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# Determination of anionic trace impurities in glycerol by capillary isotachophoresis with enlarged sample load

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**Abstract**

Glycerol of different quality classifications served as a model for a neutral excess component in the isotachophoretic determination of low-molecular-mass anionic trace impurities. Potential anionic contaminants such as nitrate, sulphate, chlorate, nitrite, oxalate, fluoride formate and phosphate were analysed up to an analyte-to-excess ratio of $1:4\cdot10^{7}$, thus providing the possibility of checking the sample for the mentioned analytes in the order of $2.5\cdot10^{-6}$–$9.5\cdot10^{-6}$%. Because we used a column-coupling isotachophoretic instrument the electrolyte system consisted of two different leading electrolytes, one for the pre-separation (10 mmol/l HCl, β-alanine, pH 3.2) in the first capillary and one for the final separation (5 mmol/l HCl, 1,3-bis[tris(hydroxymethyl)methylamino]propane, β-alanine, pH 3.6) in the second capillary. The terminating electrolyte was citric acid. Due to an increased injection volume of 300 μl, limits of detection (LODs) in the nanomolar range were realised by conductivity detection. The developed method allows simultaneous analysis without sample preparation and/or preconcentration within 25 min and is for that reason suitable for in-place process control. 

*Keywords:* Isotachophoresis; Impurities; Process control; Glycerol; Inorganic anions

## 1. Introduction

Glycerol plays an important role in the plastic, food and cosmetic industries, therefore there are different demands for a quality evaluation.

At present, only the inorganic ions chloride and sulphate are routinely analysed by turbidimetric methods [1–3]. The content of acid is monitored by titration [2,4]. Both methods, however, allow the determination of only one single species, or give as result a sum parameter without the chance to differentiate between individual acids. Moreover, the necessity of sample pre-treatment is a disadvantage, making the analysis time- and personnel-intensive.

The determination of ionic impurities in samples containing a large excess of one component is a fundamental analytical problem which is becoming more and more important also for separation techniques, as they have the benefit of a simultaneous, fast and sensitive detection.

Electrophoretic separation methods have an

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enormous advantage in that the ionic contaminants are extracted from the neutral matrix by the applied electric field. In contrast to chromatographic methods, the neutral matrix should remain at the injection position due to its non-existent electrophoretic mobility.

Capillary zone electrophoresis (CZE) normally operates with the electroosmotic flow (EOF) in the same direction as the analytes (co-EOF mode) [5,6]. In this situation, a disturbance of the separation occurs because the excess constituent is moved by the EOF towards the detector. The viscosity difference between sample and electrolyte and increased diffusion effects due to the high concentration gradient disturb the baseline and shift the migration times significantly [7]. Reversing the EOF results in a prolongation of the analysis time and leads to increasing dispersion effects with accompanying peak-broadening.

Another limiting factor in the application of CZE is the restricted injection volume, usually not higher than 1% of the total volume of the separation system [8,9]. This means that the injection volumes are in the range of 1–70 nl. Owing to the short optical path length, available for UV absorbance detection, typically 50–100 μm, the limits of detection (LODs) are more or less $10^{-6}$ mol/l [5,10–12].

The above mentioned restrictions impelled us to take into consideration another electrophoretic method: capillary isotachophoresis (ITP).

In ITP, the EOF is totally suppressed by the use of a hydrodynamically closed system, capillaries of inert polymers and suitable additives in the electrolyte system [13–16].

A column-coupling ITP instrument, introduced by Everaerts et al. [17], provides a possible solution with respect to high sample-load capacity and low limits of detection. By dividing the analysis into two stages (pre-separation and final separation) it enables a two-dimensional analysis without an appreciable increase in the analysis time.

ITP has so far been used for the determination of impurities in wet-process phosphoric acid [18] and in the products of peptide synthesis [19–22].

The purpose of this paper is to show the improvement of the LODs by more than one-order of magnitude compared to the standard set-up of the instrument and the application of the developed method to the determination of anionic trace impurities in glycerol which served as a model substance for neutral excess components.

