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Note

Decreased accuracy in isotachophoretic analysis due to septum bleed

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In capillary isotachophoresis, the sample may be introduced either by sandwiching it between the leading and terminating electrolytes with the aid of a sample tap, or by injecting it from a micro-syringe through a self-sealing rubber membrane (septum)[1]. Although the sample tap should normally be expected to give the better reproducibility, most workers have preferred the injection technique because of its simplicity and its flexibility with regard to sample volume.

In certain isotachophoretic systems, however, septum bleed, *i.e.*, the extraction of plasticizers and other additives from the septum, may cause significant analytical errors. This was discovered in a project aimed at quantitative determination of oxalic, glyoxylic and glycollic acids in reaction mixtures resulting from the cathodic reduction of oxalic acid.

EXPERIMENTAL AND RESULTS

Materials

The analyses were carried out with an LKB 2127 Tachophor (LKB, Bromma, Sweden) equipped with a 23-cm capillary tube. The leading electrolyte solution was made up from concentrated HCl (Suprapur; E. Merck, Darmstadt, G.F.R.) and a 1% dialyzed stock solution of hydroxypropyl methyl cellulose (HPMC; Dow Chemical, Midland, Mich., U.S.A.). The final concentrations were: HCl 10 m*M*, HPMC 0.3%. The HCl–HPMC solution was titrated to pH 3.30 with recrystallized β-alanine, and 5 m*M* acetic acid (p.a. quality; E. Merck) was used as the terminating electrolyte.

Evidence for septum bleed

Preliminary runs indicated that the electrolyte system contained small amounts of a number of UV-absorbing impurities with mobilities between those of the leading, sample, and terminating ions (see Fig. 1). Thus it was possible and, with regard to sensitivity, also desirable to choose the UV-absorption record (254 nm) instead of the thermometric record for quantitative analysis[2]. It was soon observed, however, that, with a given sample, the amounts of the impurities decreased from one run to the next, although the electrolyte system (in the capillary as well as in the electrode pools) had been replenished from stock solutions. (See, for example, peaks A and B in Fig. 2a and b.)

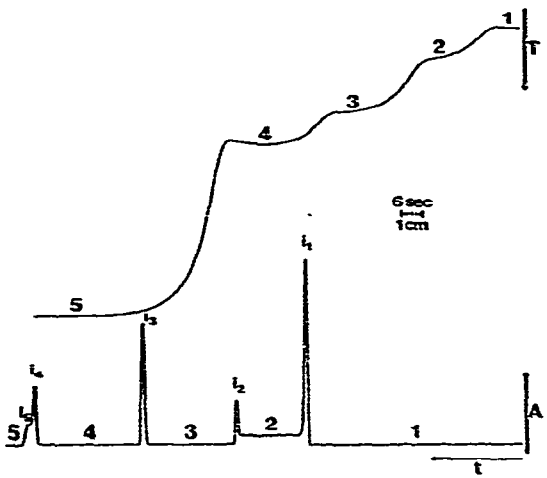

Fig. 1. Isotachopherogram obtained during analysis of a 1-μl sample of oxalic, glyoxylic and glycollic acids at 20°. Leading electrolyte: 10 m*M* HCl + β-alanine to pH 3.30; 0.3% HPMC. Terminating electrolyte: 5 m*M* acetic acid. Current: 90 μA. Paper speed: 10 cm/min. T = temperature; A = UV absorption (254 nm); t = time. 1 = chloride; 2 = oxalate (6 m*M*); 3 = glyoxylate (10 m*M*); 4 = glycollate (12 m*M*); 5 = acetate; i_1–i_5 = UV-absorbing impurities. In the record, 1 nmole of a sample species (or an impurity) gives a zone length of 4–5 mm.

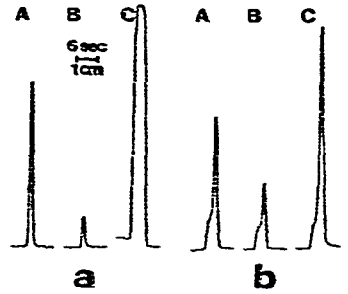

Fig. 2. Effect of condition of septum on size of impurity peaks i_1–i_5 in Fig. 1. A large number of 1-μl aliquots of the same sample (32 m*M* oxalic acid, 50 m*M* glyoxylic acid, 58 m*M* glycollic acid) were run in sequence during 2 days. The peaks shown were obtained as follows: A, in a run with a fairly fresh septum; B, with the same septum as in A, but about 30 runs later; C, in the first run with a new (cleaned) septum. The peaks in part (a) of the figure correspond to i_3 in Fig. 1; those in part (b) correspond to i_4 and i_5. Electrolytes, current strength and paper speed as in Fig. 1.

