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SPECTROPHOTOMETRIC DETECTION IN CAPILLARY ISOTACHOPHORESIS: DETERMINATION OF THE COMPOSITION OF MIXED ZONES

DANA HANIBALOVÁ, JIŘÍ VACÍK* and VLASTIMIL FIDLER
Department of Physical Chemistry, Faculty of Natural Sciences, Charles University, Albertov 2030, 128 40 Prague 2 (Czechoslovakia)

SUMMARY

An isotachophoretic apparatus with spectrophotometric detection was developed and constructed, using a Specord UV–visible spectrophotometer. The apparatus was applied to the determination of the positional isomers of naphthalenesulphonic acids in mixed zones formed after isotachophoretic separation.

INTRODUCTION

From the beginning of the development of capillary isotachophoresis (ITP), the limiting factor for its wider application has been a lack of satisfactory detection techniques. The quality of the instrumentation and the methods have since improved considerably and there now exist many detection techniques in capillary isotachophoresis, each with some advantages and drawbacks.

The existing types of detector are usually classified into universal and specific[1]. Universal detectors measure, *e.g.*, the Joule heat produced on passage of electric current through an electrolyte[1–3], the specific conductance (resistance) of the electrolyte[1,4,5] or the electric field intensity[1,6,7]. All these quantities are related to the effective mobilities of the substances present in the zones detected. Therefore, universal detectors are used for common analytical purposes and for the determination of mobilities. A general disadvantage of universal of detectors is that they yield only a single characteristic for any given zone, from which it is impossible to establish whether it represents a pure zone of a single component or a mixed zone.

Specific detectors monitor some of the properties of substances that are not dependent on the effective mobility, *e.g.*, the absorption of radiation[1,8], fluorescence[9,10], radioactivity[11] or refractive index[12]. The most common specific detector is the UV photometric detector. When this is used, the isotachophoreograms often contain neighbouring zones that absorb the incident radiation very differently. In this way, the resolution of this specific detection method is improved, as even trace impurities that accumulate at some zone boundaries and form there very short impurity zones can often be detected. These zones may escape unnoticed on a universal detector recording. This property of the UV photometric detector is utilized in the determination of very small amounts of substances[13].

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If a sample for isotachophoretic separation contains two or more substances with virtually the same effective mobilities then, in steady state, not only the pure zones, *i.e.*, the zones containing a single substance to be separated, are obtained, but also a mixed zone that behaves in the same way as a pure zone, but contains a mixture of the substances with the same effective mobilities. If the components of the mixed zone absorb the radiation differently, and a mixed zone contains n components whose relative contents are to be determined, then photometric measurement must be carried out at $n - 1$ suitable wavelengths[14] or spectrophotometric detection must be used.

In isotachophoretic separation with spectrophotometric detection[15–18], it is possible in a single experiment to separate a mixture on the basis of different effective mobilities and to obtain the absorption spectra of the individual zones.

The present work was aimed at using the apparatus developed in our Department for isotachophoretic separation with spectrophotometric detection, in order to determine the composition of mixed zones. A mixture of naphthalenesulphonic acids, formed by gradual sulphonation of naphthalene, which is an intermediate in the manufacture of dyes, was studied. The reaction mixture after the sulphonation may contain naphthalenemono-, -di-, -tri- and -tetrasulphonic acids, present in various ratios depending on the reaction conditions. For a survey of the methods used so far for the separation of a mixtures of naphthalenesulphonic acids and the identification of their components, see refs. 16 and 19.

EXPERIMENTAL

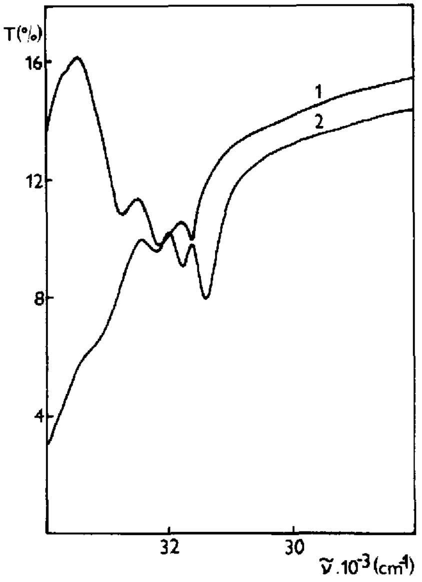
The simultaneous determination of concentrations of components in multicomponent mixtures from spectroscopic data is based on the Lambert–Beer law and on the assumption of the additivity of the absorbances of all the components of the mixture[20].

