

DETERMINATION OF ORGANIC ACIDS AND INORGANIC ANIONS IN WINE BY ISOTACHOPHORESIS ON A PLANAR CHIP

MARIÁN MASÁR^{2*}, DUŠAN KANIANSKY¹, RÓBERT BODOR¹, MATTHIAS JÖHNCK², BERND STANISLAWSKI²

¹Department of Analytical Chemistry, Faculty of Natural Sciences, Comenius University, Mlynská Dolina CH-2, SK-84215 Bratislava, Slovak Republic

²Merck KGaA, Frankfurter Straße 250, D-64293 Darmstadt, Germany

INTRODUCTION

From the works dealing with general aspects of wine analysis (ref. 1, 2) it is apparent that the determination of organic acids and inorganic anionic constituents in wine has three priority levels.

1. A top priority can be attributed to the determination of tartaric, lactic, malic and citric acids as these are, mainly, responsible for the acidity of wine. Concentrations of these acids in wine depend on the sort and mature of the grape, fermentation processes and methods of treatment and ripeness of wine. In addition, physical, chemical and biochemical processes during wine production change their concentrations. These changes are caused, e.g., by a precipitation of tartaric acid (potassium tartrate) and/or by malo-lactic fermentation.
2. A lower priority has the determination of ascorbic and acetic acids. Ascorbic acid, a natural wine component, inhibits oxidation processes in grapes and wine. Acetic acid is produced by malo-lactic and acetic fermentations. An increased

AIMS OF THE WORK

To assess various ITP electrolyte systems suitable to the separation and/or determination of anionic constituents present in wine from the point of view of their applicabilities to ITP wine analysis on a planar chip.

To elaborate analytical method suitable to the determination of anionic wine constituents and evaluate its performance parameters.

INSTRUMENTATION

A schematic arrangement of the channels of a poly(methylmethacrylate) (PMMA) chip used in this work is given in Fig. 1 (for details see design No. 2 in ref. 3). The ITP separations on the chip were performed in a laboratory constructed CE equipment (ref. 4). This equipment includes two units:

- (1) An electrolyte and sample management unit (E&SMU, in Fig. 2), connected via 300 µm I.D. FEP (Fluorinated ethylene propylene copolymer) capillary tubes to the inlets of the channels on the chip. Valves of this unit (V1, V2, VT and VS, in Fig. 2) serve to open these inlets when filling the channels and they are closed during the CE runs. Pumping syringes (P1, P2, PS, PT, in Fig. 2), connected to the inlets of the corresponding valves, deliver appropriate electrolyte solutions and the sample to the channels before the separation. An outlet channel of the chip, connected to a waste container (W, in Fig. 2), is permanently opened.
- (2) An electronic and control unit (E&CU, in Fig. 2) delivers the driving current, measures conductivity using platinum detection sensors sputtered on the channels of the chip and interfaces the CE equipment with a PC computer. This unit includes the following modules: (i) High-voltage power supply (HV, in Fig. 2), delivering the stabilized driving current in the range of 0–50 µA with a maximum voltage of 5 kV connected to the chip; (ii) High-voltage-relay (HV-relay, in Fig. 2), for the column-switching operation of the equipment; (iii) Two conductivity detectors (CD1 and CD2, in Fig. 2), decoupled from the detection sensors on the chip by transformers with PTFE insulated coils. The detector for the first channel (CD1) is provided with a comparator circuit to identify a front boundary of the ITP zone of a selected effective mobility (needed in a control of the column-switching operation of the equipment); (iv) Control unit (CU, in Fig. 2), connecting the CE unit to a PC Pentium computer.

ITP win software (version 2.31) obtained from Kascomp (Bratislava, Slovak Republic) was used for a time-programmed control of the CE runs and for the acquisition of the detection data and their processing.

