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Note

Isotachophoretic analyses of anions at high pH

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In isotachophoretic analyses of anions, performed in operational systems at high pH ($\text{pH} \geq 7$), disturbances due to carbon dioxide from the air can be expected. Even at neutral pH the isotachophoretic features can be obscured by this disturbance, because the pH of the sample zones has a tendency to increase from the leading zone towards the terminating zone in anion analysis^{1,2}. This disturbance, according to the principles of zone electrophoresis, can be caused by carbon dioxide dissolved in the terminating electrolyte. A minor disturbance can be expected from carbon dioxide dissolved in the leading electrolyte.

It was found very convenient to prepare both the leading and terminating electrolytes under nitrogen. Barium hydroxide (analytical-reagent grade) was added to the terminating electrolyte to prevent carbonate from entering the separation compartment. The terminator compartment still needed to be refreshed completely after each analysis. Complex formation between Ba^{2+} with sample anions was minimized by careful injection. Addition of barium hydroxide to the leading electrolyte is not recommended. Unwanted complex formation often occurs and precipitation of barium carbonate can block the capillary column. In this paper it is shown that isotachophoretic analyses of anions at $\text{pH} \geq 7$ can be carried out by closing the separation compartment with semi-permeable membranes of cellulose polyacetate (0.1 mm thick) and having solely doubly distilled water in the electrode compartments. Both the leading and terminating electrolytes are consequently not in direct contact with air. Carbon dioxide already dissolved in the electrolyte during preparation still has to be taken into account.

EXPERIMENTAL

Instrumental

Commonly, isotachophoretic analyses are performed in equipment such as that described in ref. 1. In our home-made equipment a PTFE capillary (I.D. 0.2 mm, O.D. 0.35 mm and length *ca.* 25 cm) is applied. This separation compartment is connected to the injection block, which has a connection, via a valve (1MM1; Hamilton, Bonaduz, Switzerland), towards an open terminating reservoir. Commercial equipment also has such an open terminating reservoir. At the end of the separation compartment a set of detectors (UV absorption, conductivity or potential gradient)

is mounted. The counter-electrode compartment is equipped with a semi-permeable membrane to prohibit any hydrodynamic flow.

The above mentioned equipment is compared with the equipment shown schematically in Fig. 1. In this device semi-permeable membranes separate the terminating and leading electrolytes from air via a barrier of doubly distilled water, which was found to conduct the electric current sufficiently. This set-up is also called a "completely closed" system.

