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PHOTOMETRIC DETECTION OF METAL CATIONS IN CAPILLARY ISO-TACHOPHORESIS BASED ON COMPLEX EQUILIBRIA

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SUMMARY

The possibilities of photometric detection of metal cations based on the formation of kinetically labile, light-absorbing complexes (chelates) during isotachophoretic (ITP) separation were studied. Xylenol orange was used as a chelating co-counter ionic constituent in the cationic mode of ITP. The metal cations formed, under the working conditions employed, light-absorbing chelates with this chelating agent and could be detected with high selectivity at 580 nm when their cationic migration was preserved.

Bleeding of the analyte zones caused by xylenol orange and probably also by chelating impurities present in its precursors were the most serious sources of systematic errors, especially at the parts per 10⁹ concentration level. At such concentrations, adsorption of the metal cations in the separation compartment also appeared important. Means of minimizing these losses of the analytes were studied. Under optimized ITP working conditions Mn²⁺ and Cd²⁺ had detection limits of ca. 10⁻⁸ mol/l for a 30-μl sample volume. These values represent almost a three orders of magnitude improvement relative to current high-resolution universal detectors. Consequently, the metals could be reliably determined at concentrations lower than 5·10⁻⁸ mol/l.

INTRODUCTION

Complex equilibria are currently employed to optimize selectively the separation conditions in the analysis of metal cations by capillary isotachophoresis (ITP). Various

* This paper is dedicated to the memory of Professor Samo Stankoviansky on the occasion of his 80th birthday.
complexing agents, mostly in the cationic mode of analysis, have been studied in this respect and a key role of these equilibria in the ITP analysis of some important groups of metals is apparent (see, e.g., refs. 1–18). However, the overall selectivity of the analysis may be low as universal, conductivity and potential gradient detectors are almost exclusively used. Indirect photometric detection has been proposed for these analytes\(^{19,20}\) but has similar disadvantages. In addition, these detection alternatives may not be sensitive enough when trace determinations are required. In spite of the fact that these disadvantages can be reduced by using a suitable sample preparation procedure before the ITP analysis\(^9,17\), developments aimed at improving the detection capability of ITP for metals are desirable.

In the determination of metals, their conversion into visible light-absorbing complexes (usually chelates) is the basis of the physico-chemical principles of various titration\(^{21–24}\) and spectrophotometric\(^{24–27}\) methods. Many of the chelating agents used for these purposes are ionic and their complexes with the metal cations are kinetically labile. In this work we performed an introductory study devoted to the use of these reactions in the photometric detection of metal cations in ITP. The detection approach is based on the following idea.

A chelating agent migrating anionically in the cationic mode of ITP and partially forming kinetically labile, light-absorbing chelates with the metal cations of analytical interest is added to the leading electrolyte. The working conditions (pH of the leading electrolyte and the concentration of the chelating agent) are chosen in such a way that the cationic migration of the analytes is preserved. In the choice of the chelating agent it is also important that the light absorption spectra of its free ionic forms prevailing under the ITP working conditions differ sufficiently from those of the metal chelates. Then, on-column photometric detection carried out at a wavelength from that part of the absorption spectra of the chelates where the light absorption by the free chelating agent is negligible provides the desired analytical information. Thus, the metal ions are detected via their light-absorbing chelates formed during the migration. It is apparent that in this instance the detection signal is proportional to the concentration of the metal ions present in the chelates and hence this is a direct mode of photometric detection.

Some of the chelating agents suitable for this detection approach are probably also applicable for work in the anionic mode (see e.g., refs. 14–18). Although in this way we could expect a higher sensitivity of detection (complete conversion of the metal cations into chelates), small differences in the effective mobilities of the chelates and/or their decomposition (bleeding) during the separation\(^{15,16}\) can considerably restrict its practical utility.

In this paper we present the results of feasibility study in which xylenol orange was used for the photometric detection of some metals (\(\text{Mn}^{2+}, \text{Cd}^{2+}, \text{Zn}^{2+}\) and \(\text{Pb}^{2+}\)) in the cationic mode of the ITP.

