Journal of Chromatography, 470 (1989) 69–78
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 265

MEASUREMENT OF LIMITING MOBILITIES BY CAPILLARY ISOTACHOPHORESIS WITH A CONSTANT TEMPERATURE AT THE SITE OF DETECTION

BOHUSLAV GAŠ, JIŘÍ ZUSKA and JIŘÍ VACÍK*
Department of Physical Chemistry, Faculty of Sciences, Charles University, Albertov 2030, 128 40 Prague 2 (Czechoslovakia)

SUMMARY

A method is described for the determination of limiting mobilities from isotachophoretic experiments. The data from an universal detector in the isotachophoretic apparatus were fed to an on-line computer and recalculated to the actual resistance. The computer simultaneously regulates (on the basis of the calculated actual resistance in the detected zone) the driving current passing through the capillary tube, so that the thermal power at the detection site is constant and the temperature of the test solution has a required (preferably standard) value. An advantage of an isotachophoretic experiment controlled in this way is the fact that the dependence of the physical and physico-chemical quantities used on the temperature need not be considered in the recalculation of the experimental data to the limiting ionic mobilities. The limiting mobility calculation considers all the substances as ampholytes. The method was verified using an apparatus with an high-frequency contactless detector.

INTRODUCTION

Production of Joule heat in the separation chamber or the separation medium is inherent to all electromigration methods. In isotachophoresis the situation is further complicated by the fact that the separation occurs in a free solution system without excess of the background electrolyte, so that the zones of the species separated have different conductivities. Therefore, different amounts of heat are produced in different zones and the zones generally have different temperatures. This offers the possibility of a simple detection of zones, and therefore several thermometric detectors have been constructed, especially at the beginning of development of instrumentation for isotachophoresis, *e.g.*, refs. 1 and 2.

The heat production has however an unfavourable effect upon the sharpness of the zone boundary. Capillary tubes of most commercial instruments are therefore thermostatted, but thermostatting of the capillary tube does not preclude different zone temperatures. It is necessary to take account of this if isotachophoresis is to be used for determination of physico-chemical constants.

The temperature influences many quantities that play a role during isotacho-

0021-9673/89/$03.50

phoretic separation, *e.g.*, the relative permitivity and viscosity of a solvent and, of course, acid dissociation constants, pK_A, and limiting ionic mobilities, u^0. For example, the temperature coefficient of the ratio of limiting mobilities, $u^0(T)/u^0(298)$ is for the majority of ions *ca.* 0.023 K^{-1}. This means that if we want to determine mobilities with an accuracy better than 1%, we must assure the temperature control better than 0.4 K.

Some isotachophoretic experiments were performed[3,4] by manual control of the driving current in order to maintain a constant potential gradient in all zones. The zones were characterized qualitatively from changes in the driving current. The temperature differences at the site of detection were slight in the constant potential gradient regime in comparison with the constant driving current regime. The same authors[3,4] tried a manually controlled constant thermal power regime, but the results did not convincing.

In our laboratory, we have carried out isotachophoretic experiments with a constant temperature at the site of detection[5]. The signal from the thermometric detector —thermocouple— was treated by a computer which controlled the driving current in such a manner as to maintain a constant signal from the detector. The changes in driving current were processed by the computer to determine the qualitative parameters of the zones.

In this contribution, a method is described for maintaining a constant temperature at the site where the universal detector senses the property of zones which is a continuous and monotonous function of the specific resistance.

THEORETICAL

During isotachophoretic experiments, radial differences in temperature exist in the zones and axial differences in temperature between the different zones. Many studies has been concerned with these problems, *e.g.*, refs. 6–9. Owing to a temperature dependence of the electrolyte conductivity, the radial temperature profile is somewhat steeper than parabolic. For our purposes, we can cope with the mean radial temperature, T_r, at the detection site of the universal detector. We intend to keep T_r constant for all zones passing through the detector.

A thermal power, p, related to the unit length, arising in the solution in all parts of the capillary tube can be expressed as

$$p = \mathrm{d}P/\mathrm{d}l = IE = I^2\rho/S \tag{1}$$

where P is the input thermal power in the capillary tube, l is the length axis of the capillary tube, I is the electric current, E is the electric field strength, S is the cross-section of the capillary tube and ρ is the specific resistance of the electrolyte. The thermostatting of the capillary tube is generally performed by keeping a constant temperature, T_0, outerside of the tube. A thermal power arising in the solution owing to a driving current entails an increase of the electrolyte temperature above the thermostatting temperature, T_0. The mean radial temperature, T_r, in any length coordinate of the capillary tube can be approximated as

$$T_r = T_0 + qp = T_0 + qI^2\rho/S \tag{2}$$

where q is the thermal transfer coefficient describing the efficiency of the thermal transfer from an electrolyte inside the capillary tube to the thermostat. The value of q depends greatly on constructional details[10] and is sometimes cited by suppliers of commercial equipment.

