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Note

Effect of a concentration cascade of the leading electrolyte on the separation capacity in isotachophoresis

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Some years ago a counter-flow technique was introduced in isotachophoresis to increase the separation power of a column without changing the detection conditions and high voltage required[1]. This technique makes it possible to obtain a complete separation of ionic species having similar mobilities or when an ionic species is present in a mixture at a sufficiently high level to cause mixed zones. The problem of mixed zones has led to the introduction of the term separation capacity[2,3] which characterizes the maximum amount of two ionic species which can be separated completely under given working conditions.

At the present time there are three ways of increasing the separation capacity in cases where mixed zones are to be resolved and complete separation obtained. (i) A change in the column length by replacing the separation capillary (Tachophor 2127 LKB). (ii) A change in the effective length of the separation path by applying a hydrodynamic counterflow[1]. (iii) An increasing of the total volume of the leading electrolyte placed between the sample and the detector[4].

The present paper describes a method[5] which employs a concentration cascade of the leading electrolyte to increase the separation capacity (and thus resolve mixed zones) without changing the detection conditions (and thus maintaining constant the length of the zones).

MATERIALS AND METHODS

The proposed method is based on isotachophoretic migration in a concentration cascade of the leading electrolyte. Its principle is shown in Fig. 1. Before the separation starts (Fig. 1a) a concentration cascade of the leading electrolyte is created in the separation capillary. This cascade consists of a section containing a low concentration (LC) of the leading electrolyte in the part of the column equipped with the detector (DET), and of a section with a high concentration (HC) of the leading electrolyte ranging from the high-concentration electrolyte inlet (HC inlet) up to the injection port (INJ). The capillary also contains the sample (S) and terminating electrolyte (T). The sample is a mixture of components a and b. On passing an electric current, the migration of ionic species occurs and the state at a certain time is shown in Fig. 1b. Here it is obvious that the pure components b and a are already partially separated from each other, while a mixed zone s still separates them. The

concentrations in all of the zones are adjusted to the high concentration of the leading electrolyte, and the zones are short and migrate slowly. The concentration boundary HC:LC at the HC inlet point moves negligibly on passage of an electric current. Under normal isotachophoretic conditions the speed of migration of the concentration boundary is less than that of the zones by *ca.* 4–5 orders of magnitude (*cf.* ref. 6). Fig. 1c illustrates the situation when the separation is complete in the section of the high-concentration leading electrolyte, and the zones of the individual components pass through the concentration boundary HC:LC into the second section of the capillary. It can be seen that behind this boundary the zones adjust to low concentrations, become longer and their migration velocity increases. It is obvious that the separation capacity of the column increases substantially due to the section containing the high-concentration leading electrolyte and in proportion to the increased electric hold-up capacity[3] of the column, while the detection sensitivity remains unchanged and corresponds to the section containing the low-concentration leading electrolyte.

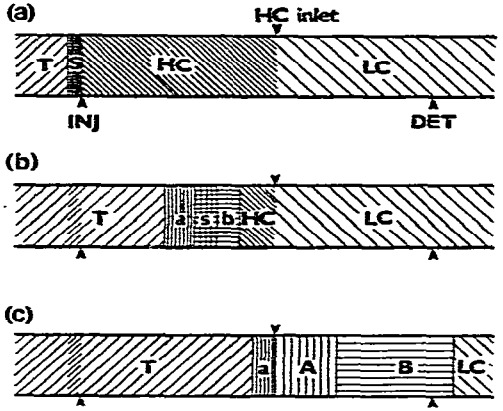

Fig. 1. Scheme showing the separation and detection in the cascade system. LC = Low-concentration leading electrolyte; HC = high-concentration leading electrolyte; S = sample containing components a and b; T = terminator; DET = detection point; INJ = injection port.

Apparatus

A flat column[7] with a 10 cm long separation capillary was adapted for the experiments by providing the separation capillary with one more opening. This opening, as shown in Fig. 2, is placed approximately halfway along the capillary and is equipped with a PTFE stopcock key. The opening permits the introduction of the leading electrolyte at various concentrations. The following procedure is used for the application of the cascade method. The column, including a three-way valve in position CD, is filled with the low-concentration leading electrolyte (LCLE) from a container joined to the LCLE inlet via an two-way valve. The two-way valve is the closed and the stopcock key is opened. The section of the capillary between the stopcock key and the three-way valve (still in position CD) is then filled with HCLE via the HCLE inlet. The stopcock is then closed, and the three-way valve in position TD is filled with the terminating electrolyte and turned into a position (TC) in which the terminator electrode is connected to the capillary. The sample is injected through