To determine the relative contents (molar fractions) of the components in a mixture, the experimental values of the absorbance of the mixture, A_{sj}, and the absorbances of pure components, A_{ij}, at a given wavenumber, $\tilde{\nu}_j$, are used:

$$A_{sj} = \sum_{i=1}^{n} x_i A_{ij} = d \sum_{i=1}^{n} \varepsilon_{ij} C_i$$

where x_i is the molar fraction of the ith component in the mixture, d is the thickness of the homogeneous medium (constant for all measurements), C_i is the concentration and ε_{ij} is the molar absorption coefficient of the ith component. The number of components in the mixture is n and number of wavenumbers is m, *e.g.*, $i \in \langle 1,n \rangle$, $j \in \langle 1,m \rangle$ and the condition $m > n$ must be satisfied. The molar fractions can be calculated statistically, by regresion analysis using the least-squares method[21,22].

When evaluating a mixed spectrum without a knowledge of the actual number of components in the mixture, several variants of the calculation must be carried out, assuming the presence of all the components in the mixture and the presence of only some of the components, and the most suitable variant must be selected on the basis of the fit of the theoretically calculated spectrum from the calculated x_i values and of the experimental spectrum of the mixture, of the confidence interval and of the results of the t-test[22].



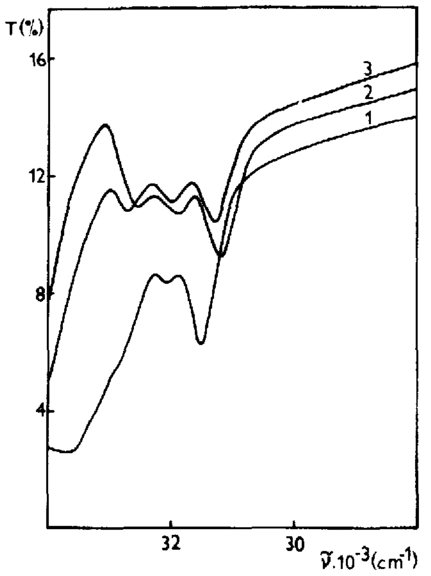


Fig. 1. Electronic absorption spectra of naphthalenemonosulphonic acids. 1 = α-NSA; 2 = β-NSA.

Fig. 2. Electronic absorption spectra of naphthalenedisulphonic acids. 1 = 1,5-Di-NSA; 2 = 1,6-di-NSA; 3 = 1,7-di-NSA.

Of twelve possible naphthalenesulphonic acid (NSA) isomers, we selected those which have the greatest importance from the point of view of the production process, namely, 1(α)- and 2(β)-naphthalenemonosulphonic acid, the 1,5-, 1,6- and 1,7-disulphonic acids (di-NSP) and the 1,3,5-, and 1,3,7-trisulphonic acids (tri-NSA). The

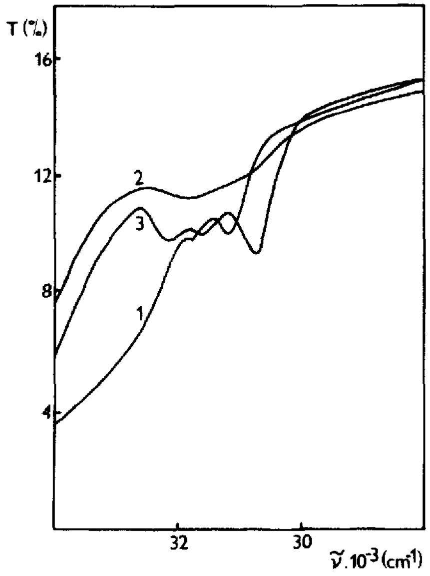


Fig. 3. Electronic absorption spectra of naphthalenetrisulphonic acids. 1 = 1,3,5-Tri-NSA; 2 = 1,3,6-tri-NSA; 3 = 1,3,7-tri-NSA.

shapes of the spectral bands (the transmittance curves) of these isomers over the region selected for the evaluation of the spectrophotometric data (34,000–28,000 cm^{-1}) are given in Figs. 1–3. The curves were obtained by measurement in the flow-through capillary cell of the apparatus described below.