## 2. Experimental

### 2.1. Instrumentation

The isotachophoretic instrument ItaChrom® EA 101 (Merck, Darmstadt, Germany) was used in the column-coupling mode. The first capillary made of fluorinated ethylene–propylene polymer (FEP) had a length of 16 cm with an I.D. of 800 μm, the second, consisting of the same material, also had a length of 16 cm but the I.D. was 300 μm. Both columns are provided with an on-column conductivity detector; additionally, the second column was equipped with a UV absorbance detector. The capillary tubes were placed in compartments made of Plexiglas, allowing heat dissipation produced on the passage of current.

Samples of 30 and 300 μl were injected with the aid of two different sample valves.

For data-evaluation and processing, ITP-WIN 2.18 software was used.

### 2.2. Chemicals

The following chemicals were used: hydrochloric acid 30% (Suprapur), citric acid (analytical-reagent grade), β-alanine (for biochemistry) all from Merck; 1, 3 - bis [tris (hydroxymethyl) methylamino] propane (BTP) (BioChemika) from Fluka (Buchs, Switzerland); purified methylhydroxyethylcellulose 1% stock solution from Comenius University (Bratislava, Slovak Republic). Anion standard solutions of nitrate, sulphate, fluoride and phosphate of 1000 mg/l were obtained from Merck; all other standard solutions were prepared from the sodium salts of the same concentration. All solutions, electrolytes and standards were produced using ultrapure water from a Seral PRO 90 C system (Seral, Ransbach-Baumbach, Germany) with a conductivity of 0.1 μS/cm.

## 3. Results and discussion

### 3.1. ITP electrolyte system

The absolute mobilities of the analytes of interest

are very close, showing only small mobility differences [23]. Owing to their chemical properties (some of them are strong acids), complete separation can not be achieved by optimisation of the pH only. Consequently, the composition of the leading electrolyte is such as to combine a suitable pH and the complexation properties of BTP in order to enhance the resolution [24,25].

Previously, we reported the separation of all mentioned anions at a pH of 3.6 and 2 mmol/l BTP added to the leading electrolyte [26].

As the absolute mobilities of the separants are high, a mobile terminating ion (citrate) could be employed also. The citrate ions were not only chosen for mobility reasons; furthermore the purity was sufficient not to give a blank value originating from this terminating ion. Such blank values, especially those deriving from the terminating ion, would complicate the interpretation of the analytical results.

It should be noted at this point that the cleanness of the electrolyte system and the separation unit is imperative. Due to the ubiquitous presence of some of the analytes, the use of high quality chemicals and freshly cleaned vessels is also a key of importance.

An isotachopherogram from an anionic standard solution and a blank run from the electrolyte system are shown in Fig. 1. The ITP system applied is listed in Table 1.

### *3.2. Sample-load capacity*

For the realisation of low LODs, undoubtedly, one possibility is to increase the injection volume. This procedure is, of course, limited to a defined amount of sample which can be resolved under the given conditions. The maximum amount of analytes which can be separated defines the separation capacity [27,28]. If the injection amount is further increased, the separation tube tends to be overloaded resulting in unresolved mixed zones. It is sometimes difficult to distinguish the existence of mixed zones, because the detector signals resemble those of the steady-state zones [29]. To avoid such underestimation, the zone length of the steps in isotachopherograms should be carefully checked by varying the amount of sample injected. We followed this procedure by recording calibration lines with successively increased concentrations of the analytes while using a constant injection volume of 300 μl. Apparently from Fig. 2, the relationship between the zone length and the concentration in the case of nitrate and sulphate, had a bend at 5 and 10 μmol/l, respective-

