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Note

Complexation with ferric ions—a potential source of error in the isotachophoretic determination of oxalate

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The purpose of this note is twofold: (a) to report how the well-known ferrioxalate species $Fe(C_2O_4)_3^{3-}$ may disturb the isotachophoretic determination of oxalate by being formed through interaction between the sample and the syringe needle during injection; and (b) to demonstrate how this disturbing effect is eliminated by the addition of ethylenediaminetetraacetic acid (EDTA).

EXPERIMENTAL

The complexation was observed in a project already described in some detail[1]. Mixtures of oxalic, glyoxylic and glycollic acids were analyzed at pH 3.30 in an LKB 2127 Tachophor (LKB, Bromma, Sweden) equipped with a 23-cm capillary tube. The samples were injected from a 1-μl syringe (type B plunger in needle syringe, Scientific Glass Engineering, North Melbourne, Australia) via the septumless syringe injector described previously[1], with the exception of some early runs in which the usual septum technique was used. The separations were monitored with a thermometric detector and a UV-absorption detector (254 nm).

RESULTS AND DISCUSSION

Evidence of complexation

Normally, the isotachopherograms were as in Fig. 1, small amounts of UV-absorbing impurities being sandwiched between the leading, sample and terminating ions. Sometimes, however, the isotachopherogram showed an additional zone, more or less broad, of a strongly UV-absorbing anion, moving in front of the oxalate ion; an example is given in Fig. 2a*.

It was observed that, whenever this zone appeared, its length gradually decreased in subsequent runs with the same sample until, finally, only a peak remained. Concurrently, the length of the oxalate zone increased correspondingly (see Figs. 2a–c). These observations indicated that the strongly absorbing zone contained oxalate. It

* The same observation has been independently made by Tschöpe *et al.*[2] in a recent study of urinary oxalate with a different electrolyte system.

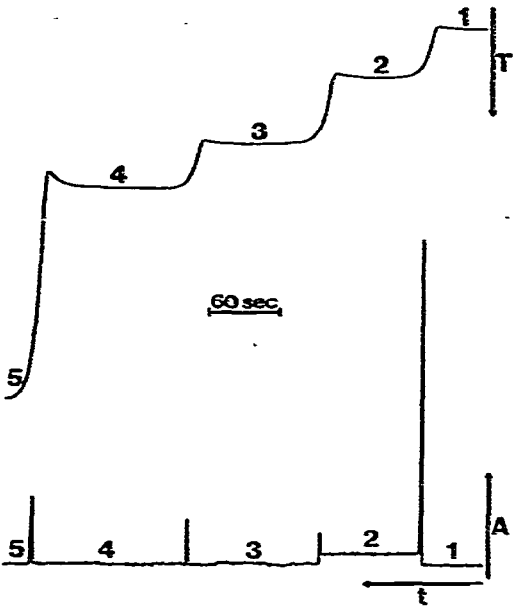

Fig. 1. Normal appearance of isotachopherogram obtained during analysis of a sample of oxalic, glyoxylic and glycollic acids at 20°. Leading electrolyte: 10 m*M* HCl + β-alanine to pH 3.30; 0.3% of hydroxypropyl methyl cellulose; terminating electrolyte, 5 m*M* acetic acid. Current: 90 μA. T = temperature; A = UV absorption (254 nm); t = time. 1 = chloride; 2 = oxalate (32 m*M*); 3 = glyoxylate (50 m*M*); 4 = glycollate (58 m*M*); 5 = acetate.

was further observed that, if the sample (after suitable dilution) was loaded into the injection port by suction through the septumless syringe injector described previously[1], the absorbing zone was directly reduced to the very small peak also present in a

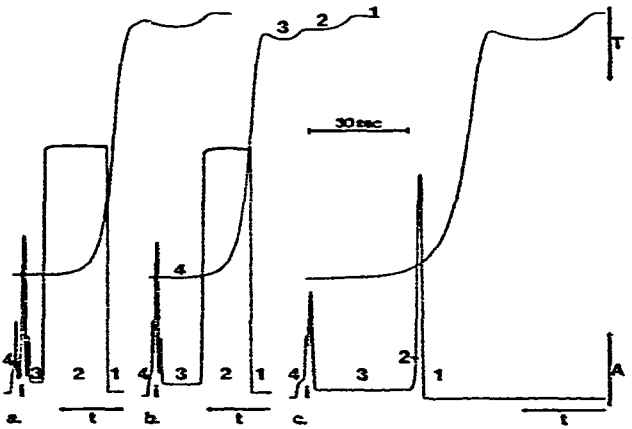

Fig. 2. Examples from a series of isotachopherograms in which a strongly UV-absorbing and oxalate-containing zone (2) is observed in front of the normal low-absorbing oxalate zone (3). Aliquots (1 μl) of the same sample of oxalic acid were injected in 10 sequential runs; the records are from the first (a), the third (b) and the tenth (c) runs. 1 = chloride; 4 = acetate; i = impurities, which in these runs occur in larger amounts due to septum bleed (*cf.* "Experimental").

"blank". Obviously, this zone contained some species formed from oxalate by interaction with the syringe needle.

