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CAPILLARY ISOTACHOPHORESIS WITH ULTRAVIOLET DETECTION SOME QUANTITATIVE ASPECTS

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SUMMARY

Equations showing that the zone length (or detection time) measured is usually not directly but only linearly proportional to the amount of sample injected, are derived. The basic theory of UV detector response is presented, and the use of UV detector response as a quantitative measure for small amounts of UV absorbing compounds is demonstrated. Using this technique complete analysis of mixtures containing components differing in relative amounts by up to four orders of magnitude were performed in one experiment with no counterflow needed.

INTRODUCTION

Recently much attention has been paid to the quantitative aspects of isotachophoresis. Since high-resolution detectors have been developed, *e.g.*, the conductivity and UV-absorption detectors, zone lengths equal to or smaller than the inner diameter of the capillary can be measured. The question can now be asked whether, for such short zones, the zone length is proportional to the amount of sample injected.

The concentration[1,2] of the consecutive zones is determined in isotachophoresis, assuming the steady state is reached, by the composition of the leading electrolyte and by the effective mobilities of the leading ion, the sample constituent and the counter ion. The boundary layer between the two consecutive zones is very thin[1,3], because in isotachophoresis a "self-sharpening" effect[1,2] is obtained. Therefore it is possible to approximate the zone profile by the equations 1–3

$$c = 0; \qquad x < x_1 \tag{1}$$
$$c = c(i) \neq 0; \qquad x_1 \leqslant x \leqslant x_2 \tag{2}$$
$$c = 0; \qquad x_2 < x \tag{3}$$

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where c is the concentration of the component in question, $c(i)$ is the concentration of this component in its zone, x is the coordinate along the capillary axis and x_1 and x_2 refer to the start and end of the zone, respectively. The zone length, l, defined by $l = x_2 - x_1$, is proportional to the amount of sample injected, as in eqn. (4)

$$l = \frac{n(i)}{c(i) \cdot S} \tag{4}$$

where S is the cross-section of the capillary.

For the detection time, t, which is equal to the time at which the zone passes the detector, and for the registered zone length, L, we can write

$$t = \frac{l}{v} + \frac{d}{v} \tag{5}$$

$$L = u \cdot t \tag{6}$$

where d is a constant, closely related to the effective dead volume of the detector, $V_d = d \cdot S$, v is the zone velocity in the capillary and u is the chart speed of the recorder. The term d/v in eqn. 5 involves the effect of finite detector dead volume. The zone boundaries are not infinitely thin, and the zone profile is influenced by electroendosmosis, by counterflow of the electrolyte (if applied) and by the temperature gradient in the capillary. Even if the zone boundaries were infinitely thin, the measuring cell has a finite volume, and a definite amount of the sample is required to fill it. We can write

$$v = U_{L(eff)} \cdot E_L = \frac{U_{L(eff)} \cdot i}{\varkappa(L)} \tag{7}$$

where $U_{L(eff)}$ is the effective mobility of the leading ion in its zone, E_L is the electric-field strength in the leading ion zone, $\varkappa(L)$ is the specific conductivity of this zone and i is the electric current density. Combining eqn. 5 with eqns. 4 and 7 we obtain

$$t = \frac{n(i) \cdot \varkappa(L)}{I \cdot c(i) \cdot U_{L(eff)}} + \frac{V_d \cdot \varkappa(L)}{I \cdot U_{L(eff)}} = \frac{A(i,L) \cdot n(i) + B}{I} \tag{8}$$

$$L = \frac{u}{I} [A(i,L) \cdot n(i) + B] \tag{9}$$

where $A(i,L)$ is a constant characteristic for the electrolyte system chosen and the component in question, B is a constant involving the detector dead volume and I is the electric current.

If the volume of the sample injected (V_0) is constant (*e.g.*, if the sample is injected by means of a sample valve), we obtain

$$L = \frac{u}{I} [A(i,L) \cdot V_0 \cdot c_0(i) + B] = \frac{u}{I} [a(i,L) \cdot c_0(i) + B] \tag{10}$$

where $c_0(i)$ is the concentration of the component in the sample. If $l \gg d$, the second term in eqn. 10 can be neglected. For the detection time, t, we then have

$$t = \frac{A(i,L)}{I} \cdot n(i) = \frac{n(i)}{c(i) \cdot K_{cal}} \tag{11}$$

where K_{cal} is the calibration constant[4]. The ratio of the detection times for two different sample components is given by

$$\frac{t(i)}{t(S)} = \frac{c_0(i)}{c_0(S)} \cdot D^*(i,S) \tag{12}$$

where $c_0(i)$ and $c_0(S)$ are the concentrations of the sample component and the standard present in the sample, respectively, and $D^*(i,S)$ is the relative correction factor[5]. When working with small quantities of the sample component (in our experiments, $\leqslant 1$ nmole), the constant B in eqns. 8–10 cannot be neglected, and the registered zone length, L, per definition need not be directly proportional to the amount of sample injected.