CHEMICALS AND SAMPLES

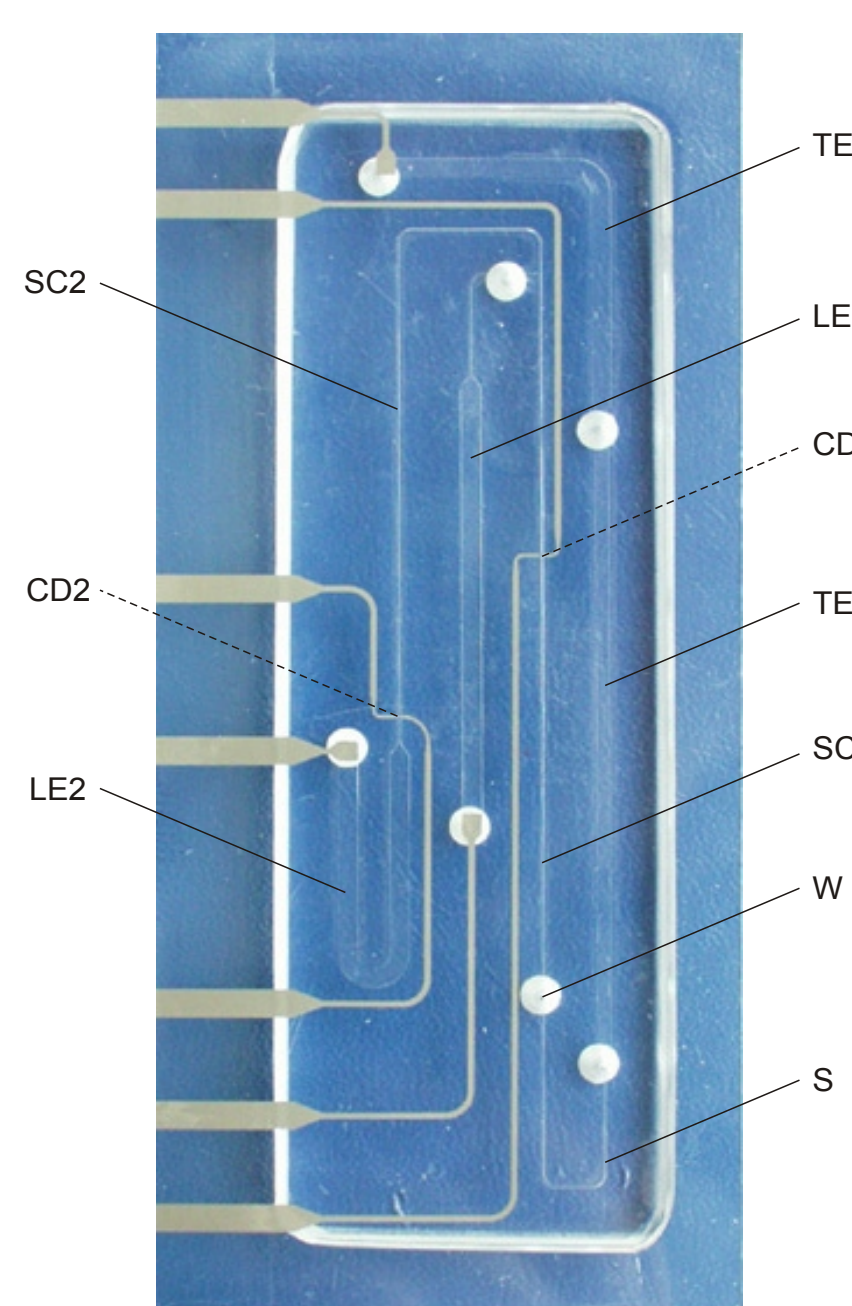


Fig. 1. A design of the PMMA chip, ver. 1.1. LE = terminating buffer reservoir (8.8 µl); TEC = terminating channel (9 µl); S = a 0.9 µl sample injection channel (21.8 x 0.26 x 0.2 mm [length x width x depth]); SC1 = the first separation channel (a 2.1 µl volume; 51.8 x 0.26 x 0.2 mm [length x width x depth]) with a platinum conductivity sensor; SC2 = the second separation channel (a 2.6 µl volume; 64.0 x 0.26 x 0.2 mm [length x width x depth]) with a platinum conductivity sensor; CD1, CD2 = conductivity detectors for SC1 and SC2, respectively; LE1, LE2 = leading buffer reservoirs