Sample introduction

In the reservoir (Fig. 1, a) a nitrogen bubble was purposely introduced: in-

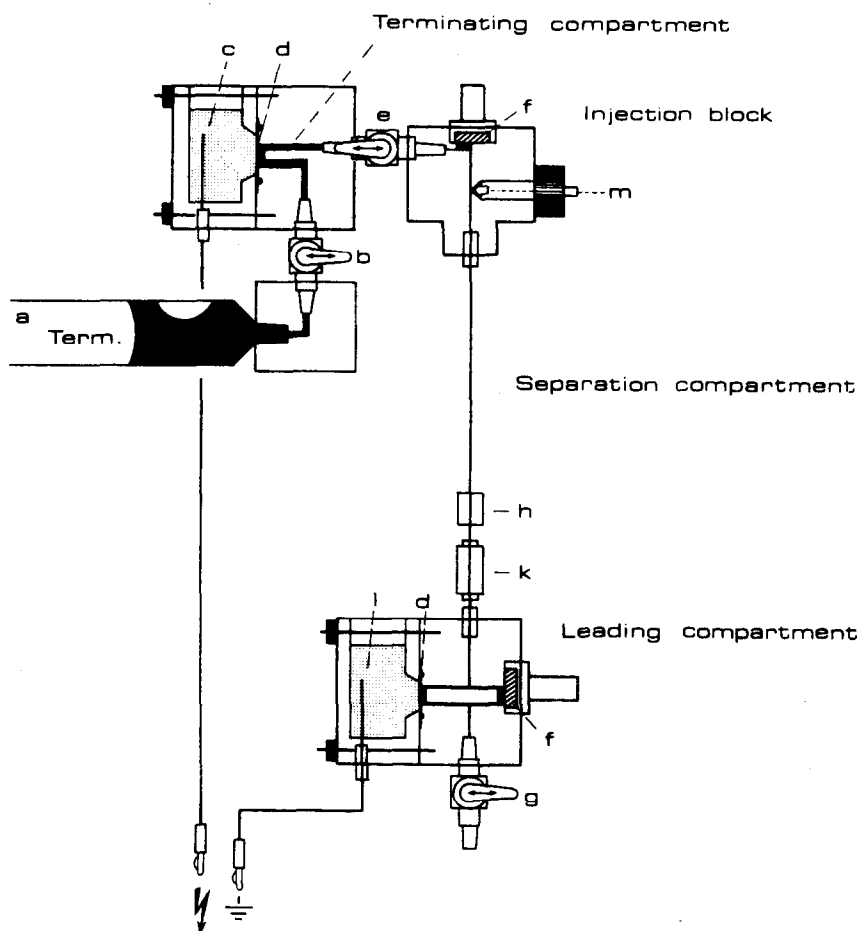


Fig. 1. Isotachophoretic equipment suitable for analysis at high pH. The conventional equipment¹ modified by adding items a-d in the terminating compartment. a = Polypropylene syringe filled with terminating electrolyte; a nitrogen-bubble was introduced before sample introduction (see text); b, e, g = two-way valves (Hamilton IMM1); c = terminating electrode immersed in doubly distilled water; d = semi-permeable membrane (cellulose polyacetate); f = silicone-rubber septum; m = home-made valve with connection towards drain; h = UV detector; k = conductivity (potential gradient) detector; l = counter electrode immersed in doubly distilled water.