We now assume that I_1 is the intensity of the light passing through the capillary cuvette of the UV detector, I_0 is the intensity of the homogeneous light illuminating the slit and that there is only one UV-absorbing zone passing the detector. For the light intensity, I_1, we have

$$I_1 = \frac{d - l}{d} \cdot I_0 + \frac{l}{d} \cdot I_0 \cdot T'; \; l \leqslant d \tag{13}$$

$$I_1 = I_0 \cdot T; \; l > d \tag{14}$$

where T' is the transmission of the capillary cuvette when filled with a sufficiently long zone of the sample component. For the experimentally determined transmission, T, we can write

$$T = 1 - (1 - T') \cdot \frac{l}{d} = 1 - D' \cdot \frac{l}{d}; \; l \leqslant d \tag{15}$$

$$T = T'; \; l > d \tag{16}$$

where D' is the absorption corresponding to T' and d is the slit width of the detector. The value of T' is related[6] to the absorption coefficient at the wavelength used and can be used as a good qualitative characteristic of the sample component (correct response). Now if $l < d$, the experimentally determined transmission, T, is no longer a characteristic constant of each ionic component, but becomes a function of the amount of sample injected

$$1 - T = D = \frac{D'}{d} \cdot l = a'_1 \cdot c_0(i) \tag{17}$$

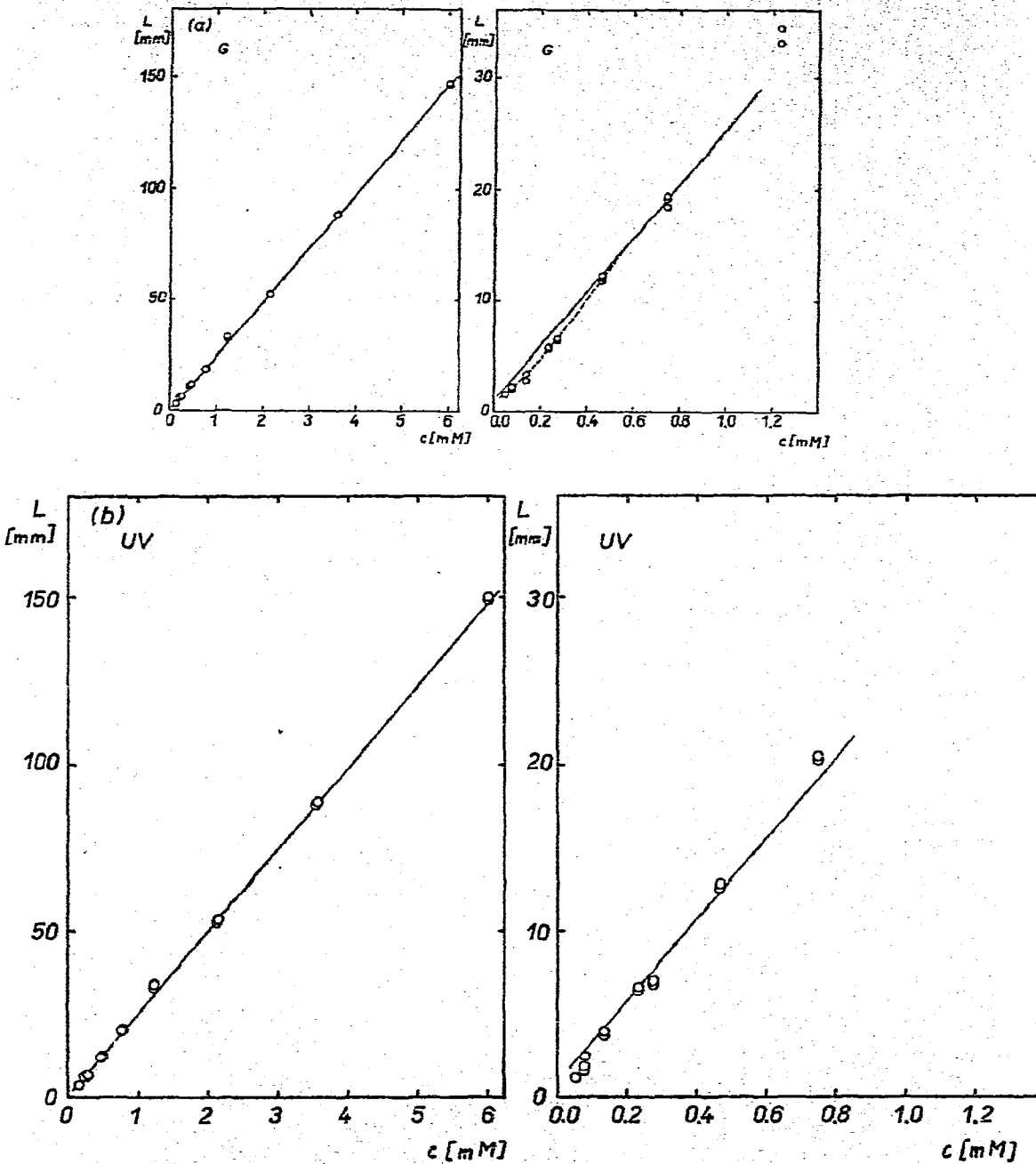

Fig. 1. Calibration graph for 1,3,5-naphthalenetrisulphonic acid: (○), experimental points; (—), least-squares fit, a, Conductivity detection; b, UV detection.

A similar equation (18) can be derived if the strongly absorbing component is spaced by an absorbing spacer.

$$1 - T = a_2' \cdot c_0(i) + b' \tag{18}$$

If the relative photometric heights, f, are used instead of absorptions, eqns. 17 and 18 can be presented in the forms

$$f = a_1 \cdot c_0(i) \tag{19}$$
$$f = a_2 \cdot c_0(i) + b \tag{20}$$

