Journal of Chromatography, 470 (1989) 105–112
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 266

CONCEPT OF RESPONSE FACTOR IN CAPILLARY ISOTACHOPHORESIS

DETERMINATION OF DRUGS IN SOLUTION FOR INTRAVENOUS INJECTION

M. M. GLADDINES*, J. C. REIJENGA and R. G. TRIELING
Laboratory of Instrumental Analysis, Eindhoven University of Technology, P.O. Box 513, 5600 MB Eindhoven (The Netherlands)
M. J. S. VAN THIEL
Department of Clinical Pharmacy and Toxicology, Eindhoven, Diaconessenhuis, P.O. Box 90052, 5600 PD Eindhoven (The Netherlands)
and
F. M. EVERAERTS
Laboratory of Instrumental Analysis, Eindhoven University of Technology, P.O. Box 513, 5600 MB Eindhoven (The Netherlands)

SUMMARY

Twenty-six drugs in solutions for intravenous injection were determined by capillary isotachophoresis with only one calibration point for each component. The maximum deviation of the labelled concentration was 2%. A new calibration constant is introduced, *viz.*, the response factor (RF, dimensionless), which is independent of the diameter of the capillary, the construction of the universal detector and the driving current used during detection. It is shown that the RF can be used on different equipment, using different currents during detection. It appears that the RF is usable for routine analysis when a deviation of 5% is acceptable. Daily one-point recalibration, however, improves this value to 1%.

INTRODUCTION

During the past 5 years, capillary isotachophoresis has been recognized as a promising technique for the determination of biochemical substances in pharmaceutical preparations and biological matrices, with studies on ephedrine alkaloids[1–6], isoquinoline alkaloids[7,8,9], sympathomimetics[10], amantadine and rimantactine[11], codeine[1,2], local anaesthetics including procaine[3,12–15], papaverine, morphine and aminophenazone[2], oral anaesthetics[16], nicotinic acid[17], minoxidil[18], amoxycillin and carboxymethylcysteine[19], aminoglycoside and lincomycin[20], cephalosporin, berberin and glycyrrhizin[21] acetylsalicylate[22], chincona alkaloids[23,24] and obidoxime[25].

Capillary isotachophoresis (ITP) is considered to be a precise and accurate method for the determination of these substances. In our view, however, capillary

0021-9673/89/$03.50

isotachophoresis offers more advantages. Usually, when a calibation graph is constructed, the zone length is plotted against the amount injected. This calibration graph depends on the equipment and the current used. If, however, the zone length is divided by the amount injected and multiplied by the driving current, a new variable is created. This variable is independent of the diameter of the capillary, the construction of the detector and the driving current used during detection. This variable, which is called the response factor (RF), is dimensionless:

$$\mathrm{RF} = \frac{ZL \cdot I}{|z| \cdot F \cdot Q}$$

where $|z|$ is the charge of the ion [equiv./mol], ZL the zone length (s), I the driving current (A), F the number of Faradays (coulombs/equiv.] and Q the amount injected (mol). For each component the RF depends only on the concentration of the leading electrolyte, which can be prepared very precisely[26]. This implies that the RF is a universal calibration constant, independent of the ITP instrument used for its measurement. The RF is the reciprocal transference number and denotes the sensitivity. The transference number equals the fraction of the driving current carried by the ions migrating in the steady state.

To demonstrate the use of the RF, 26 drugs (23 cations and 3 anions) in solution for intravenous injection were determined by capillary isotachophoresis. These solutions often contain additives, such as sodium pyrosulphite, for preservation. For each component the RF was determined by one injection of the pure component. Further, the concentration of the drug in the solution was determined by injection of the solution for intravenous injection. Qualitative parameters such as relative step height and UV absorption were also measured for identification of the drugs.

To test the reproducibility of the RF, four components (acetate, phosphate, benzoate and citrate) were determined on instruments with different capillary diameters and different driving currents on the same equipment. Also, the day-to-day variation and the variation when different analysts carried out the analyses were determined. The four components were chosen because they are more stable than the drugs.

EXPERIMENTAL

The isotachophoretic analyses were performed on equipment built by Everaerts *et al.*[27]. The separation compartment consisted of a PTFE capillary of I.D. 0.2 or 0.4 mm and a length of *ca.* 25 cm. The direct, constant driving current was taken from a modified high-voltage supply (Brandenburg, Thornton Heath, U.K.). The zones were detected by measuring the electrical conductivity and the UV absorption at 254 nm.