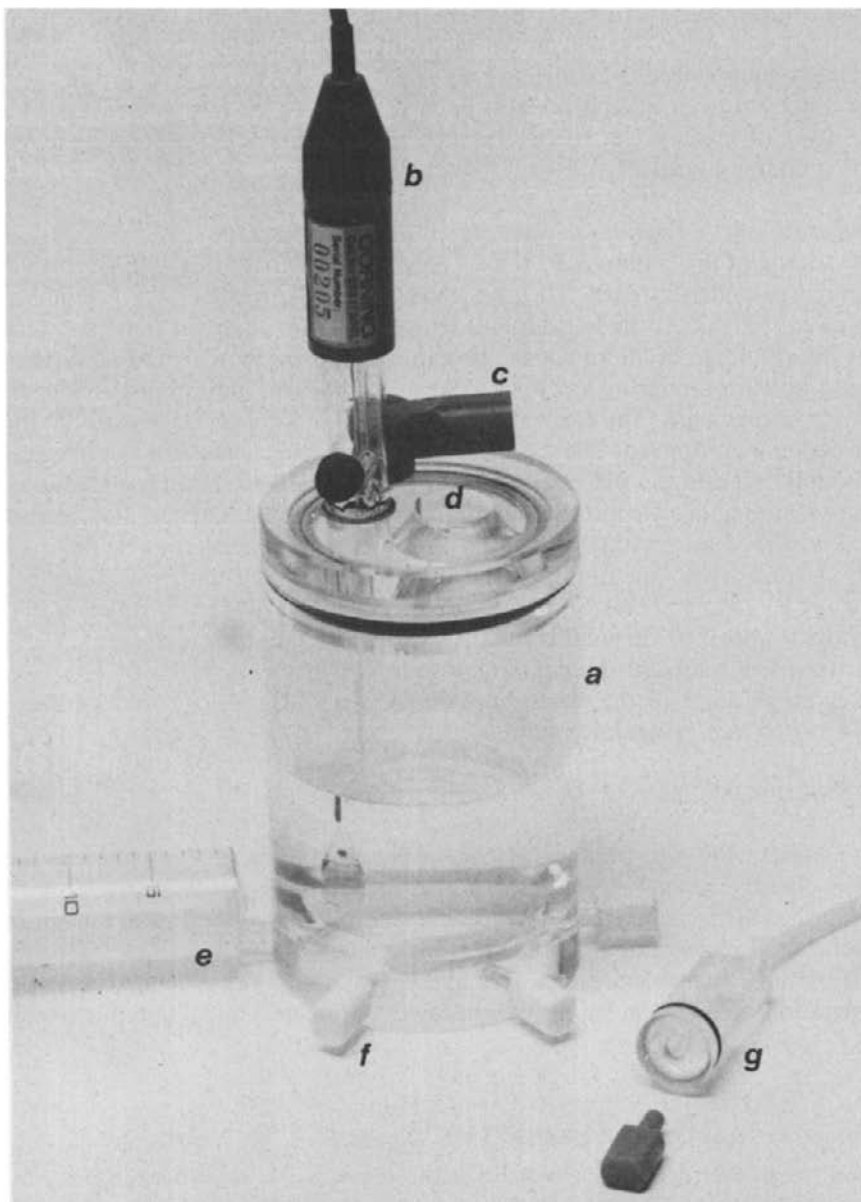


Fig. 2. Beaker for preparing electrolytes under an inert gas atmosphere. a = Beaker made of acrylic; b = combined glass pH electrode (Corning, No. 003 11 306L); c = home-made valve; d = hole for inert gas supply or addition of electrolyte constituents; e = polypropylene syringe; f = PTFE stoppers; g = connector for inert gas supply.

jecting a sample in the injection block now caused a displacement of terminating electrolyte owing to the penetration of the needle of the syringe and finally injection of the sample. Withdrawing the syringe again caused a flow of terminating electrolyte.

After sample introduction, valve b was closed to separate the reservoir a with the pressurized nitrogen bubble from the separation compartment.

If large volumes need to be injected, valve b is closed before injection and valve g at the counter-electrode compartment is opened. Injecting the sample hence causes a displacement of leading electrolyte. In such experiments a pre-separation compartment³ is commonly used.

Device for preparing electrolytes at high pH

The testing of the "completely closed" system was carried out with electrolyte systems prepared under nitrogen. The device used (Fig. 2) is made of acrylic and has a content of *ca.* 100 ml. At the bottom four connections are made for polypropylene syringes with a volume of 20 ml each. The cap is equipped with an O-ring, a two-way valve, a hole for mounting a pH electrode and a hole for mounting a connector for supply of nitrogen gas. The electrolyte was stirred via a magnetic device. During stirring nitrogen was supplied. The valve (Fig. 2, c) was open to prevent disturbances of the electrolyte inside the pH electrode due to overpressure. During addition of electrolyte constituent(s), the nitrogen supply was disconnected. Carbon dioxide that penetrated adsorbed on crystals or dissolved in liquid bases needed to be removed separately. Liquid bases can simply be purified by redistilling under vacuum in a desiccator (at room temperature) into a beaker filled with doubly distilled water. Carbon dioxide adsorbed on solid constituents can be removed under vacuum in a desiccator (at room temperature) containing sodium hydroxyde pellets.

After preparation of the electrolyte, the syringes (Fig. 2, e) could be filled, closed and stored in a refrigerator until used.

RESULTS AND DISCUSSION

To compare the performance of conventional equipment¹ with that of the "completely closed" system (Fig. 1), a series of isotachophoretic analyses was carried out with xanthosine as sample constituent. The operational conditions are given in Table I; all chemicals were of analytical-reagent grade.

Disturbances of carbonate, dissolved in the terminating electrolyte, were found in the elongation of the zone length (quantitative parameter) and the reduction of

TABLE I
OPERATIONAL SYSTEM FOR ANION ANALYSES AT pH 8.9

$I = 0.08 \text{ A cm}^{-2}$; chart speed of recorder = 6 cm sec^{-1} ; analysis time = *ca.* 9 min. β -Ala = beta-alanine; Mowiol = polyvinylalcohol: a 5% (w/v) stock solution has been purified with mixed-bed ion exchanger (Merck, Darmstadt, G.F.R.).