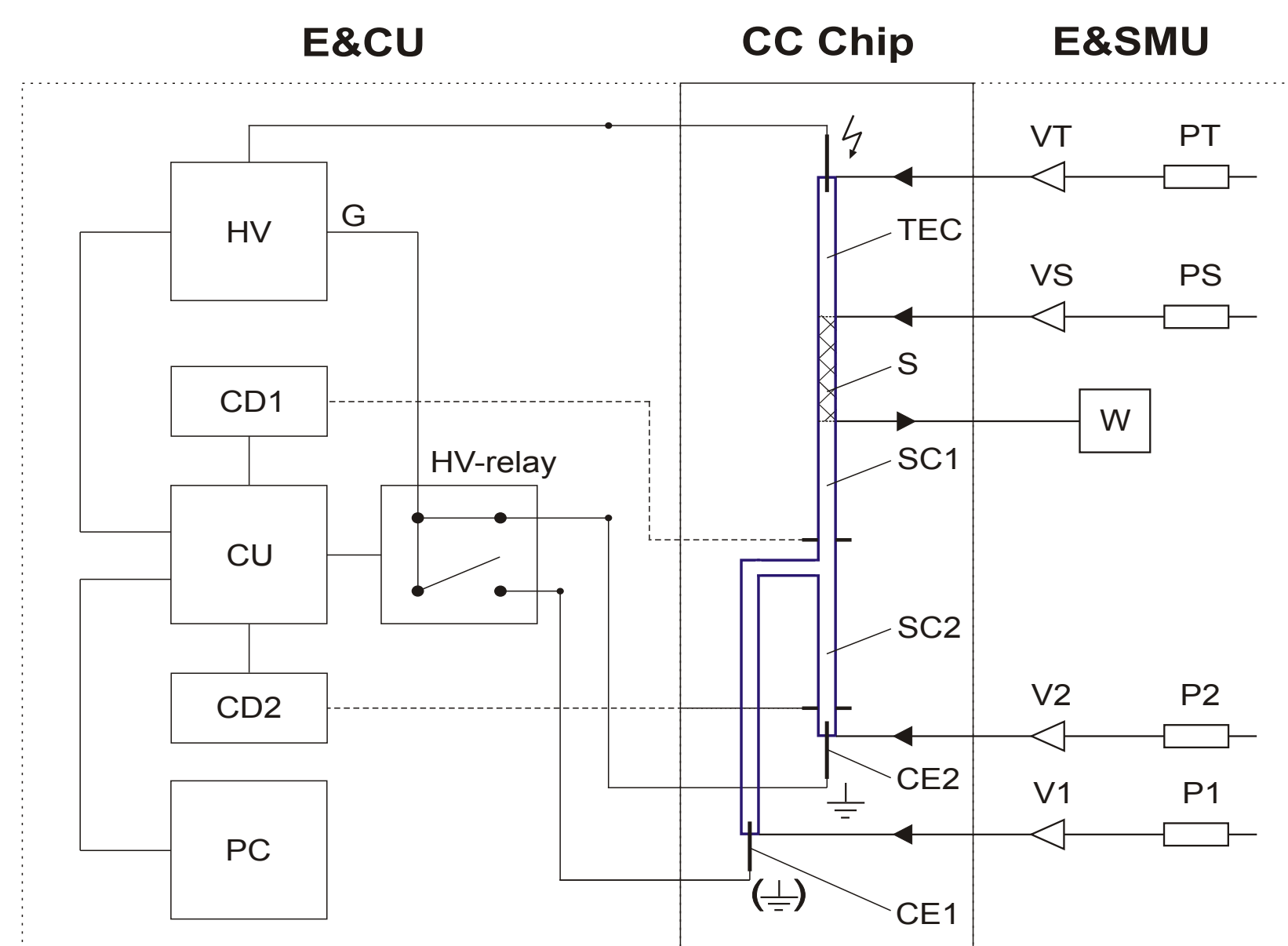


Fig. 2. A block scheme of the ITP equipment to separations with the closed separation compartment of the column-coupling configuration of the PMMA chips. E&CU = electronic and control unit; HV = high-voltage power supply; HV-relay = high-voltage relay; CE1, CE2 = counter-electrodes for the first and second separation channels, respectively; E&SMU = electrolyte and sample management unit; V1, V2, VT = needle valves for the inlets of the separation and terminating channels; VS = a pinch valve for the inlet of the sample injection channel; W = waste container. P1, P2, PS, PT = syringes for filling the first, second, sample injection and terminating channels