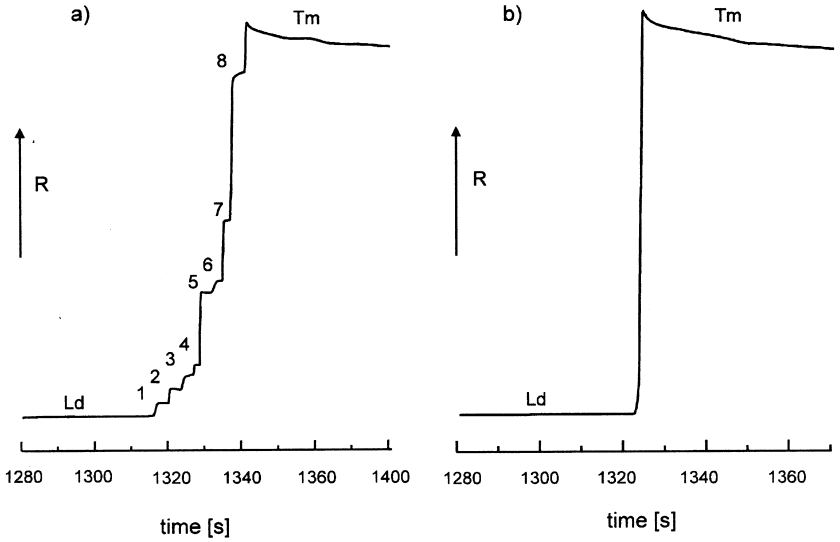


Fig. 1. (a) Isotachopherogram in the second capillary of the separation of a standard anion mixture with a concentration of 2 μmol/l of each anion. Operational system II was used. Injection: 300 μl; current: 280 μA (first capillary), 32 μA (second capillary). 1=Nitrate, 2=sulphate, 3=chlorate, 4=nitrite, 5=oxalate, 6=fluoride, 7=formate, 8=phosphate. Ld=Leading, Tm=terminating, R=resistance. (b) Isotachopherogram of a blank run (terminating injected as sample).

Table 1
Electrolyte systems applied

| | System I, 30 μl injection | | System II, 300 μl injection | | |
|---|---|---|---|---|---|
| | Leading | Terminating | First leading | Second leading | Terminating |
| Solvent | Water | Water | Water | Water | Water |
| Anion | Chloride | Citrate | Chloride | Chloride | Citrate |
| Concentration (mmol/l) | 8 | 5 | 10 | 5 | 5 |
| Counter-ion | β-Alanine | – | β-Alanine | β-Alanine | – |
| Co-counter ion | 2 mmol/l BTP | – | – | 2 mmol/l BTP | – |
| Additive | 0.1% HMEC | – | 0.1% HMEC | 0.1% HMEC | – |
| pH | 3.6 | 4 | 3.2 | 3.6 | 4 |

BTP = 1,3-bis[tris(hydroxymethyl)methylamino]propane; HMEC = hydroxymethylethylcellulose.