After systematic cleaning or exchange of those parts of the micro-syringe and the Tachophor Analyzer Unit making contact with the electrolyte system, it was finally established that the septum (LKB spare part No. 94922168) was the main source of the impurities (see Fig. 2a and b, peak C). Further evidence for a bleed of absorbing as well as non-absorbing impurities from the septum was obtained from three consecutive "blank" runs, the records of which are shown in Fig. 3. In the first run (A), small pieces of rubber were produced in the injection port by repeated violent penetration of the septum with a cannula just before the start. Before the next run (B), only the leading electrolyte solution was replenished in order not to

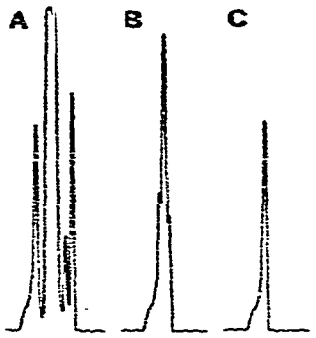

Fig. 3. UV-absorption traces for three consecutive "blank" runs: A, with small pieces of septum rubber in the injection port; B, the same pieces of rubber as in A, only with the leading solution replenished; C, the pieces of rubber rinsed away, and the septum replaced by a PTFE disc. Electrolytes, current strength and paper speed as in Fig. 1.

rinse away the pieces of rubber. In the blank run (C), the septum was replaced by a carefully cleaned disc of PTFE.

Construction and handling of a septumless syringe injector

In chromatography, where septum bleed is already a well-known drawback, either of the following remedies is being utilized: (1) The bleed is reduced by the use of commercially available, low-bleeding and/or PTFE-coated septa; (2) the bleed is eliminated by the use of so-called septumless syringe injectors, which are likewise commercially available in several designs. The same possibilities are to hand in isotachophoresis, except that, with (2), a special injector has to be built by the experimenter. The chromatography injectors, being constructed for a system involving no voltage and often high pressure, would be both unsuitable and unnecessarily complicated if used in isotachophoretic equipment. Actually, such a septumless syringe injector for the Tachophor can be made very simply, provided that the filling routine recommended by LKB is slightly changed.

The septumless injector is obtained by modifying the septum case (LKB spare part No. 94922165) according to Fig. 4. The bore of the septum case (a) is increased to a diameter of 5 mm, and a flanged plug (b) is turned from a piece of PTFE rod so as to fit into the bore. Then an axial channel (c), with a diameter only slightly larger than that of the syringe needle to be used, is drilled into the PTFE plug. The outer end of this channel is so widened that it can be closed by another PTFE plug (d). Finally, a gasket of PTFE film is inserted between the modified septum case and the injection block of the Tachophor to ensure a leak-proof connection.

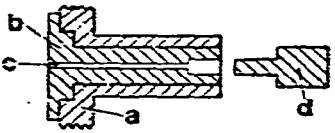

Fig. 4. Septumless syringe injector: a, septum case of the LKB 2127 Tachophor, with the bore increased to 5 mm; b, flanged PTFE plug; c, channel of diameter slightly larger than that of the syringe needle; d, PTFE plug fitting into c.

To avoid leakage of terminating electrolyte while withdrawing the needle after injection, the normal filling routine (see the LKB Tachophor 2127 Instrument Manual) is replaced by the following one: (1) Fill the leading electrolyte reservoir; (2) rinse and fill (c) by gently pressing the terminating electrolyte syringe; (3) close (c) by means of (d), rinse, and fill the injection port; (4) rinse and fill the capillary; (5) open (c), and inject as usual; after injection, slowly withdraw the syringe while giving it half turns in both directions; (6) close (c), and fill the terminating electrolyte reservoir.

DISCUSSION

The traces shown in Figs. 1–3 clearly demonstrate that the risk of a disturbing septum bleed should not be neglected in careful isotachophoretic analysis. In itself, the presence of small amounts of UV-absorbing impurities ("markers") is highly desirable when non-absorbing samples are to be analyzed in the Tachophor, as this instrument at present lacks the high-resolving conductivity detector[1]. One should remember, however, that *non-absorbing* species may also be extracted from the septum (*cf.* Fig. 3, trace A) and that these species are not disclosed by the UV-detector unless they are sandwiched between two markers. This is a serious drawback to evaluation of the isotachopherogram, as it generally means that they make unknown and varying contributions to the zone lengths of the sample species. Thus, if UV-absorbing marker species are needed to obtain high resolution, it seems far better to add them to the sample in controlled amounts than to try to take advantage of an impure electrolyte system.

It should also be pointed out that impurities resulting from septum bleed will migrate as a moving-boundary system through the terminating and sample zones, with concomitant disturbing superpositions of concentrations, until they reach a zone boundary in accordance with their effective mobilities. In other words, the interference caused by these impurities is similar to that of terminating electrolyte impurities. Consequently, the elimination of septum bleed, if it occurs, is as important as the purification of terminators already recommended by several authors (see, *e.g.*, refs. 1 and 3).

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