For our purposes, we need to know q at the site of the sensing detector. It was determined by filling the capillary tube with a calibrating solution of KCl and measuring the dependence of the solution resistance on the current flowing through the capillary tube. The detector used was an high-frequency contactless conductivity detector whose construction allows easy thermostatting of both the measuring cell and the surrounding capillary tube. The rest of the capillary tube was not thermostatted. The determination of the physico-chemical constants is convenient at a temperature of 25°C (298.15 K) at the detection site since the values of these constants are tabulated mostly for 25°C.

We need therefore to maintain the mean radial temperature, T_r, at 25°C. The thermostatting temperature, T_0, has to be

$$T_0 = T_r - qI^2\rho/S \tag{3}$$

where for ρ we take the specific resistance of the leading electrolyte and for I the starting current of the experiment. We assume a negligibly small temperature dependence of the cross-section of the capillary tube. Maintaining a constant temperature at the detection site is based on the computer control of the driving current in order to hold the thermal power term, $qI^2\rho/S$, constant, at the same value as in the leading electrolyte.

EXPERIMENTAL

Equipment

Our device is shown in Fig. 1. A reconstructed high-frequency contactless conductivity detector was used[11–16]. We have sought simplicity of the electric circuits and temperature and time stability. An autocalibrating principle was used for signal treatment. Into the signal processing circuits (SPC), both the signal from the measuring cell (MC) and the part of the alternating voltage from the exciting signal generator (ESG) are fed. The measurements were continuously calibrated for zero drift and gain. In this way, the temperature and time fluctuations of all parts of the measuring chain are efficiently suppressed, with the exception of the preamplifier (PA). However, the latter was thermostatted together with the measuring cell and the surrounding of the capillary tube.

By calculating the transfer function of the equivalent circuits of the measuring cell, we have shown a proportionality between the optimum electrolyte concentration interval and the frequency of the exciting signal[15]. Therefore, we constructed two versions of the detector, one for a frequency of 1 MHz, and the second for a frequency of 10 MHz. It was demonstrated that the former was most suitable for a leading electrolyte concentration of 0.001 M and the latter for one of 0.01 M.

The 1-MHz version was used in the equipment described. A signal from the processing circuits SPC was read by an analog-to-digital converter (ADC) and recalculated in a computer by means of a calibrating function, because the response of

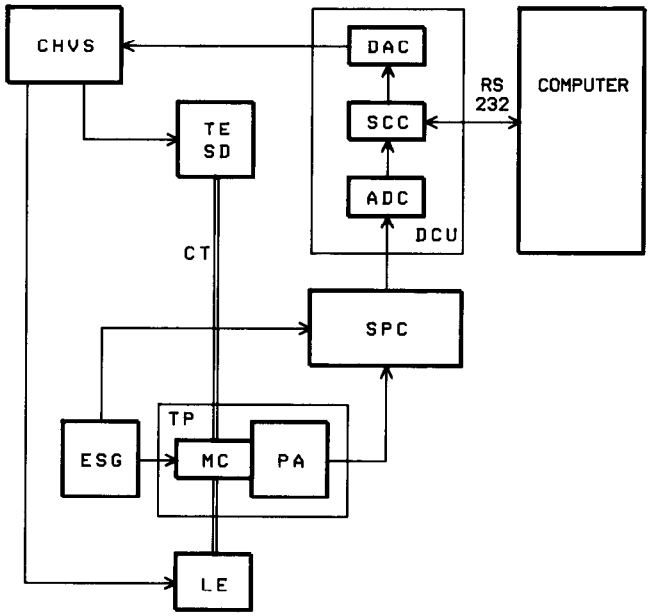

Fig. 1. Block diagram of the equipment. CHVS = Controlled high voltage supply; TE = terminating electrolyte reservoir; SD = sampling device; CT = capillary tube; MC = measuring cell; ESG = exciting signal generator; PA = preamplifier; LE = leading electrolyte reservoir; TP = thermostatted part of the equipment; SPC = signal processing circuit; ADC = 12 bit analog-to-digital converter; SCC = serial communication circuits; DAC = 12 bit digital-to-analog converter; DCU = data conversion unit.