SEPARATING CONDITIONS

A detail assessment of separabilities of organic acids and other anionic constituents to be determined in wine revealed that the separation mechanism based on differences in pK values offers a best alternative to their ITP separations on the chip.

An evaluation of various leading electrolytes implementing this separation mechanism in the pH range of 2.9–3.5 showed that the one proposed to the separations of organic acids in wine in a conventional ITP equipment by Reijenga et al. (ref. 5) provides the best results. With the exception of sulfate (migrating with the effective mobility close to that of chloride), it resolved all analytes of our interest in one ITP run

Table 1. Electrolyte systems

Parameter	
Solvent	Water
Leading ion	Chloride
Concentration (mmol/l)	10
Counter-ion	β-alanine
pH	2.9
Additive	Methylhydroxyethylcellulose
Concentration (% w/v)	0.1
Terminating ion	Capronate
	Glutamate
Concentration (mmol/l)	5
Counter-ion	Histidine
pH	6
	5

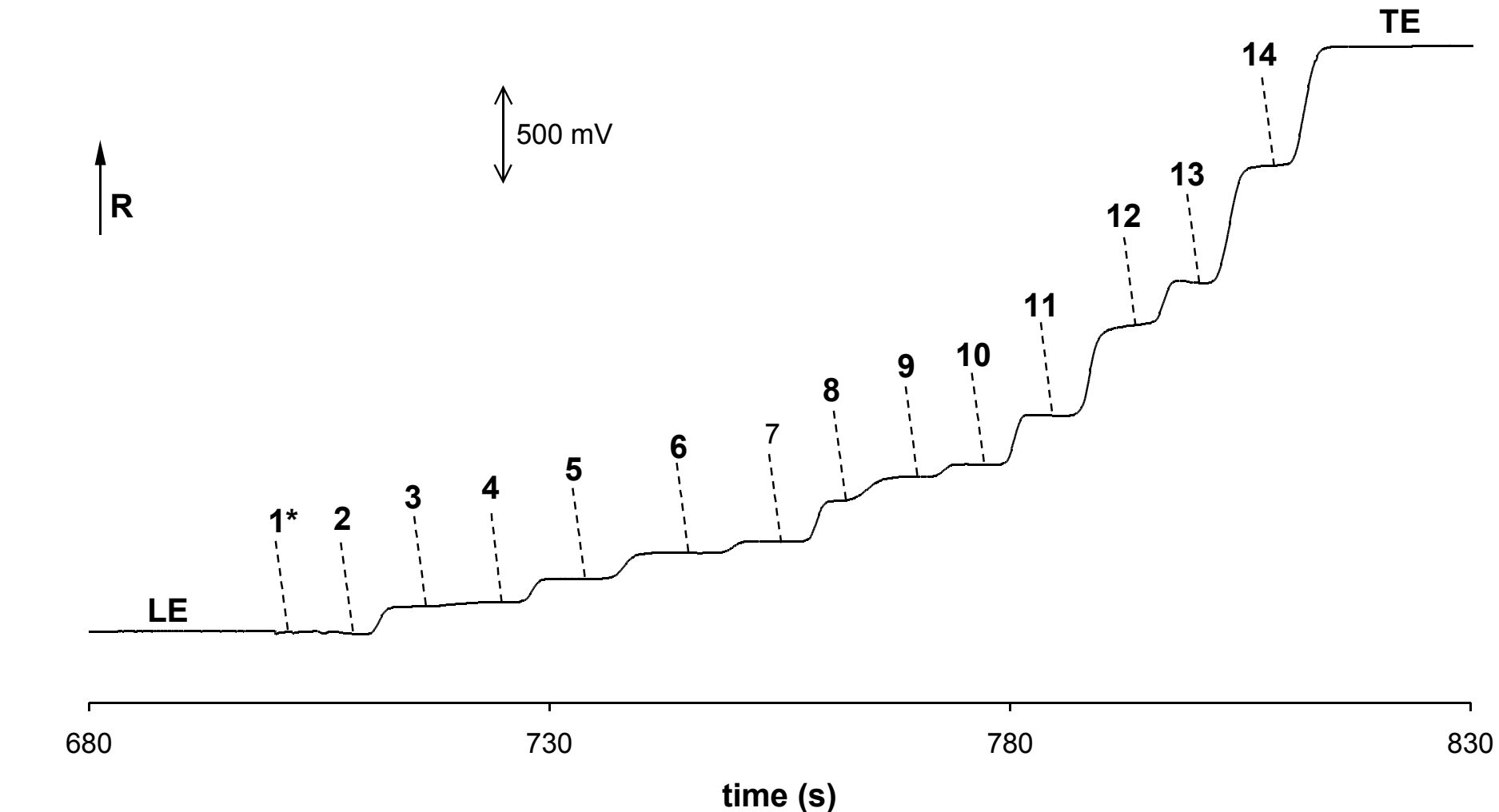


Fig. 3. Isotachophoretic separation of organic acids and three inorganic anions present in a model mixture on the PMMA chip. Zone assignments: LE = leading anion (chloride); 1* = migration position of sulphate; 2 = sulphate; 3 = phosphate; 4 = malonate; 5 = tartrate; 6 = citrate; 7 = malate; 8 = lactate; 9 = gluconate; 10 = aspartate; 11 = succinate; 12 = ascorbate; 13 = acetate; 14 = sorbate; TE = terminating anion (capronate). The separation was carried out in the electrolyte system (Table 1) using capronate as a terminating ion in both separation channels. The driving current was a 10 µA in the first and second separation channel. The concentrations of the analytes in a model sample were 12–42 mg/l. R = resistance.

QUANTITATION OF ORGANIC ACIDS