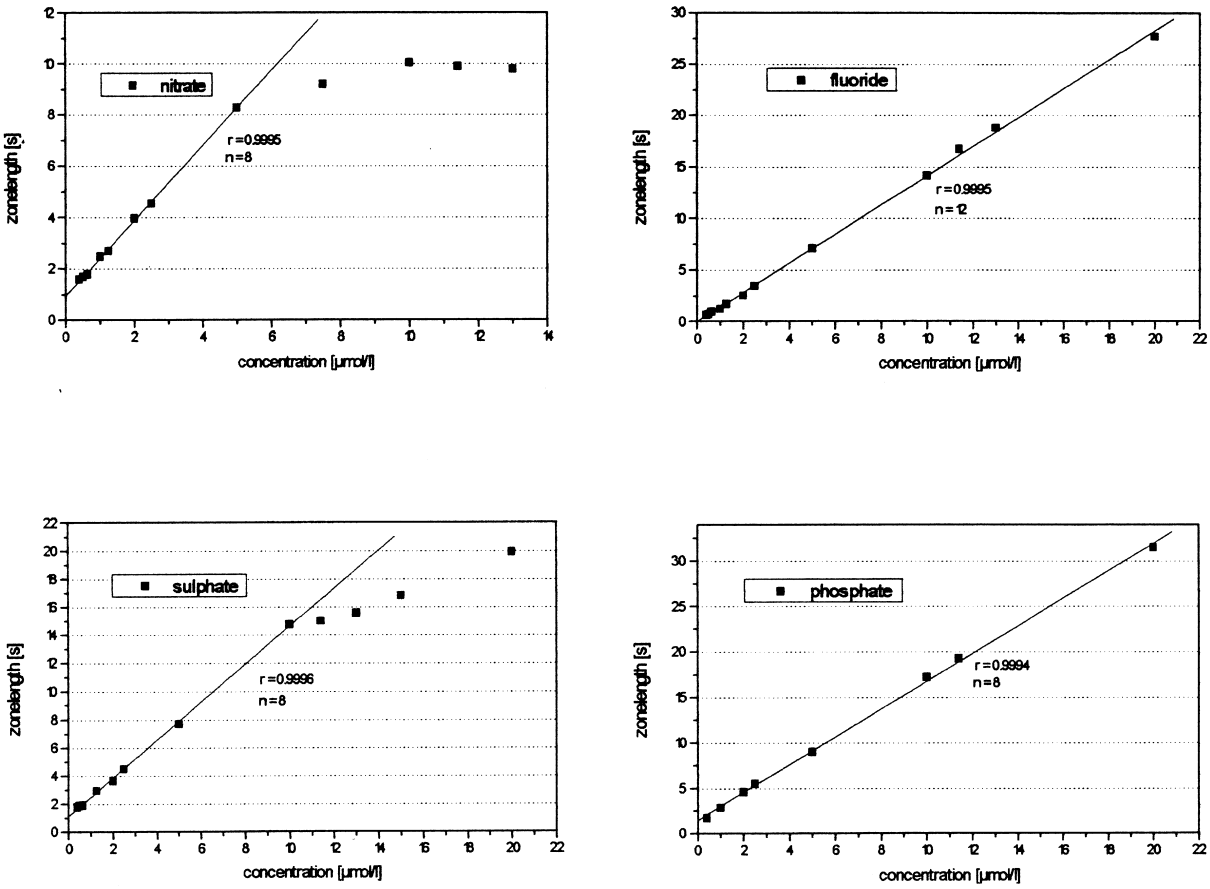


Fig. 2. Linear working range of selected ions with an injection volume of 300 μl. Conditions as in Fig. 1. $n$ = Number of calibration points used for the calibration line, $r$ = correlation coefficient.

ly. This is not surprising as this pair of analytes represents the separants with the smallest difference in the effective mobilities. In accordance with theory, resolution depends mainly on the difference in the effective mobility; therefore, column overloading is first indicated by the ions $NO_3^-$ and $SO_4^{2-}$ in the case described. In conclusion, we prescribed the maximum sample load of our system to the linear working range of each analyte (Fig. 2).

### 3.3. ITP performance parameters

A widespread calculation of the limit of detection, mainly used for chromatographic methods, is based on the work of Kaiser and Specker [30]. They suggest that a signal higher than the standard deviation of the background multiplied by a conventionally chosen factor (usually 3), should be considered as characteristic of a detectable amount of the analyte to be analysed. Actually, this concept is not applicable to ITP, operating with a discontinuous electrolyte system, inherent to the method. As quantitation is mostly carried out with the aid of calibration lines, we also estimated the LODs by considering the confidence limits of the linear calibration lines [31,32].

The LODs were calculated for the injection volume of 30 μl in the concentration range of 12.5–100 μmol/l and for the 300 μl injection with concentrations in the order of 0.4–5 μmol/l for each ion according to [33]:

$$\mathrm{LOD} = \frac{2t(P,\ f)x_{\mathrm{mean}}s/\sqrt{n_{\mathrm{i}}}}{y_{\mathrm{mean}} - a + t(P,\ f)s/\sqrt{n_{\mathrm{i}}}} \quad (1)$$

where: $t$ = Student's $t$ corresponding to $n-2$; $x_{\mathrm{mean}} = \Sigma x/n$; $y_{\mathrm{mean}} = \Sigma y/n$; $s$ = residual standard deviation; $n_{\mathrm{i}}$ = number of replicates of each measurement (2); $P$ = statistical probability (95%); $a$ = blank value and $n$ = number of calibration points.

Table 2 summarises the fixing of the LODs.