$$f = \frac{D - D_L}{D_S - D_L} \tag{21}$$

In this paper, we use these relations for trace analyses of absorbing samples.

EXPERIMENTAL

The experiments were carried out by use of equipment which will be described elsewhere[7]. The length of the capillary from the injection point to the UV detector slit was *ca.* 32 cm; the length to the conductivity detector was 38 cm. All chemicals used were of p.a. grade (Lachema, Prague, Czechoslovakia), unless mentioned otherwise. The β-alanine was p.a. quality (Koch-Light, Coinbrook, Great Britain). The naphthalenesulphonic acids and the sulphoisophthalic acid (in form of sodium salts or of potassium salts) were obtained from the Research Institute of Organic Synthesis in Pardubice-Rybitvi.

The leading electrolyte was prepared by adjusting a solution of 10 m*N* sulphuric acid to pH 4.2 with β-alanine. The terminator was phenylacetic acid. In the model experiments, an equimolar mixture of naphthalenesulphonic acids was used; in the experiments with the spacer technique, the spacers were added in concentration of *ca.* 0.6 m*M*.

RESULTS AND DISCUSSION

In order to show that capillary isotachophoresis is a very useful technique for quantitative analyses of industrial mixtures, we tried to show the reliability of eqns. 10 and 18 for a wide range of injected sample amounts. The zone lengths (driving current, 100 μA; chart speed, 80 mm/min) were measured for the following compounds present

TABLE I

CALIBRATION CONSTANTS, a (i, L) AND B (eqn. 10), FOR 1,3,5-NAPHTHALENETRISULPHONIC ACID

Method of detection	*a (i, L)* (10^{-6} C·mol^{-1}·m^3)	*B* (10^{-6} C)	*S.D.*
Conductivity	1823	37	40
UV	1823	76	30

TABLE II

SPACERS USED AND ANALYZED COMPOUNDS

δ is the relative mean deviation for 10 experiments.