ITP determination of a group of acids (tartaric, lactic, malic and citric acids) responsible for some important organoleptic characteristics of wines and their quality was a subject of a detail study performed in this work. This study was carried out under separating conditions (Table 1; electrolyte system with glutamate as a terminating ion) providing a complete resolution of the acids to be expected in wine samples at concentrations detectable by the conductivity detector on the chip.

The lowest concentrations at which the acids of our interest could be quantified were in the range of 2–10 mg/l. A mutual resolution of citrate and malate was found to be critical in reaching a complete ITP resolution of the acids present in wine samples. Under our working conditions we could resolve them when a 20 mg/l concentration of citrate was accompanied in the sample (injected by a 0.9 l volume sample loop on the chip) by a 270 mg/l concentration of malate. These amounts, in fact, determined a maximum loadability of an actual wine sample on the chip.

Reproducibilities of qualitative indices of the acids in the ITP separations (RSH values, using the leading and terminating anions as references), expressed via RSD values, were better than 2% for both the model and wine samples (see

Table 2. Reproducibilities of the RSH values and the zone lengths for the studied organic acids present in the model and real samples

Organic acid	Concentration (mg/l)	RSD of RSH (%)	RSD of zone length (%)	n	Concentration (mg/l)	RSD of RSH (%)	RSD of zone length (%)	n
Model samples					Red wine			
Tartrate	22.5	1.37	2.47	7	60.0	1.85	2.22	9
Citrate	12.6	1.19	3.10	7	33.6	1.88	2.80	9
Malate	60.3	0.99	1.99	7	160.9	1.72	2.13	9
Lactate	13.5	0.47	1.13	7	36.0	1.32	2.65	9
White wine					Red wine			
Tartrate	53.6	1.32	1.70	5	35.3	3.05	2.91	5
Citrate	24.4	1.53	1.66	5	41.1	1.65	3.55	5
Malate	178.9	0.75	1.65	5	198.8	1.97	2.39	5
Lactate	27.4	0.73	1.38	5	40.8	0.53	2.62	5