**EXPERIMENTAL**

**Instrumentation**

A CS Isotachophoretic Analyzer (VVZ PJT, Spišská Nová Ves, Czechoslovakia) was used in the column-coupling configuration of the separation unit. The analytical column of the analyser was provided with a laboratory-made on-column photometric
detector. The detection cell was placed ca. 2 cm upstream of the conductivity sensor. An LQ 1411 light-emitting diode (Tesla, Rožnov, Czechoslovakia) was employed as the monochromatic light source of the detector as its emission maximum at 580 nm agreed well with the light absorption maxima of the metal chelates. A KPX 81 phototransistor (Tesla) served as the photosensing element of the detector.

A Specord UV–VIS spectrophotometer (Carl Zeiss, Jena, G.D.R.) was used in the measurement of the absorption spectra of the chelating agents and the corresponding chelates in the preliminary choice of the agents for the ITP experiments.

**Chemicals**

The chemicals used for the preparation of the leading and terminating electrolytes were obtained from Serva (Heidelberg, F.R.G.), Sigma (St. Louis, MO, U.S.A.) and Lachema (Brno, Czechoslovakia). Some were purified by conventional methods. Hydroxyethylcellulose 4000 (Serva), after purification on an Amberlite MB-1 mixed-bed ion exchanger (BDH, Poole, U.K.), was used as an additive to the leading electrolyte.

3,3'-Bis[N,N-di(carboxymethyl)aminomethyl]-o-creosolsulphonphthalein (xylenol orange), 3,3'-bis[N-(carboxymethyl)aminomethyl]thymolsulphonphthalein (glycine thymol blue), 5,5'-nitrilodibarbituric acid (murexide) and 2-hydroxy-1-(1-hydroxynaphthyl-2-azo)-6-nitronaphthalene-4-sulphonic acid (Eriochrome Black T) were obtained in indicator-grade purities from Lachema and Merck (Darmstadt, F.R.G.). Their Na⁺ and NH₄⁺ salts were converted before the use into the free acids on a column packed with Dowex 50W-X8 (H⁺) cation exchanger (Serva). Of these chelating agents only xylenol orange was stored as a 10⁻² mol/l stock solution.

Stock solutions of metal cations (10⁻² mol/l) were prepared from their analytical-reagent grade salts obtained from Lachema and Merck.

Water delivered by a Rodem-1 two-stage demineralization unit (OPP, Tišnov, Czechoslovakia) was further purified by circulation through laboratory-made polytetrafluoroethylene (PTFE) cartridges packed with a mixed-bed ion exchanger (Amberlite MB-1; BDH). The solutions were prepared from freshly recirculated water.

**Sample and solution handling**

To avoid problems due to contamination of the solutions with heavy metals and/or to minimize the losses of the analytes, the following precautions were taken: (i) The solutions (leading and terminating electrolytes, sample solutions containing the metal cations at concentrations of 10⁻⁴ mol/l and less) were stored in polyethylene or quartz vessels cleaned as recommended in the literature (see, e.g., refs. 29 and 30). (ii) In the work on the concentration level of the analytes at 10⁻⁵ mol/l and less, only the samples prepared immediately before a series of the experiments were used. (iii) The samples were transferred into the injection valve of the analyser with polyethylene disposable syringes. The syringes were cleaned before use by storing them for several days in 1 mol/l nitric acid. They were washed with demineralized water immediately before use. (iv) Those parts of the injection valve of the analyser which came into contact with the solutions were made of PTFE. In addition, we employed a terminating compartment made of PTFE and any contact of the terminating electrolyte solution present in this compartment with the laboratory environment was avoided.
RESULTS AND DISCUSSION

Choice of the chelating agent

As is clear from the previous description of the detection principle, the choice of the chelating agent had to follow requirements important for both the separation of metal cations and their detection. Obviously, general requirements concerning the constituents used for the preparation of the leading electrolyte solutions in ITP also had to be considered. In summary, the chelating agent suitable for our detection purposes should fulfill the following criteria: kinetic lability of the complexes formed during the migration; desired separating effect or no disturbances to the separation when other constituent(s) is (are) responsible for the resolution of the separands; its use must not introduce a systematic bias into the quantitation due to zone bleeding; different light absorption spectra of the chelate and the free chelating agent; high molar absorbptivities of the metal chelates at the detection wavelength; chemical stability; and availability in a sufficient degree of purity.