Apparatus

The isotachophoretic apparatus with conductivity and spectrophotometric detection, developed in our Department, consists of two parts: the separation part, with a conductivity detector, and the spectrophotometric detector. The isotachophoretic separation apparatus is composed of common functional elements: (a) electrode chambers made of Umaplex with a system of stop-cocks for filling the capillary and forming a boundary between the leading and terminating electrolytes and injecting samples into the boundary; (b) a PTFE separation capillary, 300 × 0.8 mm O.D. × 0.45 mm I.D.; and (c) a conductivity detector[5].

This isotachophoretic apparatus is situated in the cell space of the spectrophotometer and fixed in the cell holders. The separation capillary is placed in the measuring beam and forms a flow-through cell. A scheme of the apparatus is shown in Fig. 4.

A Specord UV-visible spectrophotometer (Carl Zeiss, Jena, G.D.R.) is used as the spectrophotometric detector without any modification (after removing the ITP apparatus and replacement of the original cells it can be used for common spectrophotometric measurements). In place of the relatively large quartz cell, the part of

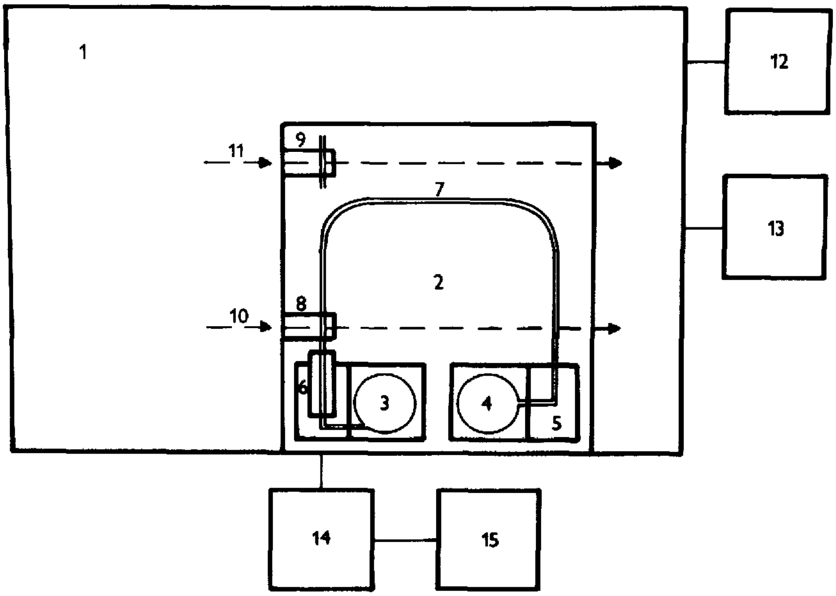


Fig. 4. Schematic diagram of the isotachophoretic apparatus with spectrophotometric detection. 1 = Spectrophotometer; 2 = cell space of the spectrophotometer; 3 = leading electrolyte vessel; 4 = terminating electrolyte vessel; 5 = injection port; 6 = conductivity detector; 7 = separation capillary; 8 = slits defining the capillary section for detection; 9 = slits for the reference capillary; 10 = measuring beam; 11 = reference beam; 12 = spectrophotometer recorder; 13 = data output for the computer (tape puncher); 14 = electronic circuitry of the conductivity detector; 15 = conductivity detector recorder.

the PTFE separation capillary defined by screens is placed in the measuring beam (*i.e.*, a flow-through cell with dimensions of *ca.* 0.3 × 0.3 + 0.3 mm), so the amount of light available for detection is decreased considerably. Therefore, in the measurement of spectra in the isotachophoretic apparatus the noise of the measuring system is much more pronounced, especially in the short wavelength region, where the PTFE capillary also absorbs. The resultant spectra can be recorded by the built-in recorder; for computer handling of the results, the secondary output of the instrument was connected through a suitable interface to a Celatron tape puncher.

