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Note

Deoxygenation of small samples for reductive mode electrochemical detection in high-performance liquid chromatography

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A common difficulty in the use of reductive mode electrochemical detection in high-performance liquid chromatography is due to the sensitivity of the detector to oxygen. This produces a strong chromatographic peak if it is not removed from a sample: the presence of oxygen is not infrequently seen in published chromatograms, for example.

Sample deoxygenation is usually effected in some sort of inert-gas purging vessel, from which the sample is transferred by syringe or directly through a transfer line to an injection valve. An illustration is given by Bratin *et al.*[1]. A much easier technique is to deoxygenate the sample in the syringe that might otherwise be used for the transfer, either to the valve or to the deoxygenation vessel, as described below. For small samples this is particularly valuable, because the loss of sample due to entrainment in the deoxygenation assembly and its associated pipe-work is minimized. The technique is rapid and is readily set-up. All that is required is a modified hypodermic syringe. The following refers to a Rheodyne injector, but presumably others might equally well be used.

EXPERIMENTAL

The bottom of a 1-ml glass hypodermic syringe is tapered down, in a glass-working torch, to give a thick-walled capillary that will accommodate a syringe needle appropriate to the valve injector used, *e.g.*, for the Rheodyne Model 7125 injector the Scientific Glass Engineering needle, part number 1OARL2(N), is suitable. The capillary is shortened to a length of about 5 mm, and the end of the needle, similarly shortened, is fixed into the capillary with epoxy cement. To avoid the needle's becoming plugged, its end is pushed into the capillary before the application of the cement. This is applied around the exterior of the joint and then, with a slight vacuum, sucked into the space between the walls of the capillary and the needle whilst the latter is slowly rotated in the former. A lengthwise notch, about 5 mm long, is filed in the inside wall of the open end of the syringe barrel to act as a gas vent. The syringe is then silanized, preferably with a non-halogenated reagent, *e.g.*, bis(trimethylsilyl)acetamide (an electrochemical response is produced from some halogenated compounds, which may prove troublesome to remove from freshly sila-

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nized glassware), and approximate graduations corresponding to the injector loop volume are marked on the tapered part of the syringe.

The injector is set in an upright position, and a nitrogen supply (fitted with an oxygen trap and a solvent presaturator) is connected to the vent line from the "inject" position of the injector. A polytetrafluoroethylene ferrule is used in the connection so that the vent line may be released and resealed conveniently by finger pressure in order to wash out the needle port and vent as necessary.

The sample, preferably slightly in excess of the loop volume, but see below, is drawn into the tapered part of the dry syringe, which is then presented to the injector in the "inject" setting and purged with nitrogen at *ca.* 5 ml min^{-1}. The initial flow may be accelerated by the withdrawal of the syringe plunger up to the notch in the barrel. After 2 min the injector is set in the "load" position, and the sample pushed into the loop and injected in the usual way.

RESULTS

Some results from chromatograms run under conditions insignificantly different from those of Bratin *et al.*[1], with a Bioanalytical Systems mercury film electrode operated at a potential of −1.0 V *vs.* Ag/AgCl, are shown in Fig. 1. Chromatogram C is from an aerated solution containing 5 ng of nitrobenzene in the injected 20 µl of sample. Only the massive response due to oxygen is seen. After this injection the baseline required over 30 min to stabilize. The same sample, after deoxygenation as described above, gave chromatogram B, which shows the nitrobenzene peak free from oxygen interference. Chromatogram A is from a deoxygenated sample of the

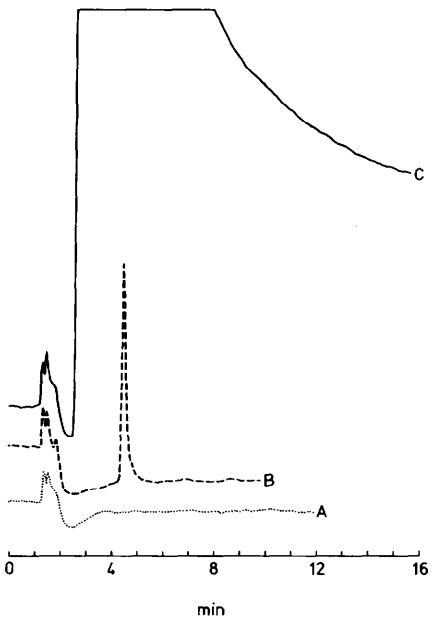

Fig. 1. Chromatograms of deoxygenated solvent blank (A), 5 ng of nitrobenzene in 20 µl deoxygenated solvent (B) and as B but aerated (C). Further details are given in the text.

solvent (buffered aqueous methanol). The same sensitivity setting was used for each chromatogram.

As little as 2 μl of sample is retained in the syringe after the deaeration and transfer, provided the sample is not allowed to wet the syringe barrel. Usually this only occurs with samples that tend to foam. In this circumstance the nitrogen flow-rate should be reduced to allow more time for bubble-drainage, and, if acceptable chromatographically, the level of organic solvent in the sample may be increased to suppress the foaming.

It should be noted that the actual volume transferred to the sample loop in the injector will be less than the nominal volume when only small volumes of sample are available[2]. However, the effect has not been of significance relative to other variables in the detection and analysis of materials in trace amounts, for which the technique was designed, and good reproducibility is obtained. Thus, the peak heights of eight replicate injections of 5-ng amounts of nitrobenzene, in 20 μl of solvent, were distributed with a coefficient of variation of 2.4%.

When less than a loop-volume of sample is available, the loop can be deliberately underfilled. Although this leaves a bubble of nitrogen in the loop, from the purge gas underlying the sample in the syringe and its needle, chromatographic performance is unaffected. The bubble dissolves when the loop is pressurized, and does not emerge at the detector because, with a heated solvent reservoir[1], the concentration of nitrogen in the effluent is well below the solubility limit at ambient temperature and pressure.

REFERENCES

1 K. Bratin, P. T. Kissinger and C. S. Bruntlett, *J. Liquid Chromatogr.*, 4 (1981) 1777.
2 *Technical Note No. 1*, Rheodyne, Cotati, CA, September 1979.