As our experiments were intended to study the detection of metals we did not evaluate in detail the separating capabilities of the chelating agents used in the investigation. Obviously, their influence on the ITP separations of metal cations cannot be neglected (also at low concentrations of the agents) when the corresponding values of the stability constants are taken into consideration (refs. 21, 33 and references cited therein).

Among the group of chelating agents potentially applicable for our detection purposes, we preferred metallochromic indicators as their complexes are usually kinetically labile. Our preliminary choice was based on measurements of the absorption spectra in a wide pH range. In this way we obtained relevant data for the indicators, i.e., their pH working range and optimum detection wavelength for ITP. The chemical purities of the indicators used in further investigations were evaluated via their ITP anionic profiles. In general, the profiles showed low chemical purities of the available preparations (see, e.g., Fig. 1). The use of simple purification procedures as recommended did not lead to improvements in this respect. Therefore, we had to take into account that mixtures of chelating agents (probably with different chelating properties) rather than individual agents were employed in our experiments.

The low chemical stabilities of some indicators (murexide, Eriochrome Black T) further restricted our choice. This undesirable property of the indicators led us to evaluate regularly the actual content of the chelating agent(s) present in the leading electrolyte. Ba²⁺ served for this purpose and the amplitude of the signal of the photometric detector for its zone was taken as a measure of the concentration of the indicator present in the leading electrolyte.

Of the metallochromic indicators studied we chose xylene orange for a detailed investigation because, with the exception of its lower chemical purity, it best met the remainder of the above criteria. Its light absorption maximum was at 445 nm (under experimental conditions close to those employed in ITP) whereas the maxima for the metal chelates ranged from 570 to 580 nm. In addition, at the detection wavelength (580 nm) the contribution of the free chelating agent to the light absorption was negligible.

The composition of the leading electrolyte is given in Table I. Here, both the concentration of xylene orange and pH were chosen to illustrate the analytical possibilities of the detection method and problems encountered in its use.
Fig. 1. Anionic profile of xylenol orange at pH\textsubscript{L} = 3.5. 10 mM HCl buffered to pH\textsubscript{L} = 3.5 with β-alanine was used as the leading electrolyte [HEC at 0.1% (w/v) concentration served as the additive]. 5 mM acetic acid was used as the terminating electrolyte. A 30-pl volume of the leading electrolyte solution used for the separation of metals (Table I) containing 1.5 mM xylenol orange was injected. The isotachopherograms from (a) conductivity and (b) photometric detectors were recorded at a 45-μA driving current. \( R, A \) = increasing resistance and light absorption at 580 nm, respectively.

<table>
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<th>Parameter</th>
<th>Electrolyte</th>
<th>Leading</th>
<th>Terminating</th>
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</thead>
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<td>( \text{H}_2\text{O} )</td>
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</tr>
<tr>
<td>Cation</td>
<td>( \text{NH}_4^+ )</td>
<td>( \text{H}^+ )</td>
<td></td>
</tr>
<tr>
<td>Concentration (mM)</td>
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<td>1</td>
<td></td>
</tr>
<tr>
<td>Counter ion</td>
<td>( \text{OAc}^- )</td>
<td>( \text{NO}_3^- )</td>
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</tr>
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<td>Co-counter ion</td>
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<td></td>
</tr>
<tr>
<td>Concentration (mM)</td>
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<td>–</td>
<td></td>
</tr>
<tr>
<td>pH</td>
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<td>\text{ca.} 3.0</td>
<td></td>
</tr>
<tr>
<td>Additive</td>
<td>HEC\textsuperscript{a}</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Concentration (%, w/v)</td>
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<td>–</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} OAc = Acetate; XO = xylenol orange; HEC = hydroxyethylcellulose.
Migration behaviour of the metal cations and their photometric detection with xylenol orange