It is obvious that the LODs could be improved by a factor of 7–40 based on the enlargement of the injection volume by one-order of magnitude. On the one hand, this improvement is founded in the decrease of the concentration of the leading electrolyte, but on the other hand, the main reasons for the improvement in system II are: (i) a higher number of calibration points and (ii) a lower or equal residual standard deviation (except for phosphate).

Table 2
Results of the calibration of the anions with the second conductivity detector in the analytical capillary

| Anion | $n$ | Calibration equation | $r$ | $s$ (s) | $x_{\mathrm{mean}}$ (μmol/l) | $y_{\mathrm{mean}}$ (s) | LOD (μmol/l) |
|---|---|---|---|---|---|---|---|
| *System I, 30 μl injection* | | | | | | | |
| Nitrate | 5 | $y = 0.446 + 0.118x$ | 0.9993 | 0.1883 | 41.5 | 5.37 | 7.79 |
| Sulphate | 5 | $y = 1.324 + 0.189x$ | 0.9995 | 0.2383 | 41.5 | 9.18 | 6.07 |
| Chlorate | 5 | $y = -0.904 + 0.167x$ | 0.9994 | 0.2078 | 41.5 | 6.02 | 6.95 |
| Nitrite | 5 | $y = 0.157 + 0.097x$ | 0.9996 | 0.1080 | 41.5 | 4.18 | 5.33 |
| Fluoride | 4 | $y = -0.027 + 0.096x$ | 0.9996 | 0.1399 | 46.87 | 4.84 | 6.55 |
| Formate | 5 | $y = 0.273 + 0.096x$ | 0.9994 | 0.1369 | 41.5 | 4.27 | 6.92 |
| Phosphate | 5 | $y = 0.647 + 0.118x$ | 0.9998 | 0.0902 | 41.5 | 5.54 | 3.59 |
| *System II, 300 μl injection* | | | | | | | |
| Nitrate | 8 | $y = 0.959 + 1.465x$ | 0.9995 | 0.0768 | 1.659 | 3.390 | 0.192 |
| Sulphate | 7 | $y = 1.147 + 1.348x$ | 0.9992 | 0.0928 | 1.754 | 3.496 | 0.279 |
| Chlorate | 5 | $y = -0.319 + 1.813x$ | 0.9997 | 0.0925 | 2.125 | 2.435 | 0.346 |
| Nitrite | 6 | $y = 0.300 + 0.765x$ | 0.9985 | 0.0818 | 1.813 | 1.687 | 0.459 |
| Fluoride | 7 | $y = 0.007 + 1.407x$ | 0.9990 | 0.1091 | 1.610 | 2.275 | 0.265 |
| Formate | 6 | $y = 0.882 + 0.852x$ | 0.9991 | 0.1295 | 2.085 | 2.346 | 0.563 |
| Phosphate | 6 | $y = 1.271 + 1.407x$ | 0.9995 | 0.1980 | 3.483 | 6.822 | 0.502 |
| Oxalate | 6 | $y = 0.457 + 1.513x$ | 0.9996 | 0.0828 | 1.539 | 2.832 | 0.400 |

$n$ = Number of calibration points, calibration relates the step length in s ($y$) to the concentration ($x$) of the anion in μmol/l, $r$ = correlation coefficient, $s$ = residual standard deviation, $x_{\mathrm{mean}} = \Sigma x/n$, $y_{\mathrm{mean}} = \Sigma y/n$, LOD = limit of detection.

For the ions nitrate, sulphate and phosphate there is, according to the calibration equations in Table 2, a blank value of about 1 s marking the lower limit of the linear working range.

A further improvement of the LODs is therefore limited by this kind of chemical noise, reflected by the purity of the chemicals used and the surrounding environment.

### *3.4. Analysis of real samples*

Our experiments were aimed at the investigation of possible applications concerning the quality and process control of industrial chemicals. Glycerol was chosen as a model substance for the following reasons: (i) it is a very important chemical and raw material; (ii) it is a difficult matrix component with respect to its high viscosity and (iii) no separation method so far has been applied, especially for the determination of anionic impurities in the ppb range.