The zone lengths and step heights on the electropherograms were measured with an IBM-XT computer (IBM, Boca Raton, FL, U.S.A.), which was connected via an ADC (Labmaster, Scientific Solutions, OH, U.S.A.) with the conductivity detector. The program used was written in Turbo Pascal (Borland International, Scotts Valley, CA, U.S.A.)[28].

The reproducibility of the RF was tested by injection of four components (acetate, phosphate, benzoate, and citrate) on equipment with different capillary diameters and using different currents. These analyses were carried out on three different days with leading electrolyte systems prepared freshly each day. On the third day the analyses were also carried out by a second analyst. The electrolyte system used is listed in Table I (system 1).

The currents used were 20 and 30 μA for the 0.2 mm I.D. capillary and 70 and 85 μA for the 0.4 mm I.D. capillary. Fig. 1 shows an isotachopherogram of the four-component mixture.

For the analyses of drugs anionic and cationic electrolyte systems were used, as listed in Table I. The driving current was 30 μA for anions. For the cations it was 40 μA during the first 6 min of the analysis and 30 μA during detection. Both analyses took *ca.* 10 min.

The RF was calculated by injection of one standard solution containing 100 mg per 100 ml of the pure component. For the quantitative measurement no internal standard was used. The samples were injected with a 10-μl syringe (Hamilton, Bonaduz, Switzerland) equipped with a fixed-volume accessory. The determinations of both the pure component and the component in the solution were made in duplicate. A difference in the two measurements of less than 1% was considered sufficiently precise. If this difference was greater than 1% a third injection was made.

The relative step height (RSH) was calculated using the equation

$$\mathrm{RSH} = \frac{h_X - h_L}{h_{IS} - h_L}$$

where h_X is the height of the step of the component, h_L is the height of the step of the leading ion and h_{IS} is the height of the step of the internal standard. The internal standards used were chlorate (sodium chlorate, Merck, Darmstadt, F.R.G.) for anions

TABLE I

OPERATIONAL SYSTEMS USED FOR THE ISOTACHOPHORETIC EXPERIMENTS

MES = 2-(N-Morpholino)ethanesulphonic acid (Sigma, St. Louis, MO, U.S.A.); HEC = hydroxyethylcellulose (Polysciences, Warrington, PA, U.S.A.); PVA = poly(vinyl alcohol) (Hoechst, Frankfurt, F.R.G.); HIBA = hydroxyisobutyric acid (Fluka, Buchs, Switzerland). All other chemicals from Merck (Darmstadt, F.R.G.). For system 3, see also ref. 29.

Parameter	*System*		
	1	*2*	*3*
Leading ion	Chloride	Potassium	Potassium
Concentration (*M*)	0.01	0.01	0.01
pH	6.0	5.0	4.2
Complex ion	–	–	HIBA to pH 5.2
Counter ion	Histidine	Acetate	Acetate to pH 4.2
Additive	0.2% HEC	0.05% Mowiol	0.05% Mowiol
Terminating ion	MES	H^+	H^+
Concentration (*M*)	0.005	0.005	0.005

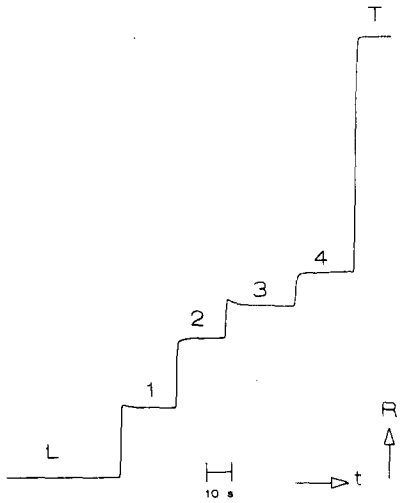


Fig. 1. Isotachopherogram of the four-component mixture analysed in system 2 (Table I). L = Leading ion; 1 = citrate; 2 = acetate; 3 = phosphate; 4 = benzoate. T = Terminator; t = time; R = Resistance.

and sodium for cations. The linearity of the conductivity detector electronics was tested by applying ten resistances in the range from 100 kΩ to 1 MΩ. The response of the conductivity detector was plotted against the resistance. The correlation coefficient of the regression line was 0.9999.

The UV absorption was expressed as a percentage of full absorption:

$$\text{UV absorption} = \frac{H_x}{H_{100\%}} \cdot 100\%$$

where H_x is the height of the UV signal of the component and $H_{100\%}$ is the height of the UV signal at 100% absorption.