The isotachopherograms in Fig. 2 were obtained from the analysis of a model mixture containing Mn$^{2+}$, Zn$^{2+}$, Cd$^{2+}$ and Pb$^{2+}$ at $10^{-4}$ mol/l concentrations. It can be seen that for the cations that form light-absorbing chelates with xylenol orange the expected responses were obtained. It is also apparent that their migration order followed the stabilities of the chelates (Mn$^{2+}<\text{Cd}^{2+}<\text{Zn}^{2+}<\text{Pb}^{2+}$). Pb$^{2+}$ formed to a great extent PbHL$_3^{-}$ species (HL represents the singly protonated ionic form of the chelating agent) with xylenol orange at pH values close to our pH$_L$ (ref. 27, p. 292) and was retarded into the terminating zone. As the decomposition of the Pb chelate in this zone was not sufficient to achieve a higher effective mobility of Pb relative to that of the H$^+$ ion (owing to a lower pH and a lower steady-state concentration of the free chelating agent), the Pb injected was completely lost during the separation. Such behaviour of the metal cations must be considered when the complex equilibria are involved in the ITP separation$^{31,32}$. In this particular instance we investigated the possibility of eliminating these losses of Pb by the use of a competitive metal cation. Cu$^{2+}$ was tested for this purpose, with positive results. Unfortunately, the concentration of Cu$^{2+}$ that had to be added to the sample was critical. A concentration of Cu$^{2+}$ below a very narrow optimum concentration range only partly prevented the

![Fig. 2. ITP separation of Mn$^{2+}$, Cd$^{2+}$, Zn$^{2+}$ and Pb$^{2+}$ with xylenol orange used as a chelating co-counterion. A 30-μl volume of the injected sample contained the metal cations at $10^{-4}$ mol/l concentrations. The isotachopherograms from (a) conductivity and (b) photometric detectors were recorded at a 100-μA driving current. Composition of the operational system as in Table 1 and symbols as in Fig. 1.](image-url)
losses of Pb. On the other hand, at a higher Cu\textsuperscript{2+} concentration the detection of Pb\textsuperscript{2+} was disturbed by a strongly light-absorbing copper chelate. As such a solution was impractical for use in the detection of very low concentrations of the metals it was not studied further.

Organic bases, cationically migrating amino acids, tetraalkylammonium cations and alkali metal cations were considered as spacing constituents. Here, unexpected migration behaviours of the metal cations were observed. The isotachopherogram in Fig. 3 serves as an illustration of this behaviour. It can be seen that Tris (having a lower effective mobility than Zn and Cd) not only spaces the Mn zone but also acts as a carrier for the injected Zn and Cd. Moreover, these metal constituents, spread along the rear part of the Tris zone, were mutually separated by a sharp boundary. The corresponding response from the conductivity detector also indicates that part of the Tris zone occupied by the metals and suggests a higher resistance. However, when the measuring electrodes were cleaned electrochemically\textsuperscript{1} this enhanced response disappeared. Hence it can be ascribed to a higher sensitivity of the coated measuring electrodes to the multiple charged ions\textsuperscript{1,34}. Further, the observed migration behaviours of Zn and Cd can again be explained in terms of the bleeding of the zones of metals into the Tris zone. At the same time, competition of the metal ions for the ligand explains their resolution in this zone. These results also suggest that in the ITP analysis

Fig. 3. Carrying and spacing effects of Tris in the separation of metal cations. A 30-\mu l volume of the injected sample contained Tris (5 \cdot 10^{-4} \text{ mol/l}), Mn\textsuperscript{2+} (10^{-4} \text{ mol/l}), Cd\textsuperscript{2+} (10^{-4} \text{ mol/l}), Pb\textsuperscript{2+} (10^{-4} \text{ mol/l}) and Zn\textsuperscript{2+} (5 \cdot 10^{-2} \text{ mol/l}). ITP working conditions and symbols as in Fig. 2. The length of the Tris zone increased by ca. 5\% in comparison with the run without metal cations.
of metals present in samples containing non-complexing cationically migrating constituents the choice of the complexing agent must consider this behaviour of the metal ions also in instances when their bleeding into the terminating zone does not occur\textsuperscript{31,32}.