the detector is not linearly dependent on the specific resistance of the electrolyte. A digital-to-analog converter (DAC) controls the driving current supplied to the capillary tube by a controlled high-voltage supply (CHVS). Communication between the computer and the isotachophoretic equipment was realized by means of an optically isolated RS 232 serial line. Before measurement, the thermostat was set at the temperature, T_0 calculated according to eqn. 3. After sample introduction, the control of the experiment was passed to the computer. The control program operates in a conversational manner. The flow chart of that part of the computer program that carries out the temperature control is shown in Fig. 2. A requested starting current together with other data are entered via the keyboard and the computer switches on the current to capillary tube. Subsequently, the computer calculates the thermal power generated in the leading electrolyte. The computer then drives the current in the capillary tube so as to attain the same thermal power in the course of the whole experiment. A programmable timer in a program loop permits variation of the clock frequency of the measurement from 10 Hz to an arbitrarily low value. At the same time, the specific resistance measured is stored in a memory and displayed on a CRT screen in the form of an isotachopherogram.

After the termination of the measurement, any part of the experiment can be graphically presented in any way. Values of the specific resistance or the time can be read with a 12-bit accuracy by means of a cursor. In addition, it is possible to store any

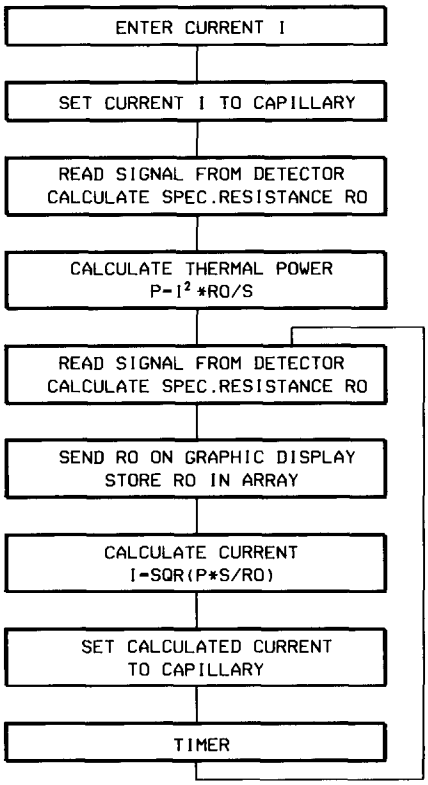

Fig. 2. Flow chart of the control program.

part of or the whole experiment on a floppy disc. An illustration of the isotachopherograms is shown in Fig. 3.

The above described control of the driving current is rapid and without any overshoots. This is due to the small time constant and small open loop gain of the feedback loop. In our earlier work[5], temperature was measured by means of a thermometric detector and the computer controlled the current so as to achieve a constant signal from the detector. To attain a small control error, the open loop gain was higher and, in addition, the time constants in a loop were higher due to thermometric detection. Therefore, the transfer function of the control had to be properly chosen in order to maintain rapid and stable control. We used a so-called self-adaptive control algorithm.

Measurements and calculations

In order to confirm the general applicability of the method and equipment proposed, the limiting mobilities of some anions were determined. Our model[17,18] of the isotachophoretic steady state has been reported previously. In this study, the computational method[18] was modified for evaluation of limiting mobilities from constant temperature isotachophoretic experiments. The model is based on the same