RESULTS AND DISCUSSION

The reproducibility of the RF

Table II gives the results for the determination of acetate. The mean value and standard deviation of five measurements are indicated by a line (mean value) and a hatched box (standard deviation). The difference between the highest and the lowest mean value is 0.053 or 3.0%. For the other components these values are in the same range: 3.8% for phosphate, 3.5% for benzoate and 4.8% for citrate. It appears that the RF is usable for routine analysis when a deviation of 5% is acceptable. Daily one-point recalibration, however, improves this value to 1%.

Analysis of drugs for intravenous injection

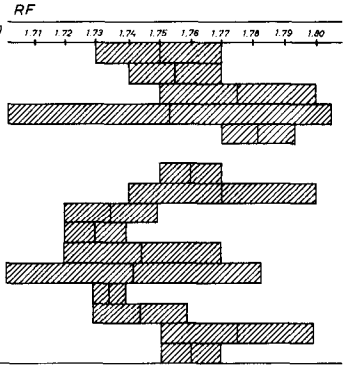
Fig. 2 shows an isotachopherogram of codeine sulphate. Table III gives the results for the determination of drugs in solution for intravenous injection. For each component the following parameters are given: RF, the electrolyte system used, the

TABLE II

MEAN VALUE (LINE) AND STANDARD DEVIATION (HATCHED BOX) (n = 5) OF THE RF OF ACETATE ON DIFFERENT DAYS, BY TWO ANALYSTS, USING DIFFERENT CURRENTS AND CAPILLARY DIAMETERS

Electrolyte system 1 (Table I) was used. I = Current; I.D. = inner diameter.

Analyst	Day	I (μA)	I.D. (mm)	RF
A	1	20	0.2	
A	1	30	0.2	
A	1	70	0.4	
A	1	85	0.4	
A	2	20	0.2	
A	2	30	0.2	
A	2	70	0.4	
A	2	85	0.4	
A	3	20	0.2	
A	3	30	0.2	
A	3	70	0.4	
A	3	85	0.4	
B	3	20	0.2	
B	3	30	0.2	
B	3	70	0.4	
B	3	85	0.4	



concentration determined (C_{DE}), the labelled concentration (C_{LA}), the deviation, calculated using the equation

$$\text{Deviation} = \frac{C_{DE} - C_{LA}}{C_{LA}} \cdot 100\%$$

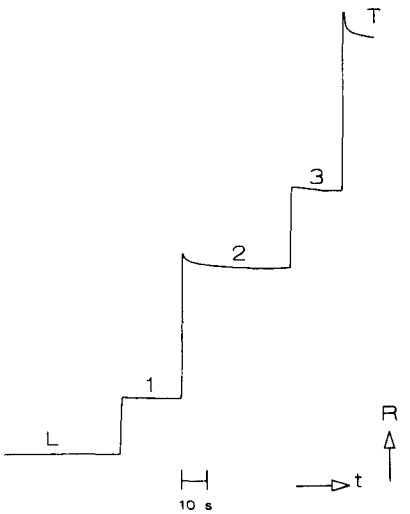


Fig. 2. Isotachopherogram of codeine sulphate analysed in system 1 (Table I). L = Leading ion; 1 = sodium (internal standard for qualitative analysis); 2 = Tris (not used); 3 = codeine. T = Terminator; t = time; R = Resistance.

TABLE III

RESULTS OF DETERMINATION OF DRUGS IN SOLUTION FOR INTRAVENOUS INJECTION

System numbers according to Table I. For definitions of deviation, RSH and UV absorption, see text.