We further investigated in detail the migration behaviours of Cd and Zn in multi-component mixtures of non-complexing cations. In these experiments the concentrations of Zn\textsuperscript{2+} and Cd\textsuperscript{2+} in the samples were varied while the concentrations of the non-complexing cations were kept constant. Isotachopherograms from such experiments for Zn are shown in Fig. 4. The isotachopherograms show that Zn was retarded by xylenol orange into the rear part of the BALA zone (the chelate was decomposed at the front of the terminating zone) when its concentration in the sample was 10\textsuperscript{-5} mol/l (Fig. 4b). At a 5-fold higher concentration Zn was spread along the zones of BALA and EACA and along the rear part of the Tris zone (Fig. 4c) whereas at a 1.5 \cdot 10\textsuperscript{-4} mol/l concentration it was present along all of these zones and also, partially, in its own zone characterized by the same effective mobility as in Fig. 2. These isotachopherograms suggest that in each of the zones of non-complexing constituents the degree of complexation of Zn\textsuperscript{2+} was different and decreased stepwise in the direction of the terminating zone. This is an expected agreement with a decrease in the pH of the zones and with a decrease in the concentration of the chelating co-counter ion. It is also apparent that each of the zones of non-complexing constituents (migrating behind the zone of the metal) had, therefore, a certain carrying capacity for Zn under given working conditions. This carrying capacity will be zero for the zone in which the chelate is decomposed to such an extent that the effective mobility of the

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**Fig. 4.** Migration behaviour of Zn present in a multi-component mixture of non-complexing cationic constituents. A blank solution (No. 1; Table II) (see the isotachopherogram) (a) was spiked with Zn at (b) 10\textsuperscript{-5} mol/l, (c) 5 \cdot 10\textsuperscript{-5} mol/l and (d) 15 \cdot 10\textsuperscript{-5} mol/l concentrations. 1 = Ba\textsuperscript{2+}; 2 = Na\textsuperscript{+}; 3 = TMA\textsuperscript{+}; 4 = Li\textsuperscript{+}; 5 = Tris; 6 = EACA; 7 = BALA. Zn/BALA, Zn/EACA, Zn/Tris and Zn\textsuperscript{*} indicate Zn present in the zones of BALA, EACA, Tris and in its own zone, respectively. The isotachopherograms were recorded at a 100-\mu A driving current. Composition of the operational system as in Table I and symbols as in Fig. 1.
metal is higher than that of the constituent forming this zone. The terminating constituent acts in this way for Zn. On the other hand, when the areas of the signals obtained for different concentrations of Zn are compared, some loss of this cation is clear. Bleeding of Zn into the terminating zone is a possible explanation. The losses of the metal were much lower than for Pb. They could be detected only with difficulty with the aid of the photometric detector because Zn$^{2+}$ was present as an impurity in

Fig. 5. Migration behaviour of Cd present in a multi-component mixture of non-complexing cationic constituents. The composition of the blank solution (a) was the same as in Fig. 4 except Zn$^{2+}$ was added at $2.5 \times 10^{-6}$ mol/l concentration. Cd$^{2+}$ was present in the injected samples at $10^{-6}$, $5 \times 10^{-6}$, $2 \times 10^{-5}$ and $8 \times 10^{-5}$ mol/in runs b, c, d and e, respectively. Isotachopherograms from (A) photometric and (B) conductivity detectors. Other details as in Fig. 4.
the chemicals used for the preparation of the leading electrolytes. Consequently, the losses of the injected Zn\(^{2+}\) could not be distinguished from the fluctuations of the signal due to the varying amount of the impurity present in the separation compartment.

Cd\(^{2+}\) forms with xylenol orange a chelate with a lower stability constant than those of Pb\(^{2+}\) and Zn\(^{2+}\). Therefore, its decomposition was detected at the front of the EACA zone (Fig. 5). It can be seen that either BALA or EACA can be used to space the migrating positions of Zn and Cd. In terms of the previous discussion, this is due to the fact that both amino acids act as carriers for Zn and the fronts of their zones decompose the Cd chelate.