RSD = relative standard deviation; RSH = relative standard height calculated on the terminating anion (glutamate, Table 1); n = number of parallel measurements

Table 3. Parameters of the regression equations (y = a + bx) for the studied organic acids

Organic acid	a (s)	b (s/mg)	r	n	Δx (mg/l)
Tartrate	-0.55	0.370	0.9980	20	15.0–105.1
Citrate	0.16	0.315	0.9988	20	8.4–58.8
Malate	-3.16	0.341	0.9981	20	40.2–281.6
Lactate	1.82	0.240	0.9975	20	9.0–63.1

a = intercept; b = slope of the calibration line; n = number of data points; x = concentration of the analyte; y = zone length; Δx = concentration interval for which the calibration data were measured.

ANALYSIS OF WINES

Typical isotachophoregrams as obtained from the analyses of white and red wines performed in this work are given in Figs. 4 and 5, respectively. Relevant data characterizing the quantitation of the acids of our interest in these particular samples are summarized in Table 4. From these data and from the ones obtained for model samples (Table 2) it is apparent that the reproducibilities attained in the analysis of practical samples did not deviate from those

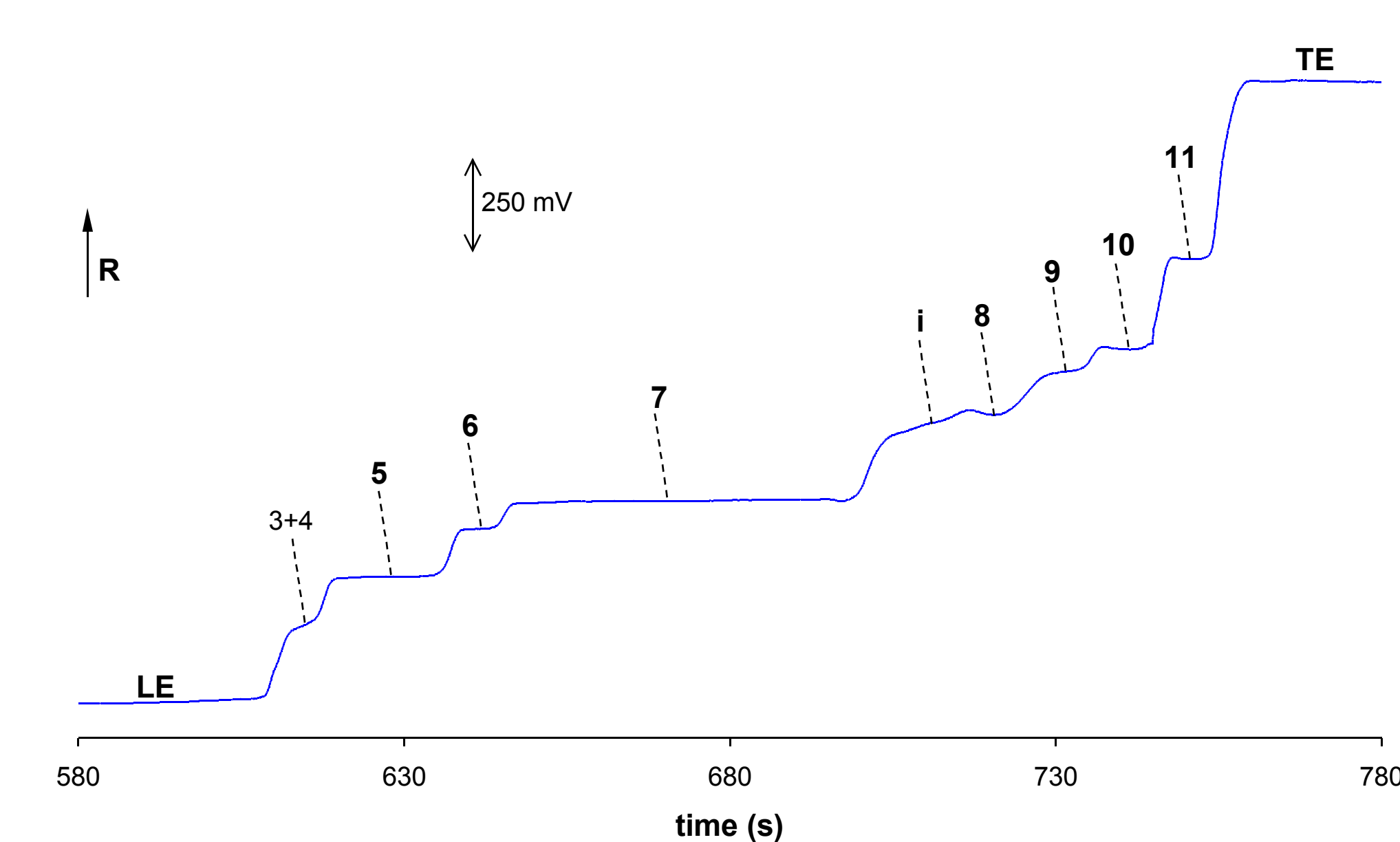


Fig. 4. Isotachophoregram from the separation of the organic acids and inorganic anions present in a 20 times diluted white wine on the PMMA chip. The separation was carried out in electrolyte system (Table 1) using glutamate as a terminating ion in both separation channels. The driving current was 20 µA in the first separation channel, in the second channel it was 10 µA. LE = leading anion (chloride); TE = terminating anion (glutamate); i = impurities from the sample. For the other zone

Table 4. Determination of organic acids in wines

Organic acid	White wine			Red wine		
	Determined (mg/l)	RSD (%)	n	Determined (mg/l)	RSD (%)	n
Tartrate	1072.7	1.65	5	1243.6	2.84	5
Citrate	488.2	1.69	5	821.3	3.59	5
Malate	3576.9	1.57	5	3976.8	2.28	5
Lactate	547.4	1.76	5	814.9	2.08	5