Operational system II was used for the complete anionic analysis of different batches of glycerol. We focused our investigations on the glycerol batches 100 and 87% ''extra pure'' and ''pro analysis''.

The purity of the electrolytes used as working system were checked before and after a series of analyses by running a blank with terminating electrolyte injected as sample.

Experimentally, we found that it is of advantage to dilute the glycerol samples 100 and 87% by factors of 2 and 1.5 with terminating electrolyte, respectively. Otherwise, the injection of undiluted glycerol leads to a too low conductivity for the ITP run. After dilution, the concentration of the two glycerol types (100 and 87%) was fixed to be 6.89 and 7.75 mol/l. These concentrations can be directly injected.

Taking into account the attainable limits of determination of the analytes (Table 3) one can calculate the ATER (analyte-to-excess ratio), this being in the range of $1:1\cdot10^{7}$–$1:4\cdot10^{7}$.

The limits of determination resulted from the accurate zone length measurement capability of the conductivity detector. We found that a zone length of 1 s is sufficient for a precise zone determination. The LODs for the ions nitrate, sulphate, formate and phosphate correspond to a zone length higher than 1 s, for this reason, the LOD is equal to the limit of determination. For the remaining analytes the limit of determination was adjusted to a zone length of 1 s.

Table 3 gives an impression of the resulting purity control values obtainable.

Fig. 3 shows representative isotachopherograms of different glycerol samples. It is obvious that the glycerol batches contained varying contents of low-molecular-mass impurities, namely; formate, phosphate and sulphate.

Identification of the impurities contained was executed by comparing the RSHs (relative step heights) in the sample with the RSHs in standard solutions and, for confirmation, by adding the appropriate ion to the sample. For quantification, the mean value of three independent measurements were put in the already recorded calibration equation.

Table 3
Limit of determination, ATER and the attainable purity control of the corresponding ions in glycerol

| Anion | Limit of determination | | ATER, glycerol | | Purity control, glycerol | |
|---|---|---|---|---|---|---|
| | (μmol/l) | (ppb) | 100% | 87% | 100% | 87% |
| Nitrate | 0.192 | 11.9 | $1:3.56\cdot10^{7}$ | $1:4.03\cdot10^{7}$ | $2.8\cdot10^{-6}$% | $2.5\cdot10^{-6}$% |
| Sulphate | 0.279 | 26.8 | $1:2.45\cdot10^{7}$ | $1:2.77\cdot10^{7}$ | $4.1\cdot10^{-6}$% | $3.6\cdot10^{-6}$% |
| Chlorate | 0.616 | 51.4 | $1:1.11\cdot10^{7}$ | $1:1.25\cdot10^{7}$ | $9.0\cdot10^{-6}$% | $8.0\cdot10^{-6}$% |
| Nitrite | 0.653 | 30.0 | $1:1.05\cdot10^{7}$ | $1:1.18\cdot10^{7}$ | $9.5\cdot10^{-6}$% | $8.5\cdot10^{-6}$% |
| Fluoride | 0.420 | 8.00 | $1:1.62\cdot10^{7}$ | $1:1.84\cdot10^{7}$ | $6.2\cdot10^{-6}$% | $5.4\cdot10^{-6}$% |
| Formate | 0.563 | 25.3 | $1:1.36\cdot10^{7}$ | $1:1.54\cdot10^{7}$ | $7.2\cdot10^{-6}$% | $6.5\cdot10^{-6}$% |
| Phosphate | 0.502 | 47.6 | $1:1.21\cdot10^{7}$ | $1:1.37\cdot10^{7}$ | $8.3\cdot10^{-6}$% | $7.3\cdot10^{-6}$% |
| Oxalate | 0.400 | 35.2 | $1:1.71\cdot10^{7}$ | $1:1.71\cdot10^{7}$ | $5.8\cdot10^{-6}$% | $5.2\cdot10^{-6}$% |

ATER = Analyte-to-excess ratio; ppb = parts per billion (μg/kg).