Anion	$U/U_{L(eff)}$	δ (%)	U_{eff} ($10^{-5} \cdot V^{-1} \cdot cm^2 \cdot s^{-1}$)
Sulphoisophthalate	0.816	0.2	54.3
Malonate	0.700	0.2	46.5
β-Chloropropionate	0.466	0.3	31.0
Phenylacetate	0.347	0.3	23.2
1,3,5-Naphthalenetrisulphonate	0.904	0.2	60.1
1,5-Naphthalenedisulphonate	0.762	0.3	50.4
1-Naphthalenesulphonate	0.521	0.4	34.6

in concentrations of between 0.05 and 6 mM: 1-naphthalenesulphonic acid; 1,5-naphthalenedisulphonic acid and 1,3,5-naphthalenetrisulphonic acid. In Fig. 1 the experimental results with the UV detector and the conductivity detector are compared. The parameters $a(i,L)$ and B in eqn. 10 were computed by a least-squares method (linear regression) and are listed in Table I. The standard deviation (S.D.) is also listed, showing the distribution of the experimental points. Eqn. 10 is valid for the UV-detected zone lengths over the whole concentration range examined; for the conductivity-detected zone lengths, it is approximately valid from 0.2 mM (0.8 nmole under our experimental conditions). Thus for small amounts of sample the conductivity detector seems to give poor results. The reasons are to be found in the operational conditions chosen, for while the detection limit of the conductivity detector is *ca.* 50 μM (corresponding to 0.2 nmole), the UV detector is so specific that it can be used for concentrations which are ten times lower.

The relations between the UV-detected zone lengths, L, the relative photo-

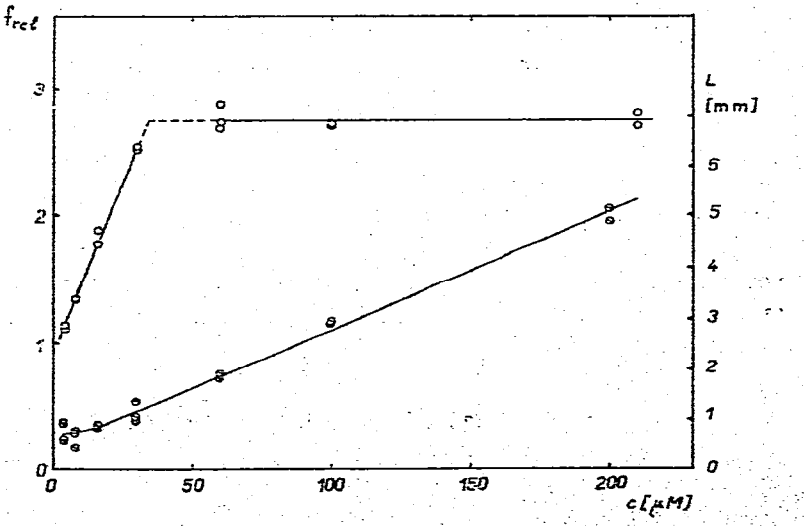

Fig. 2. The relations between f and L and amount of sample injected (as measured by the concentration of the component in the sample; injected volume, 4 μl) for 1,3,5-naphthalenetrisulphonic acid. The constants in eqns. 19–21 are $a_2 = 52 \cdot 7.10^3$ mol/l and $b = 0.96$ (S.D. = 0.05).

metric height, f, and the amount of sample injected were examined for sample concentrations in the range 4–200 μM. Such short UV-absorbing zones cannot be distinguished from each other unless they are spaced by non-absorbing components. The spacers used are listed in Table II. The relations are shown in Fig. 2. It can be seen, that the predicted linear relation between the measured zone length and amount of sample injected does not hold for certain concentrations, and that the detected zone length cannot be used as a reliable measure of quantity for certain concentrations. However, Fig. 2 shows that for approximately the same concentration (35 μM) the linear relation holds between the relative photometric height, f, and the amount of sample injected. The parameters of this relation (described by eqn. 20) were determined by a least-squares method (linear regression) and are listed under Fig. 2.

Using the equations given it is possible to determine the amounts of naphthalenesulphonic acids down to concentrations (in the original sample) of 4 μM, *i.e* a total amount injected of 16 pmole (Fig. 3). Using the equipment described by us, a total amount of the sample (if naphthalenesulphonic acids are to be separated) of at least $140 \cdot 10^{-9}$ g equiv. can be separated. This means, of course, that mixtures containing trace amounts of the naphthalenesulphonic acids (or of other components having similar differences in mobilities) can be analyzed quantitatively even if they amount to only *ca.* 0.02% of the total amount of the major component. An example is given in Fig. 4. The major component was 1-naphthalenesulphonic acid at a concentration of *ca.* 20 mM; the other components were naphthalenedi- and naphthalenetri-sulphonic acids. Unknown impurities were also observed. The results of this analysis are given in Table III.

