In the above experiments, the temperature of the injection port had been set at the 'normal' value of 180°C. It was therefore decided to investigate the effect of injection port temperature on the magnitude of the intercept, since it may be argued that the effect should be reduced with decreasing port temperature. Blank runs were accordingly performed with syringe X using injection port temperatures of 180, 160, 140, 120, 100 and 90°C. The results are shown in Fig. 4, and provide final confirmation of the idea that evaporation from the needle is responsible for the intercept.

Turning now to the calibration for cyclohexanol, this was performed with the same syringe X, using an injection port temperature of 180°C, and a residence time for the needle in the port of 2 s in all cases. The results are shown in Fig. 5, from which it can be seen that the intercept, if any, is within the experimental scatter. From this it is clear that within the 2-s residence time of the needle, cyclohexanol is sufficiently involatile for the anomaly to be undetect-

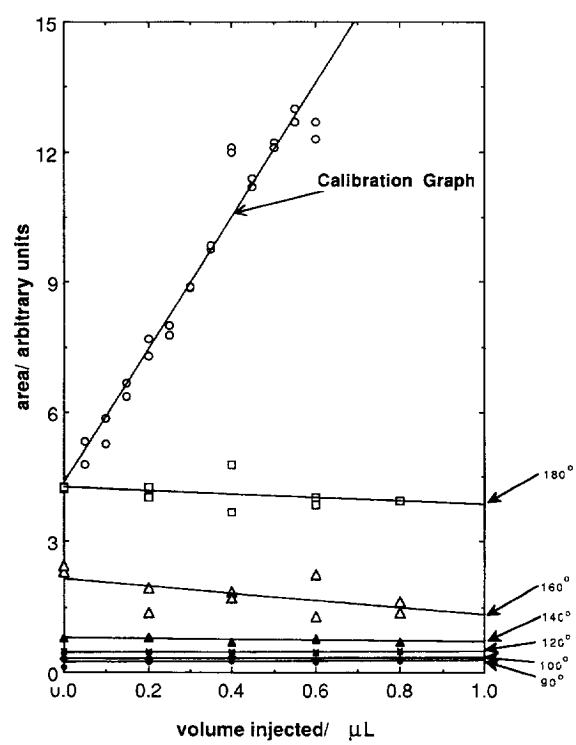

Fig. 4. Repeat of the procedure used in Fig. 3, except that the injection port temperatures were as indicated on the figure.

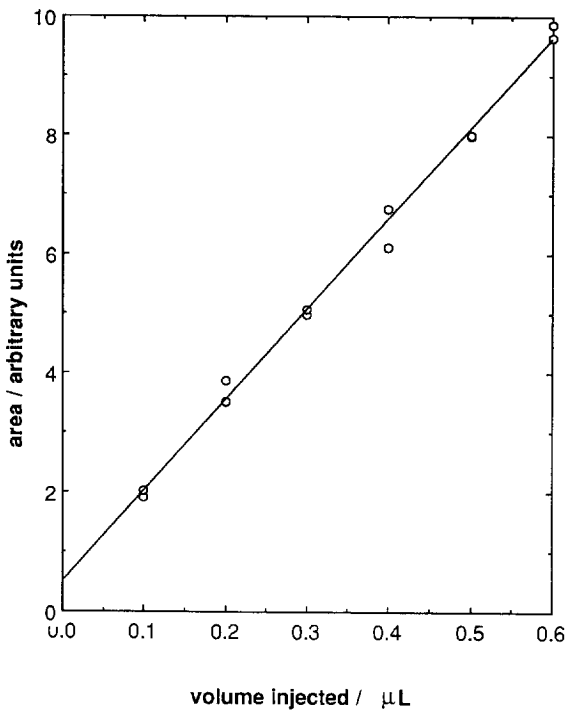

Fig. 5. Cyclohexanol calibration performed with syringe X, using the same procedure as that used for the cyclohexane calibration shown in Fig. 1. For cyclohexanol, the intercept is either zero or within the experimental scatter.

able. (The boiling points of cyclohexane and cyclohexanol at atmospheric pressure are ca. 80 and 160°C, respectively).

It is therefore now apparent that the attempts (mentioned in the introduction) to calibrate with known ratios of cyclohexane/cyclohexanol were doomed to failure, because as the ratio changes, the bonus by evaporation from the needle changes too.

The whole of the problem encountered in this calibration work should be absent if a syringe of the type in which the piston passes down the needle were to be used, such as syringe type Y. Fig. 6 shows the results obtained with this syringe for a cyclohexane calibration, using an injector port temperature of 180°C. The absence of any positive intercept (indeed, there is a suggestion of a very small negative intercept in the least squares line) provides very strong support for the explanation of the anomaly presented above.

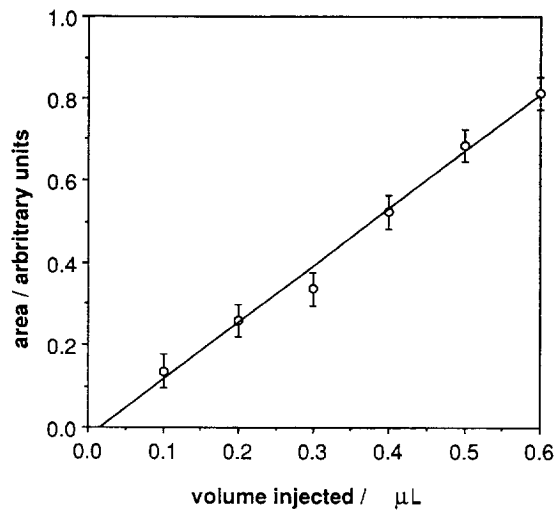

Fig. 6. Cyclohexane calibration performed with syringe Y, using the same procedure as that used for the calibration shown in Fig. 1. This plot shows that the intercept is either zero or within the experimental scatter.

4. Conclusions

On the basis of the evidence in this paper, the authors conclude that a conventional (open-needle) syringe is inappropriate for quantitative work on liquids, because of evaporation of bonus material from the needle during its residence time in the heated injection port. The possibility of evaporation from the needle has indeed been discussed, for example, by Grob and Bronz [8], by Lebbe [17], and in the book by Nogare and Juvet [18] where it is mentioned that needle corrections of up to 0.3 μl may be required because of losses by evaporation. It will be noted that this is of the same order as implied by the intercept in our original cyclohexane calibration. The anomaly increases with (a) increasing temperature of the port, (b) residence time of the needle in the port, (c) the volatility of the liquid being injected, and (d) the speed with which the syringe plunger is depressed, as would be expected from previous recommendations in the literature [8,16].

The anomaly, when present, also results in unmeaningful calibrations or estimations using mixtures of components of differing volatility. It could also have serious implications when using an internal

standard in similar situations. However, it has been demonstrated that the problem completely disappears if the syringe is of the type where the piston passes down the needle, and syringes of this type are therefore essential for quantitative work.

Acknowledgments

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