Optimal measuring conditions

For the isotachophoretic separation of suitable NSA solutions, histidine was selected as the leading electrolyte (0.01 mol dm^{-3}) with histidine hydrochloride (0.01 mol dm^{-3}). Glutamic acid was used as the terminating electrolyte (0.01 mol dm^{-3}). The NSA concentration in the separated zones is then suitable for spectrophotometric detection. When 40-μl NSA samples are introduced through a valve (0.01 mol dm^{-3}), the components are completely separated in the capillary of the given length and the zones are sufficiently long for spectrophotometric detection. The separation and the detection with the conductivity detector at a current of 50 μA take 33 min; for the recording of the spectra, taking 32.7 sec, the current was decreased to 10 μA, thus increasing the time of the experiment to 40 min. The mixture is isotachophoretically separated according to the individual sulphonation steps (Fig. 5) and it is thus possible to determine mono-, di- and tri-NSA in the sample. From the subsequently

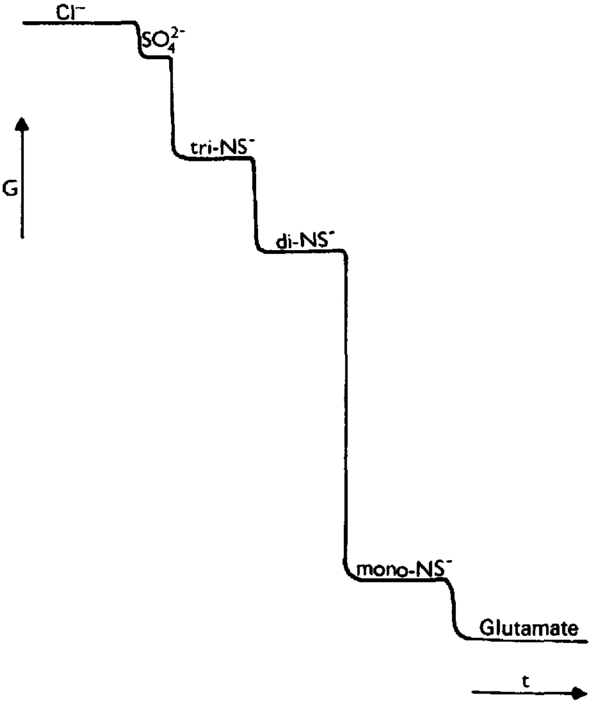


Fig. 5. Isotachopherogram of a model mixture of NSA obtained with a conductivity detector. SO_4^{2-} Is residue after sulphonation.

obtained spectra the contents of the individual positional isomers in the mixed zones of mono-, di- and tri-NSA can then be found.

Additives of the partial absorbances

The principle of the additivity of the partial absorbances was verified by comparing the experimental spectra of a mixture of 80% α-NSA and 20% β-NSA with the theoretical spectrum, calculated assuming additivity of the spectra of pure α- and β-NSA.

Evaluation of the UV spectra

A suitable section of the spectrum was selected for the evaluation, considering the shape of the spectra of the pure components (regions where the differences are greatest) and the quality of the recording (the noise nicreases towards shorter wavelengths). The region from 34,000 to 28,000 cm^{-1} was selected, in which all the components have different spectra and a sufficient number of transmittance values can be recorded (for 76 points in the wavenumber field).

A family of programs in BASIC was designed for the quantitative evaluation of the spectra of the mixed zones on a Hewlett-Packard HP 9830 calculator. The first, preparatory program serves for the creation of a set of standard spectra of pure components. The input data consists of multiply (more then 10 times) measured spectra of the pure components under conditions identical with those in the separation (subsequently may be used for the spectra of the mixed zones). The computation yields average spectra of the pure components and their basic statistical characteristics, allowing the evaluation of the quality (reliability) of this standard spectrum.