RSD = relative standard deviation; n = number of parallel determinations

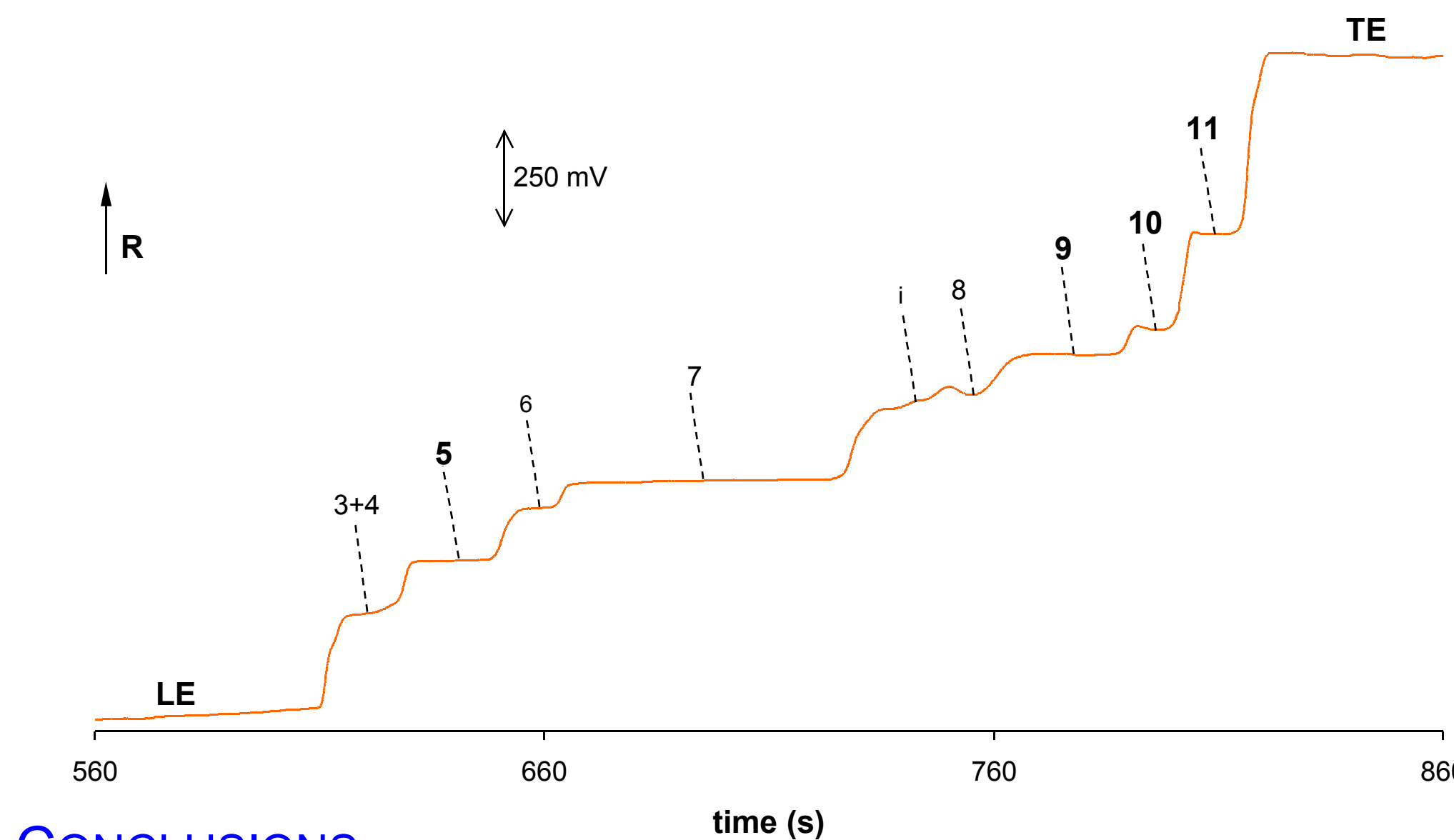


Fig. 5. Isotachophoregram from the separation of the organic acids and inorganic anions present in a 20 times diluted red wine on the PMMA chip. The separation was carried out in electrolyte system (Table 1) using glutamate as a terminating ion in both separation channels. The driving current was 20 µA in the first separation channel, in the second channel it was 10 µA. LE = leading anion (chloride); TE = terminating anion (glutamate); i = impurities from the sample. For the other zone

CONCLUSIONS

Isotachophoresis performed on a PMMA chip with a 94 mm total length of the separation channel and the conductivity detection of the zones was found suitable to the separation of thirteen anionic constituents (organic acids and inorganic anions), currently occurring in wines. An optimum electrolyte system in this respect (pH = 2.9), separating these constituents via their differences in pK values, provided the analysis times of 10–15 minutes.

Although a sample loadability of the present chip is significantly lower in comparison to conventional ITP equipment, it was sufficient to separate anionic constituents present in 0.9 l volumes of 20–100 times diluted wine samples. A maximum sample loadability in the analysis of wine samples was set by the resolution of citrate and malate. Nevertheless, this pair of the analytes could be still resolved and quantified when a citrate to malate molar concentration ratio was 1:20.

Reproducible ITP determination of tartaric, lactic, malic and citric acids (the acids responsible for some

REFERENCES

1. J. Farkaš, Technologie a biochemie vína, SNTL / ALFA, Praha, 1980.
2. E. Verada Alonso, A. García de Torres, A. Rivero Molina, J.M. Cano Pavon, Quim. Anal., 17 (1998) 167–175.
3. B. Grass, A. Neyer, M. Jöhnck, D. Siepe, F. Eisenbeiss, G. Weber, R. Hergenroder, Sens. Actuators B, submitted.
4. D. Kaniánsky, M. Masár, J. Bieličková, F. Iványi, F. Eisenbeiss, B. Stanislawski, B. Grass, A. Neyer, M. Jöhnck, Anal. Chem., 72 (2000) 3596–3604.