Component	*RF*	*System*	C_{DE} *(mg/ml)*	C_{LA} *(mg/ml)*	*Deviation (%)*	*RSH*	*UV absorption (%)*
Isoniazide	3.8	2	100.1	100.0	0.1	10.7	44
Neostigmine methylsulphate	2.6	2	0.49	0.50	−2.0	4	8
Suxamethonium chloride	4.0	2	50.4	50.0	0.8	1.5	0
Isoprenaline sulphate	5.9	2	0.98	1.0	−2.0	5.2	7
Lidocaine hydrochloride	2.8	2	10.0	10.0	0.0	4.9	8
Codeine sulphate	2.8	2	29.9	30.0	−0.3	5	25
Gallamine triethiodide	5.4	2	20.4	20.0	2.0	1.7	0
Levarterenol tartrate	2.4	2	2.0	2.0	0.0	3.4	8
Atropine sulphate	5.7	2	0.25	0.25	0.0	5.2	3
Apomorphine hydrochloride	2.4	2	4.9	5.0	−2.0	4.7	79
Quinine gluconate	2.8	2	80.4	80.0	0.5	4.3	7
Adrenaline tartrate	2.6	2	1.9	1.8	5.5	4.5	8
Scopolamine hydrobromide	3.1	2	0.25	0.25	0.0	4.8	4
Chlorpromazine hydrochloride	2.4	2	5.7	5.6	1.8	4.5	93
Promethazine hydrochloride	2.9	2	24.5	25.0	−2.0	4.9	92
Imipramine hydrochloride	2.9	2	12.5	12.5	0.0	5.2	80
Procaine hydrochloride	2.8	2	19.6	20.0	−2.0	5.2	55
Thiamine hydrochloride	3.5	2	24.8	25.0	−0.8	3.4	86
Tetracaine hydrochloride	3.0	2	9.9	10.0	−1.0	6.1	25
Fysostigmine salicylate	2.8	2	0.98	1.0	−2.0	5.1	79
Promazine hydrochloride	2.8	2	24.6	25.0	−1.6	4.7	92
Fluorescein	2.5	1	199.8	200.0	−0.1	11.8	91
Ascorbate	2.0	1	101.1	100.0	1.1	–	86
Citrate	2.2	1	32.5	32.3	0.6	2.7	0
Magnesium sulphate	5.0	3	149.0	150.0	−0.7	–	0
Calcium chloride	5.8	3	37.1	36.8	1.0	1.2	0

the RSH and the UV absorption. It can be seen that the quantitative deviation is 2% or less, with one exception (adrenaline tartrate, 5.5%). In view of the accuracy and precision of the RF, only the adrenaline tartrate concentration differs significantly from the labelled concentration.

It must be stressed that one-point calibration can only be used when the zones are sufficiently large. To determine the minimum zone length for which the RF may be used, several amounts of the four-component test mixture were analysed. Fig. 3 shows the calibration graph for acetate. On the ordinate the factor $(ZL \cdot \mathrm{I})/F$ is plotted, so that the slope of the calibration line represents the RF. This slope amounts to 1.72 (regression coefficient 0.9999).

In Table IV the RFs are given for each amount injected (one-point calibration). A systematic deviation of the RF from the true value is encountered only with a 2.3-s zone length, an effect associated with differences in the front and rear zone boundary profiles[30]. The other components show similar deviations with zone lengths of 2.1 s for phosphate and 2.9 s for benzoic acid and citrate. The minimum zone length for which

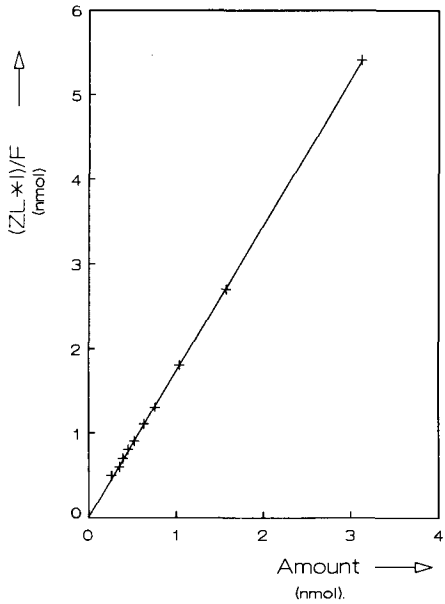


Fig. 3. Calibration graph for acetate. The slope represents RF. Slope = 1.72; intercept on ordinate = 0.0095 (nmol); $r = 0.9999$.

TABLE IV

RF OF ACETATE, MEASURED IN SYSTEM 1 (TABLE I), FOR DIFFERENT AMOUNTS INJECTED

Amount (nmol)	*Zone length (s)*	*RF*
3.12	26	1.73
1.57	13.1	1.73
1.04	8.5	1.70
0.78	6.5	1.73
0.63	5.3	1.75
0.52	4.4	1.74
0.45	3.7	1.71
0.39	3.3	1.74
0.35	2.9	1.71
0.26	2.3	1.84

the RF can be used, in a one-point calibration, is approximately 3 s if a 20-μA driving current is applied (*ca.* 1 mm in a 0.2-mm I.D. capillary).