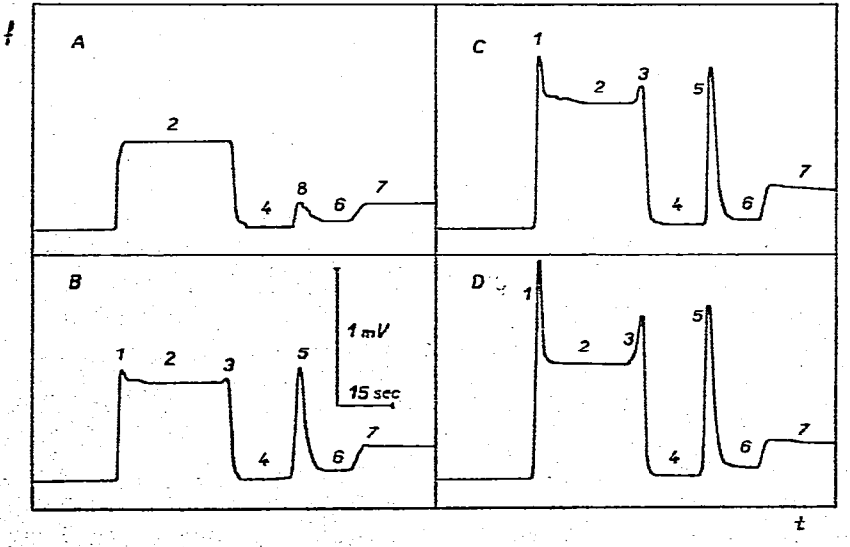

Fig. 3. Trace analyses of naphthalenesulphonic acids. Zones: 1 = 1,3,5-naphthalenetrisulphonic acid; 3 = 1,5-naphthalenedisulphonic acid; 5 = 1-naphthalenesulphonic acid; 2 = sulphoisophthalic acid; 4 = malonic acid; 6 = β-chloropropionic acid; 7 = phenylacetate; 8 = unidentified impurity. Concentration of individual compounds in the sample: A, no sample; B, 4 μM; C, 8 μM; D, 16 μM.

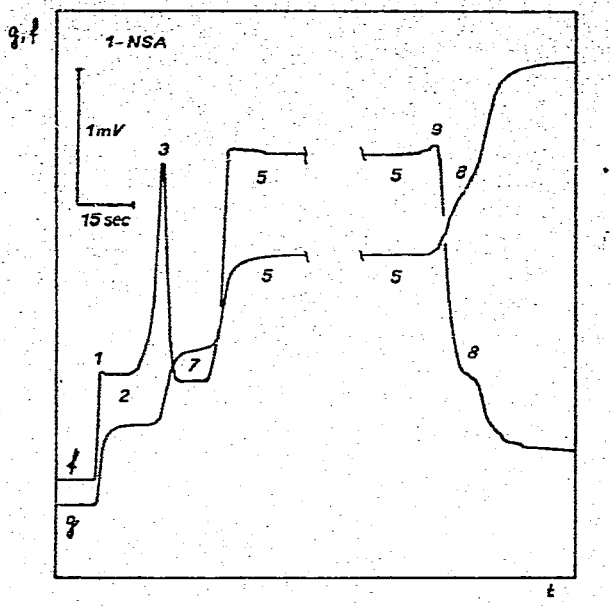

Fig. 4. Analyses of 1-naphthalenesulphonic acid (1-NSA). Zones: 1 = naphthalenetrisulphonic acids; 2 = sulphoisophthalate; 3 = naphthalenedisulphonic acids; 5 = naphthalenesulphonic acids; 7–9, mixed zones of impurities and sample components.

TABLE III

ANALYSIS OF POTASSIUM 1-NAPHTHALENESULPHONATE

Anion	*Concentration in original sample (mM)*
Monosulphonate	20
Disulphonate	0.2
Trisulphonate	0.004
Number of unidentified impurities	3

The results show clearly that an apparatus which has a universal detector, *e.g.*, a conductivity detector, and also a specific detector, *e.g.* a UV detector, is preferred not only for qualitative analysis but also for quantitative analysis. The UV detector can be used for quantitative determination of very small amounts.

CONCLUSIONS

If the length of the zone in the tube of narrow bore is longer than the slit width of the UV detector, then the UV-detected zone length is linearly related to the amount of sample injected. The correct response of the UV detector is reached and can be used as a reproducible qualitative characteristic. However, if the zone length (in the capillary) is shorter than the slit width of the UV detector, then the response of the UV detector is no longer a good qualitative characteristic, but it can be used as a quantitative measure.

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