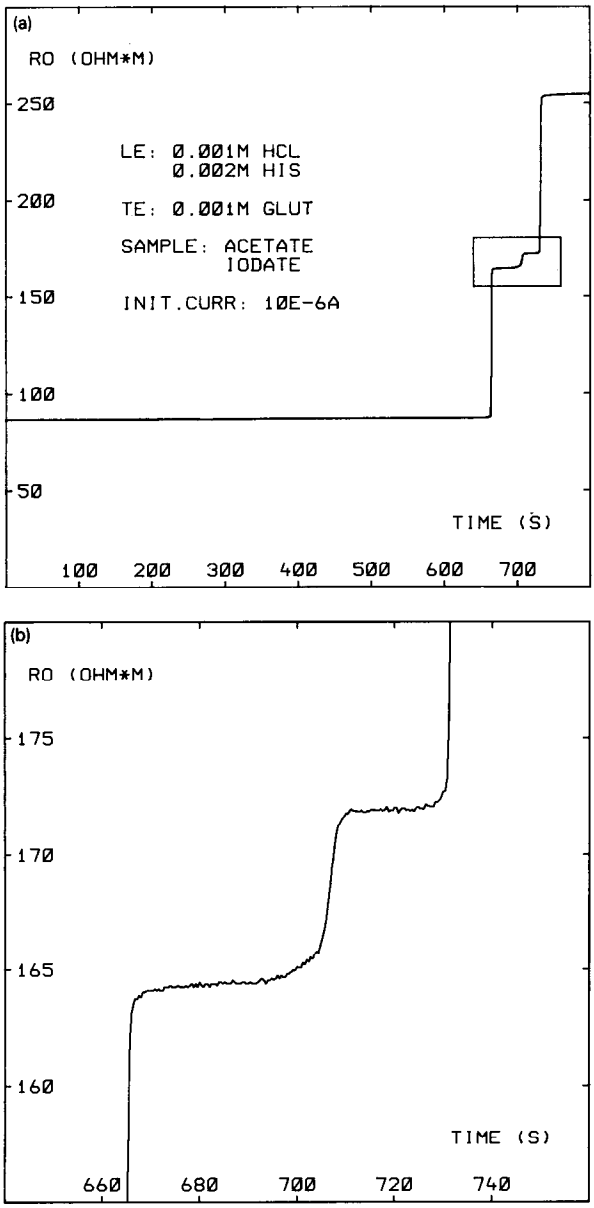

Fig. 3. Illustration of an isotachopherogram and of its computer treatment. (a) Separation of acetate and iodate obtained with the operational system His/His · HCl. The terminating electrolyte was glutamic acid. The initial current was 10 μA. (b) Amplified part of the isotachopherogram.

presumptions as those used for a model described by Everaerts[2], Hirokawa and Kiso[19,20] and other authors and has the following aspects:

(a) All substances can be considered as ampholytes.

(b) The Onsager–Fuoss theory[21] (taking into account the so-called "mixture effect") is used for the relationship between actual and limiting mobilities.

(c) The limiting mobilities, dissociation constants, relative permittivity and viscosity of the solvent can be considered as temperature dependent. In the method proposed, it is not necessary however, because the experiments are carried out at constant temperature.

(d) Acid dissociation constants are not corrected for the ionic strength. (This will be improved on in the near future by using Debye–Hückel equations[22,23].)

The leading electrolyte was 0.002 *M* hydrochloric acid with 0.003 (pH 5.82) and 0.004 *M* (pH 6.12) histidine as a counter ion. The terminating electrolyte was 0.002 *M* glutamic acid. The separation tube had 0.45 mm I.D. The starting driving current was 15 μA. All chemicals used were of analytical reagent grade. The water used was twice distilled. The limiting mobilities of the following acids were determined: strong acids, nitric, iodic, chloric and perchloric; weak monovalent acids, acetic and propionic; weak divalent acids, oxalic, succinic, adipic and maleic; amphiprotic glutamic acid. For oxalic, succinic and adipic acids, the limiting mobilities of the mono- and divalent components could not be obtained simultaneously, because they are almost completely dissociated to the divalent forms. Therefore, the limiting mobilities of the monovalent components were entered into the computer as input data. Similarly, for glutamic acid, the mobility of the ion $glut^+$ is considered to be the same as that of the ion $glut^-$ and the mobility of the ion $glut^{2-}$ is entered as an input datum. For maleic acid, the mobilities of both forms were obtained.

In Table I are listed both the input data for calculation and the data evaluated

TABLE I

LIMITING MOBILITIES EVALUATED (25°C)

pK_a = Thermodynamic acid dissociation constant from refs. 30 and 33. U^0 = evaluated limiting mobility ($m^2\ V^{-1}\ s^{-1}$) · 10^9. σ = Standard deviation of a single measurement of mobility ($m^2\ V^{-1}\ s^{-1}$) · 10^9. U^0(lit) = Limiting mobility from ref. 30.