CONCLUSIONS

It has been shown that capillary ITP is suitable for the determination of drugs in solution for intravenous injection. For the quantitative quality control of these

components the use of only one calibration point yields a maximum deviation of 2%. For each component a new calibration constant, the response factor (RF), is determined, which is independent of the diameter of the capillary, the construction of the universal detector and the current used during detection. The RF is an universal calibration constant, which is sufficiently accurate for routine analysis.

REFERENCES

1 J. Pospichalova and V. Jokl, *Pharmazie*, 42 (1987) 55.
2 I. Valka, J. Minarcik, J. Pumpria and D. Walterova, *Cesk. Farm.*, 35 (1986) 81.
3 S. Fanali, F. Foret and P. Boček, *J. Chromatogr.*, 330 (1985) 436.
4 H. Klein and R. Teichman, *Pharm. Ztg.*, 8 (1982) 447.
5 Y. Kasahara, H. Hikino and T. Hine, *J. Chromatogr.*, 324 (1985) 503.
6 A. Barcuchova and V. Jokl, *Cesk. Farm.*, 1 (1983) 12.
7 D. Walterova, Z. Stransky, V. Preininger and V. Simanek, *Acta Univ. Palacki. Olomuc., Fac. Med.*, (1985) 23.
8 D. Walterova, Z. Stransky, V. Preininger and V. Simanek, *Electrophoresis*, 6 (1985) 128.
9 D. Walterova, V. Preininger and V. Simanek, *Planta Med.*, 50 (1984) 149.
10 W. Schollack, M. Tkaczyk and J. Koerbl, *Zentralbl. Pharm. Pharmakother. Laboratoriumsdiagn.*, 126 (1987) 1.
11 R. Jannasch, *Pharmazie*, 41 (1986) 478.
12 J. Kostelecka and A. Haller, *Cesk. Farm.*, 36 (1987) 198.
13 H. Klein and R. Teichman, *Ernährung*, 10 (1986) 608.
14 P. Boček, in *Topics in Current Chemistry*, Springer, Berlin, Heidelberg, New York, 1981, p. 131.
15 H. Klein, *Arzneim.-Forsch/Drug. Res.*, 11 (1982) 795.
16 J. Cizmarik and Z. Stransky, *Collect. Czech. Chem. Commun.*, 51 (1986) 993.
17 E. Kenndler and R. Katzenberger, *Mikrochim. Acta*, I (1986) 415.
18 S. Fanali, M. Cristalli and P. Catellani, *J. Chromatogr.*, 405 (1987) 385.
19 S. Fanali, M. Sinibaldi and M. G. Quaglia, *J. Chromatogr.*, 408 (1987) 441.
20 H. Klein and R. Teichmann, *J. Chromatogr.*, 250 (1982) 152.
21 H. Miyazaki and K. Katoh, in K. Nobusawa (Editor), *Electrofocusing and Isotachophoresis*, Yoritsu Shuppan, Tokyo, 1978, p. 173.
22 F. M. Everaerts, M. Geurts, F. E. P. Mikkers and Th. P. E. M. Verheggen, *J. Chromatogr.*, 119 (1976) 129.
23 J. C. Reijenga, G. V. A. Aben, A. A. G. Lemmens, Th. P. E. M. Verheggen, C. H. M. M. De Bruin and F. M. Everaerts, *J. Chromatogr.*, 320 (1985) 245.
24 H. Klein and R. Teichmann, *Pharm. Ztg.*, 132 (1987) 1131.
25 R. Jannasch, *Pharmazie*, 41 (1986) 511.
26 N. Groot, *Graduation Report*, Eindhoven University of Technology, Eindhoven, 1980.
27 F. M. Everaerts, J. L. Beckers and Th. P. E. M. Verheggen, *Isotachophoresis—Theory, Instrumentation and Applications* (*Journal of Chromatography Library*, Vol. 6), Elsevier, Amsterdam, 1976.
28 B. J. Wanders, A. A. G. Lemmens, F. M. Everaerts and M. M. Gladdines, *J. Chromatogr.*, 470 (1989) 79.
29 F. M. Everaerts, Th. P. E. M. Verheggen, J. C. Reijenga, G. V. A. Aben, P. Gebauer and P. Boček, *J. Chromatogr.*, 320 (1985) 263.
30 J. C. Reijenga, A. A. G. Lemmens, Th. P. E. M. Verheggen and F. M. Everaerts, *J. Chromatogr.*, 320 (1985) 67.