<i>Electrolyte</i>	<i>Leading</i>	<i>Terminating</i>
Anion	Chloride	β -Ala/OH ⁻
Concentration	0.0075 <i>M</i>	<i>ca.</i> 0.01 <i>M</i>
Cation	Ammediol	(Ba ²⁺)
pH	8.9	<i>ca.</i> 10
Additive	Mowiol; 0.05%	None

the step height (qualitative parameter) of xanthosine, both due to the "diluting" effect (zone electrophoresis).

In Figs. 3 and 4 the influence of carbonate clearly is shown. Both qualitative and quantitative information is poor in analyses carried out at high pH in conventional equipment. In the experiments the terminating reservoir was purposely not refreshed completely after each analysis to show the disturbance of carbonate. In the "completely closed" system a straight calibration line (correlation coefficient = 0.9999) for xanthosine was obtained (Fig. 3). The carbonate zone had a constant length of 7 mm, which means it no longer obscured the analytical result. In fact, the terminating electrolyte was simply refreshed completely after each analysis.

From Fig. 4, it can be seen that in conventional equipment the zone length of carbonate is elongated (*ca.* 15 mm) in the experiment in which solely leading electrolyte *versus* terminating electrolyte was analysed. The zone length of carbonate increased in a series of analyses. Experiments with the "completely closed" system (Fig. 1) proved that addition of Ba^{2+} to the terminating electrolyte was no longer necessary. Unwanted complex formation with sample constituents thus could be excluded.

The amount of terminating electrolyte between the injection point and the

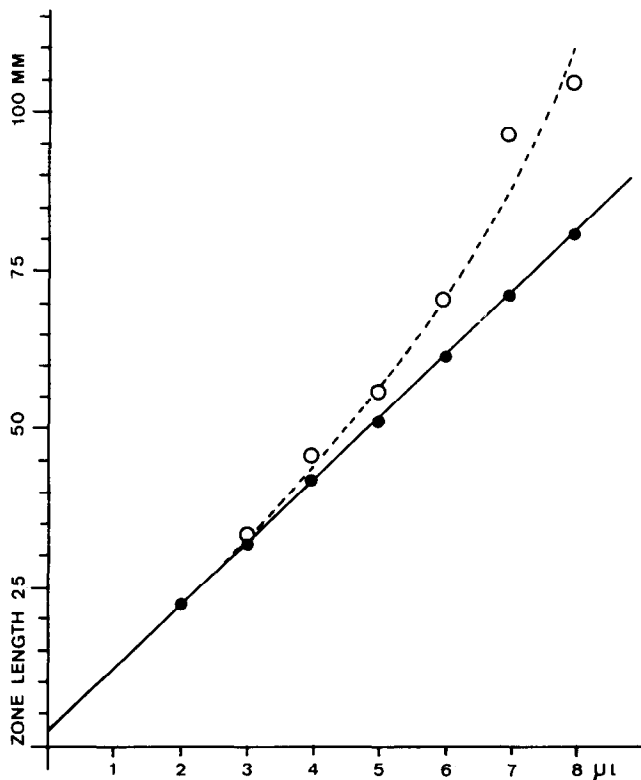


Fig. 3. Calibration line of xanthosine in the operational system (Table I). The analyses were performed (○) in conventional equipment¹ and (●) in the equipment shown in Fig. 1. The correlation coefficient in the latter line is 0.9999. The deviation of the other line (○) is due to the carbonate originating from the terminating electrolyte (see text).