Anion of acid	pK_a	U^0	σ	U^0(lit)
Hydrochloric (1−)	−6.1[a]	79.1[a]	–	79.1
Nitric (1−)	−1.34[a]	74.5	0.1	74.1
Iodic (1−)	0.77[a]	42.1	0.2	42.0
Chloric (1−)	−2.70[a]	66.9	0.1	67.0
Perchloric (1−)	−7.3[a]	70.0	0.1	69.8
Acetic (1−)	4.76[a]	41.9	0.1	42.4
Propionic (1−)	4.87[a]	36.5	0.1	37.1
Oxalic (1−)	1.27[a]	42.4[a]	–	42.4
Oxalic (2−)	4.27[a]	77.8	0.1	77.0
Succinic (1−)	4.21[a]	33.0[a]	–	33.0
Succinic (2−)	5.64[a]	60.2	0.1	60.9
Adipic (1−)	4.43[a]	24.6[a]	–	24.6
Adipic (2−)	5.41[a]	52.7	0.5	52.4
Maleic (1−)	1.92[a]	40.7	0.4	41.3
Maleic (2−)	6.22[a]	62.0	0.4	62.4
Glutamic (1−)	4.38[a]	29.1	0.2	28.9
Glutamic (2−)	9.96[a]	49.6[a]	–	49.6
Glutamic (1+)	2.16[a]	29.1[b]	–	28.9

[a] Input data for evaluation.

[b] Value set the same as for glutamic acid (1−).

TABLE II

CALCULATED AND EXPERIMENTAL MOBILITIES OF K^+ AND Cl^- (25°C)

U_i = Mobility of ion i calculated by means of Onsager–Fuoss theory[21]. U_i^0 = Limiting mobility of ion i. $U_{K^+} + U_{Cl^-}$(exptl.) = Experimental value of mobility[31,32]. All mobilities in $(m^2\ V^{-1}\ s^{-1}) \cdot 10^9$.

Concentration	U_{K^+}	$U^0_{K^+} - U_{K^+}$	U_{Cl^-}	$U^0_{Cl^-} - U_{Cl^-}$	$U_{K^+} + U_{Cl^-}$	$U_{K^+} + U_{Cl^-}$*(exptl.)*
0	76.2		79.1		155.3	155.3
0.0001 *M* K^+, 0.0001 *M* Cl^-	75.7	0.5	78.6	0.5	154.3	154.6
0.001 *M* K^+, 0.001 *M* Cl^-	74.6	1.6	77.5	1.6	152.1	152.5
0.01 *M* K^+, 0.01 *M* Cl^-	71.3	4.9	74.1	5.0	145.4	146.9

TABLE III

CALCULATED MOBILITIES OF K^+ AND SO_4^{2-} (25°C)

Symbols as in Table II.

Concentration	U_{K^+}	$U^0_{K^+} - U_{K^+}$	$U_{SO_4^{2-}}$	$U^0_{SO_4^{2-}} - U_{SO_4^{2-}}$
0	76.2		82.9	
0.0001 *M* K^+, 0.00005 *M* SO_4^{2-}	75.4	0.8	81.7	1.2
0.001 *M* K^+, 0.0005 *M* SO_4^{2-}	73.7	2.5	79.1	3.8
0.01 *M* K^+, 0.005 *M* SO_4^{2-}	68.4	7.8	71.0	11.9

according to our model. The data reported by Hirokawa *et al.*[24–30] are also given for comparison. A very good agreement with published limiting ionic mobilities is apparent from the table, especially for the anions of strong acids.

In earlier versions of our models[17,18] we calculated the temperatures of the zones by means of eqn. 2 and recalculated temperature-dependent quantities to 25°C. In the method described, the temperature of 25°C is maintained automatically, so that no temperature corrections are necessary. This results in a better accuracy of the physico-chemical quantities evaluated.

An autocalibrating contactless detector having a long-term thermal stability has been used. It follows that the sensing electrodes do not sustain any chemical and physical changes and thus the measurement is not disturbed.

Furthermore, the 1-MHz version of the detector facilitates the use of the electrolyte systems in millimolar range. The lower the concentration of the electrolyte, the lower is the correction of ionic mobilities to infinite dilution carried out by the Onsager–Fuoss theory. The electrolyte system is roughly speaking closer to infinite dilution. For comparison, the calculated mobilities of Cl^- and SO_4^{2-} with a common counter ion K^+ are given in Table II. The difference between the limiting mobility and the mobility at a given concentration together with the values of $u_{K^+} + u_{Cl^-}$ obtained from the experimentally measured dependence of the conductivity on the concentration[31,32] for KCl are also shown. The decrease in the mobility with increasing concentration of di- and polyvalent ions is higher than for monovalent ions (see Table III), so that, for example, SO_4^{2-} cannot be isotachophoretically determined with a 0.001 *M* Cl^- leading electrolyte system, due to the fact that SO_4^{2-} is more mobile than Cl^- at this concentration. It is seen also that the correction required by the theory at 0.01 *M* is more than three times higher than for that of 0.001 *M* as follows from the square root dependence of the mobility on the ionic strength. Even for the most simple case of an uni-univalent electrolyte, the calculated mobility deviates by 1% from the experimentally obtained value at 0.01 *M* (compare the values 145.4 and 146.9).