This species was suspected to be ferrioxalate for the following reasons: (i) all steel surfaces in contact with air, even those of stainless steel, are covered by a more or less thick layer of Fe_2O_3 and FeOOH; (ii) ferric ions have a high affinity for oxalate ions; (iii) the resulting complex, $Fe(C_2O_4)_3^{3-}$, is known to have high molar absorptivity in the UV region; and (iv) although bulky, the ion $Fe(C_2O_4)_3^{3-}$ would be expected to have a mobility not much lower than that of Cl^-. [*Cf.* the ion $Fe(CN)_6^{4-}$, which is even faster than Cl^- at concentrations less than *ca.* 5 m*M* (ref. 3)].

The suspicion was confirmed when ferric nitrate was added to a sample of oxalic acid. The resulting isotachopherogram (see Fig. 3a) essentially agreed with that of Fig. 2a. If more ferric nitrate was added, the length of zone 2 increased correspondingly, whereas the length of zone 3 decreased (not shown in the figure). By contrast, no such effect was observed when ferrous nitrate was added.

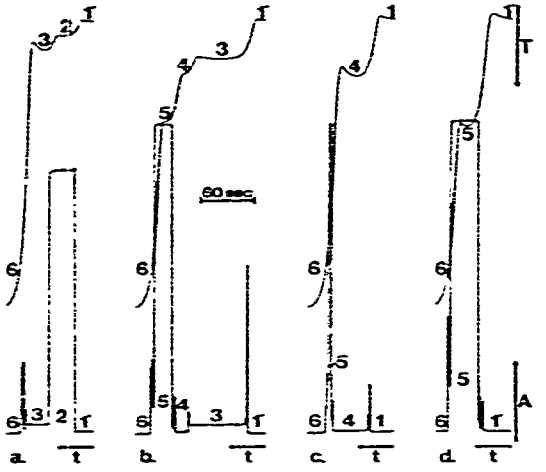

Fig. 3. Identification and elimination of the complex formed between oxalate ions from the sample and ferric ions from the syringe needle. Samples: (a) 25 m*M* oxalic acid + 4 m*M* $Fe(NO_3)_3$; (b) 25 m*M* oxalic acid + 4 m*M* $Fe(NO_3)_3$ + 10 m*M* Na_2 EDTA; (c) 10 m*M* Na_2EDTA; (d) 9 m*M* Na_2 EDTA + 9 m*M* $Fe(NO_3)_3$. Sample volume: 1 μl. 1 = chloride [shown in (c) only]; 1′ = nitrate; 2 = ferrioxalate complex; 3 = oxalate; 5 = ferric–EDTA complex; 6 = acetate.

Remedies

There are at least three methods of eliminating such disturbing complexation: (1) the sample can be introduced into the capillary via a sample tap[3], which can be loaded by suction; (2) the septumless syringe injector[1] can be used, together with a syringe equipped with a PTFE (or glass) cannula (*e.g.*, the Ultra Micro Sampler from Oxford Laboratories, Athy, Eire); (3) the ferrioxalate complex can be decomposed by means of competitive binding.

In practice, the last-mentioned method seems to be the most attractive, as it requires no additional equipment. The formation of the ferrioxalate complex is simply and effectively prevented by adding to the sample an 0.2 *M* solution of Na_2EDTA to give a final concentration of *ca.* 10 m*M*. This is evident from Fig. 3b. The broad

square peak (2) in the UV detector trace in Fig. 3a has decreased to a spike, whereas the low-absorption zone characteristic of oxalate has increased correspondingly*.

The isotachopherogram in Fig. 3b also shows two new zones. As expected, these zones proved to contain pure EDTA and the ferric–EDTA complex, respectively (*cf.* the traces b–d in Fig. 3). According to the literature[4], EDTA has the following pK values: 2.0, 2.7, 6.2 and 10.3. Moreover, it is known to form 1:1 complexes with ferric ions. This means that, in the actual experiment, the average negative charge of the free EDTA should be close to 2, and the complex ion should have the formula $Fe(EDTA)^-$. The order of the zones in Fig. 3b thus seems reasonable.

As regards Fig. 3c, it is interesting to note the presence of peak 5. On the one hand, it offers indirect evidence for the supposed interaction of oxalate ions with the syringe needle; on the other, it emphasizes the inherent risk of contamination when strong complexing agents are injected through a metal needle (eventually, the accompanying gradual corrosion of the needle may also be of importance).

Further, it should be noted that the ferric–EDTA complex and the excess of EDTA may disturb the isotachophoretic pattern if oxalic acid and other acids are to be determined simultaneously. In the system shown in Fig. 1, the EDTA moves between the oxalic and glyoxylic acids and causes no problems. In contrast, the ferric–EDTA complex moves ahead of the glycollic acid and shows a tailing into the zone of the latter. This tailing might be eliminated if the length of the capillary was increased, but this possibility was not examined, as it was more convenient to make two runs with a given sample, if necessary. In the first run, no EDTA was added; if the ferrioxalate zone appeared, EDTA was added and a second run was performed. The amount of each acid could thereby be evaluated from at least one of the runs.

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* The EDTA method has the further advantage of being effective also when the sample itself contains ferrioxalate. For instance, this may be the case with urine from a sick person.