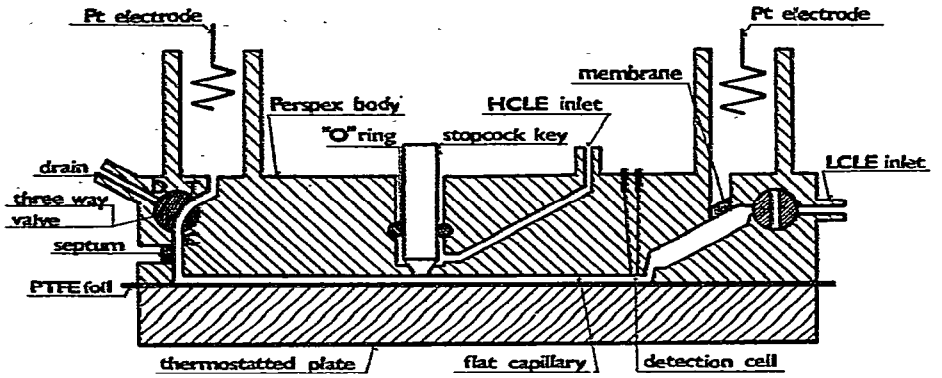

Fig. 2. Sectional view of the isotachophoretic column adapted for the cascade method.

the septum and the analysis is started by connecting the platinum electrodes to a stabilized current supply.

Materials

A mixture of 0.01 *M* HCl and 0.02 *M* urotropine (hexamethylenetetramine) at pH 5 was used as the LCLE, and a mixture of 0.05 *M* HCl and 0.1 *M* urotropine at the same pH served as the HCLE for creating the cascade system. 0.01 *M* glutamic acid was the terminating electrolyte. All the chemicals were of analytical grade (Lachema, Brno, Czechoslovakia). The liquid fertilizer sample under analysis was of the N–P type (North Bohemian Chemical Works, Lovosice, Czechoslovakia). The separations were carried out at a constant current of 400 μA and at a temperature of 22°.

RESULTS AND DISCUSSION

The separations of a sample of the liquid fertilizer (containing ortho- and pyrophosphate) are illustrated in Fig. 3. Fig. 3a shows the isotachopherogram ob-

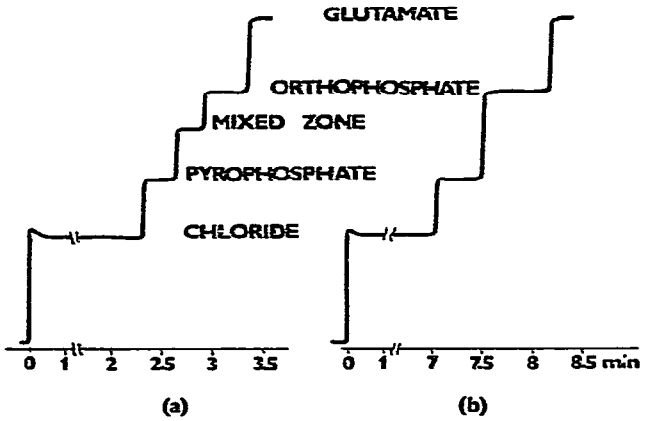

Fig. 3. Comparison of isotachopherograms of samples of liquid fertilizer obtained by the normal (a) and cascade method (b). In both cases the same samples were separated (*ca.* 37 and 18 nmole of ortho- and pyrophosphate, respectively). For separation conditions see text.

tained by a procedure in which the entire separation capillary was filled with one leading electrolyte, *i.e.*, the low-concentration electrolyte. The separation capacity of the column was obviously not high enough to achieve complete separation of ortho- and pyrophosphates and a mixed zone appeared on the record. (By decreasing the amount charged for the separation, a complete separation could be obtained; however, the zones would then be short.) Fig. 3b shows the separation by the cascade method of the same amount of the sample and at the same electric current as in Fig. 3a. It can be seen that a complete separation can be thus simply achieved. The step heights are the same as in Fig. 3a, which means that the composition of the low-concentration leading electrolyte in the detection part of the column remains unchanged, *i.e.*, the mixing of the HCLE and LCLE is not disturbed by diffusion or by a shift of the concentration boundary between them caused by the electric current.

CONCLUSIONS

A leading-electrolyte cascade can be used in isotachophoresis to optimize the separation capacity of the column used. It enables a complete separation in cases where normal procedures give mixed zones, while the detection conditions remain unchanged. The method can easily be adopted in any present isotachophoretic instrument and technique by providing the separation capillary with one more inlet for creating the required concentration cascade of the leading electrolyte.

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