The main program serves for the actual evaluation of the experimental spectrum of a mixed zone by the procedure described in the previous section (linear regression analysis by the least-squares method). The input data are the experimental spectra of the mixed zone and the type of mixture assumed, *i.e.*, a mixture of mono-, di- or tri-NSA. The output is the theoretical content of the components in the mixture (the molar fractions) and the appropriate statistical characteristics (standard deviation, variance, the result of the *t*-test) of all mixture types.

The other programs are auxiliary programs for data transfer and manipulation.

RESULTS AND DISCUSSION

The reproducibility of the spectra was poorer than that in common measurements of absorption spectra in view of the instrumentation used (it amounted to 1–2% of the transmittance), but the series of analyses of a mixture of positional isomers of NSA yielded results in good agreement with those obtained by more complicated separation techniques. The results of model analyses are given in Tables I–III. The positional isomers can be determined with satisfactory precision even when present at concentrations of 5% for mixture of α- and β-NSA; 1,5-di-NSA in a mixture with 1,6-di-NSA and 1,7-di-NSA; and 1,3,5-tri-NSA in a mixture with 1,3,6-tri-NSA and 1,3,7-tri-NSA. For 1,6-di-NSA, 1,7-di-NSA, 1,3,6-tri-NSA and 1,3,7-tri-NSA, whose spectra are similar, the precision of the determination is poorer. Considering the

TABLE I

EVALUATION OF THE UV SPECTRA OF MIXTURES OF NAPHTHALENEMONOSULPHONIC ACIDS

Taken (%)		*Found (%)*	
α-NSA	*β-NSA*	*α-NSA*	*β-NSA*
0	100	–	99.5 ± 1.1
5	95	6.7 ± 1.8	95.1 ± 2.8
10	90	9.4 ± 0.8	90.8 ± 1.8
50	50	51.0 ± 1.1	50.6 ± 2.7
90	10	88.0 ± 1.5	11.4 ± 3.6
95	5	94.0 ± 3.9	6.5 ± 3.5
100	0	99.7 ± 0.8	–

TABLE II

EVALUATION OF THE UV SPECTRA OF MIXTURES OF NAPHTHALENEDISULPHONIC ACIDS

Taken (%)			*Found (%)*		
1,5-di-NSA	*1,6-di-NSA*	*1,7-di-NSA*	*1,5-di-NSA*	*1,6-di-NSA*	*1,7-di-NSA*
5	50	45	4.1 ± 1.2	56.0 ± 15.8	39.4 ± 14.0
30	40	30	28.3 ± 3.5	38.7 ± 13.5	35.6 ± 10.1
80	10	10	80.6 ± 3.6	9.8 ± 13.2	12.6 ± 9.2
85	5	10	83.3 ± 1.8	0.0	17.4 ± 4.8
85	10	5	84.7 ± 5.6	15.7 ± 5.6	0.0
94	1	5	93.0 ± 0.9	0.0	6.9 ± 2.8

TABLE III

EVALUATION OF THE UV SPECTRA OF MIXTURES OF NAPHTHALENETRISULPHONIC ACIDS

Taken (%)			*Found (%)*		
1,3,5-tri-NSA	*1,3,6-tri-NSA*	*1,3,7-tri-NSA*	*1,3,5-tri-NSA*	*1,3,6-tri-NSA*	*1,3,7-tri-NSA*
2	49	49	1.8 ± 0.7	51.4 ± 2.6	47.7 ± 1.9
5	5	90	5.2 ± 2.3	0.0	94.5 ± 3.2
5	15	80	4.8 ± 0.7	13.8 ± 2.7	81.7 ± 1.9
45	45	10	44.8 ± 1.8	46.4 ± 2.8	9.1 ± 2.0
45	10	45	42.9 ± 3.8	12.8 ± 2.7	43.7 ± 2.1
90	9	1	88.9 ± 1.3	10.2 ± 1.1	0.0
90	1	9	87.9 ± 2.2	0.0	12.0 ± 0.6

complicated character of other applicable analytical methods on the one hand and the rapidity of the isotachophoretic separation with spectrophotometric detection together with the minimal requirements for sample preparation on the other, the usefulness of the combined detection described here is obvious.

The results indicate that the use of any modern microprocessor-controlled spectrophotometer would permit automation and acceleration of the measurements and data handling and the attainment of a better precision even for mixtures containing components with similar spectra.

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