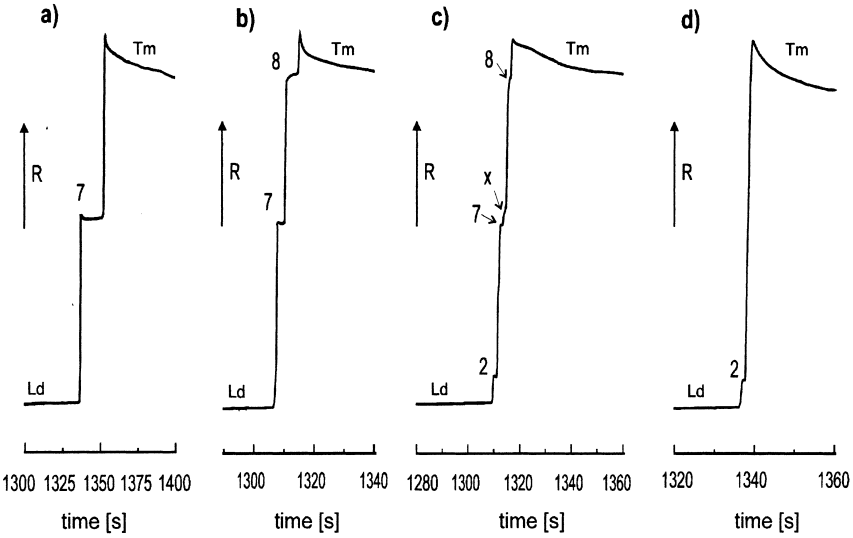


Fig. 3. Isotachopherograms of different batches of glycerol. (a) 87% 'extra pure', 7=formate $3.1\cdot10^{-4}$%, (b) 100% 'extra pure', 7=formate $5.9\cdot10^{-5}$%, 8=phosphate $6.3\cdot10^{-6}$%, (c) 87% 'pro analysis', 2=sulphate $4.8\cdot10^{-6}$%, 7=formate $7.8\cdot10^{-6}$%, ×=not identified, 8=phosphate below $7.3\cdot10^{-6}$%, (d) 100% 'pro analysis', 2=sulphate below $4.1\cdot10^{-6}$%. Conditions as in Fig. 1. Ld=Leading, Tm=terminating, R=resistance.

The quality classifications are easy to distinguish; moreover, it can be seen that glycerol 87% has a higher impurity content than the glycerol 100%.

The phosphate ion in Fig. 3c and the sulphate ion in Fig. 3d were only recorded qualitatively, both corresponding average zone lengths (1.7 and 1.22 s) were below the linear working range determined by the calibration equation (Table 2); therefore, a content lower than the blank value is stated.

In the case of the glycerol 100% ''extra pure'', we examined the precision for the determination of phosphate and formate by injecting the sample six times. The resulting standard deviations were 0.057 and 0.076 s, giving R.S.D.s of 2.4 and 3.8%, respectively.

With respect to the classical turbidimetric determination which is executed for the ions sulphate and chloride in the range down to $1\cdot10^{-4}$–$5\cdot10^{-4}$% [2], it should be emphasised that the ITP separation offers the possibility of analysing anions in a significantly lower concentration range (Table 3).

Additionally, it should be mentioned that chloride cannot be detected in that low concentration range in our system. Nevertheless, the ITP determination offers the further advantage of a simultaneous and fast determination of all mentioned analytes without sample preparation.

## 4. Conclusions

It has been shown that ITP provides a powerful tool for the analysis of low-molecular-mass impurities in glycerol. In dependence of the analyte concentration in the sample, the injection volume could be increased by a factor of 10; in this way, LODs in the nanomolar range were achieved.

Hence, glycerol could be checked for the content of contaminants in the order of $2.5\cdot10^{-6}$–$9.5\cdot10^{-6}$%, which is beyond the ability of turbidimetric measurements.

It can be assumed that the described method can be transferred to nearly all neutral matrix components.

Due to the fact that no sample preparation is needed and analytical results are obtained within 25

min, ITP is suitable for in-place quality and process control.

Additionally, regarding accuracy and long-term reliability, further experiments are in progress.

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