REFERENCES

1 J. Vacík, J. Zuska, F. M. Everaerts and Th. P. E. M. Verheggen, *Chem. Listy*, 66 (1972) 545.
2 F. M. Everaerts, J. L. Beckers and Th. P. E. M. Verheggen, *Isotachophoresis*, Elsevier, Amsterdam, Oxford, New York, 1976.
3 J. Pospíchal, M. Deml, Z. Žemlová and P. Boček, *J. Chromatogr.*, 320 (1985) 139.
4 J. Pospíchal, M. Deml and P. Boček, *J. Chromatogr.*, 390 (1987) 17.

5 C. Szitás, J. Vacík and J. Zuska, *Fifth International Symposium on Isotachophoresis, Maastricht, Sept. 3–5, 1986.*
6 M. Coxon and M. J. Binder, *J. Chromatogr.*, 95 (1974) 133.
7 M. Coxon and M. J. Binder, *J. Chromatogr.*, 101 (1974) 1.
8 M. Coxon and M. J. Binder, *J. Chromatogr.*, 107 (1975) 43.
9 V. Kašička, *Diploma Thesis*, Charles University, Prague, 1977.
10 Z. Ryšlavý, P. Boček, M. Deml and J. Janák, *J. Chromatogr.*, 144 (1977) 17.
11 M. Demjaněnko, *CS Pat. Certification*, A0 169,577 (1974).
12 B. Gaš, *CS Pat. Certification*, A0 204,104 (1979).
13 B. Gaš, *CS Pat. Certification*, A0 206,186 (1979).
14 B. Gaš, M. Demjaněnko and J. Vacík, *J. Chromatogr.*, 192 (1980) 253.
15 B. Gaš and J. Vacík, *Chem. Listy*, 74 (1980) 652.
16 J. Vacík, J. Zuska and I. Muselasová, *J. Chromatogr.*, 320 (1985) 233.
17 V. Kašička, J. Vacík and Z. Prusík, *J. Chromatogr.*, 320 (1985) 33.
18 V. Zadražil and J. Vacík, in P. Boček (Editor), *Isotachophoresis, Basic Course, Advanced Course, ITP-84, Hradec Králové*, Ústav radioekológie a využitia jadrovej techniky, Spišská Nová Ves, 1984.
19 T. Hirokawa and Y. Kiso, *J. Chromatogr.*, 242 (1982) 227.
20 T. Hirokawa and Y. Kiso, *J. Chromatogr.*, 248 (1982) 341.
21 L. Onsager and R. M. Fuoss, *J. Phys. Chem.*, 36 (1932) 2689.
22 P. Debye and E. Hückel, *Phys. Z.*, 24 (1923) 185.
23 P. Debye and E. Hückel, *Phys. Z.*, 24 (1923) 305.
24 T. Hirokawa and Y. Kiso, *J. Chromatogr.*, 252 (1982) 33.
25 T. Hirokawa, M. Nishino and Y. Kiso, *J. Chromatogr.*, 252 (1982) 49.
26 T. Hirokawa, S. Kobayashi and Y. Kiso, *J. Chromatogr.*, 318 (1985) 195.
27 T. Hirokawa, T. Gojo and Y. Kiso, *J. Chromatogr.*, 369 (1986) 59.
28 T. Hirokawa, T. Gojo and Y. Kiso, *J. Chromatogr.*, 390 (1987) 201.
29 T. Hirokawa, T. Tsuyoshi and Y. Kiso, *J. Chromatogr.*, 408 (1987) 27.
30 T. Hirokawa, M. Nishino, N. Aoki, Y. Kiso, Y. Sawamoto, T. Yagi and J. Akiyama, *J. Chromatogr.*, 271 (1983) D1.
31 R. Brdička and J. Dvořák, *Základy fysikální chemie*, Academia, Praha, 1977.
32 R. Brdička and J. Dvořák, *Grundlagen der physikalischen Chemie*, VEB Deutscher Verlag der Wissenschaften, Berlin, 1988.
33 J. A. Dean (Editor), *Lange's Handbook of Chemistry*, McGraw-Hill, New York, 1973.