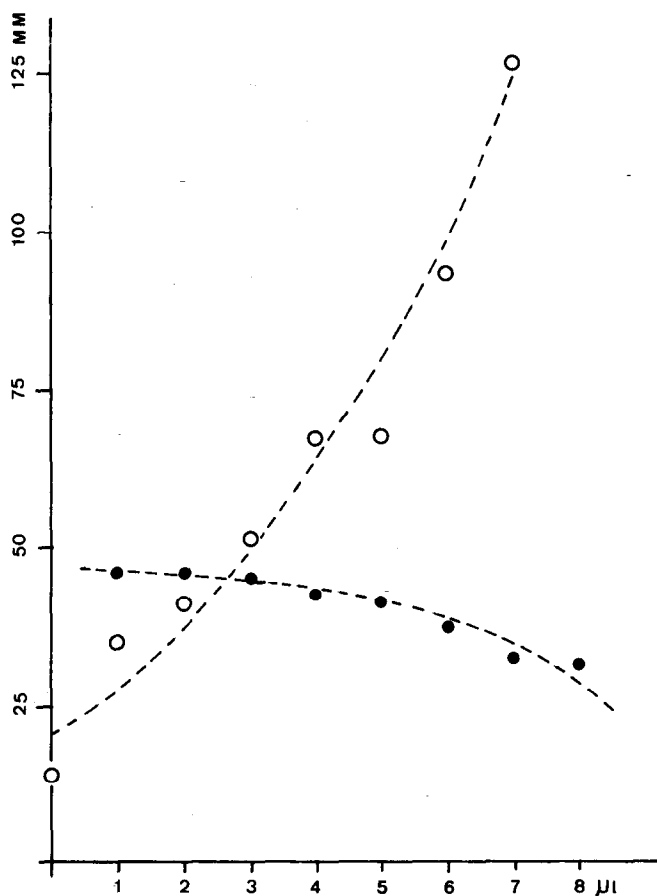


Fig. 4. The zone lengths (○) of carbonate in a series of analyses in conventional equipment, in which increasing amounts of xanthosine were injected. These zone lengths were taken from isotachopherograms from which the calibration line (○) in Fig. 3 was constructed. The reduction of the step height of xanthosine (●) is also shown. For further explanation, see text.

membrane needs to be chosen properly, to prevent the terminator becoming exhausted, especially if a high sample load is applied (long analysis time). Twice the total volume of the separation compartment was found to be sufficient, assuming the concentration of the terminating electrolyte was of the same order of magnitude as the leading electrolyte.

Fig. 5 shows an isotachopherogram of xanthosine, allopurinol, guanosine and adenine (for operational conditions, see Table I). The terminator was in the reservoir of the equipment for *ca.* 15 h before the analysis. The zone length of carbonate still did not show any elongation.

CONCLUSIONS

Analysis of, *e.g.*, purines bases, peptides and proteins can be carried out at pH ≥ 7 with no disturbances from Ba^{2+} or carbonate. Most commercial equipment can

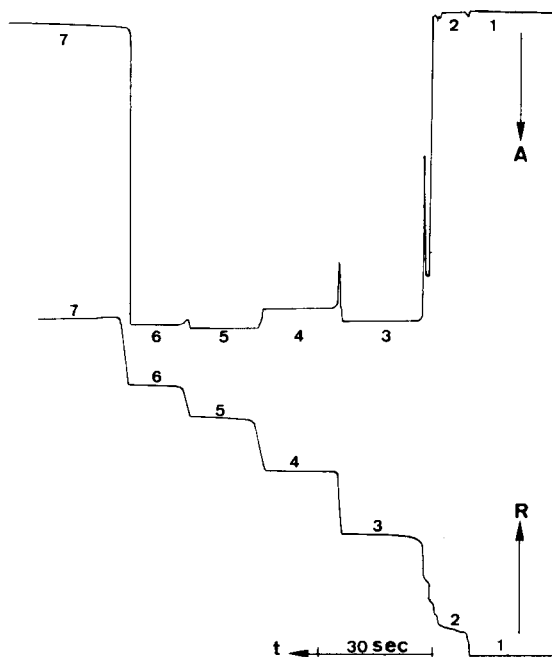


Fig. 5. Isotachopheric analysis of xanthosine, allopurinol, guanosine and adenine in the equipment shown in Fig. 1. Operational conditions are listed in Table I. Note the small zone of carbonate, which was found to be constant from one analysis to another. 1 = Chloride; 2 = carbonate; 3 = xanthosine; 4 = allopurinol; 5 = guanosine; 6 = adenine; 7 = β -alanine. R = increasing electric resistance; t = time; and A = UV absorption.

be simply modified with a semi-permeable membrane at the terminating reservoir. "Completely closed" systems are recommended if electrode reactions with terminator and/or additives are expected.

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