When our detection approach was employed for the concentrations of metals below 10\(^{-6}\) mol/l their losses were typical. The isotachopherograms in Fig. 6 show such a situation. At this concentration level of the analytes we found that Ba\(^{2+}\) and Li\(^{+}\) had a positive effect in eliminating these losses. In this respect comparisons of the isotachopherograms b, d and e in Fig. 6 are illustrative. The cations were originally added to the samples as a spacer (Li\(^{+}\)) and as an internal standard (Ba\(^{2+}\)) for the evaluation of the concentration of the chelating agent. Nevertheless, these results suggest that bleeding of the analytes may not be the only cause of their losses. In spite of the fact that in this particular instance there is no experimentally based evidence, it seems appropriate to expect that adsorption phenomena in the separation compartment can also be important at such sample concentrations. Then, competition of Ba\(^{2+}\) and, partially, also Li\(^{+}\) with the analytes for the adsorption sites can provide an explanation of the positive effects of these cations.

When the isotachopherograms in Fig. 6b and c are compared it can be seen that an increase in the concentration of Zn\(^{2+}\) in the sample (the Zn stock solution was

![Fig. 6. Influence of Ba\(^{2+}\) and Li\(^{+}\) on the detectability of Mn\(^{2+}\) and Cd\(^{2+}\) at low ppb concentrations. The isotachopherograms from the photometric detector were obtained for 30-µl volumes of samples of the following compositions: (a) blank solution No. 1 (Table II); (b) same as (a) but with Cd\(^{2+}\), Mn\(^{2+}\) and Zn\(^{2+}\) added at 2 \(\times\) 10\(^{-7}\) mol/l; (c) same as (b) but the concentration of Zn was 8 \(\times\) 10\(^{-6}\) mol/l; (d) and (e) sample compositions as in (b) but the blank solution without Ba\(^{2+}\) and Li\(^{+}\), respectively, were used. Other details as in Fig. 4.](image-url)
photometric detection of metal cations in ITP proved to be free of Cd) led to a higher signal for a $2 \cdot 10^{-7}$ mol/l concentration of Cd$^{2+}$. This result implies that in the run shown in Fig. 6b part of the Cd was present along the EACA zone (and to a smaller extent also in the BALA zone) and/or adsorbed in the separation compartment. Then, on increasing the concentration of Zn$^{2+}$ in the sample (Fig. 6c) it was displaced into its migration position. This displacement effect of Zn can be ascribed either to its preferential trapping on the adsorption sites or to its higher complexing ability, leading (via competition with Cd$^{2+}$ for the ligand) to a higher effective mobility of Cd in the zones of non-complexing constituents.

From the previous discussion it is apparent that at the concentrations of the metal cations detectable by the conductivity detector, the bleeding of their zones [either to the terminating zone or to the zone(s) of non-complexing cation(s)] is potentially the most serious source of the analytical errors. At parts per $10^9$ (ppb) concentrations the losses cannot be explained only in this way. Adsorption of the metal cations in the separation compartment (hardly detectable by the conductivity detector) could provide a reasonable explanation in our particular case. However, uncertainty concerning the chelating properties of the impurities present in the precursors of xylenol orange used in these experiments (see Fig. 1) means that such an interpretation must be also treated with caution. This discussion implies that a further study with xylenol orange of a higher purity could be helpful in clarifying these results.

**Detection limits and quantitative analysis**

The above results clearly show that our approach to the photometric detection of metal cations provides considerably lower detection limits than current alternatives. However, to exploit its potential in trace ITP analysis it is necessary to minimize system errors due to the losses of the analytes during the ITP separation. In spite of the fact that origin of these losses is not yet exactly known, the experiments performed at concentrations of the analytes below $10^{-6}$ mol/l suggest that one way to solve this problem is to add suitable cationic constituents, such as Ba$^{2+}$, to the sample and to compensate for the losses of the analytes associated with the use of non-complexing spacing (carrying) constituents by adding the corresponding amounts of the metal cations to the sample.

In determining the detection limits for Mn$^{2+}$ and Cd$^{2+}$ we followed this solution, obtaining the values from fluctuations of the heights of the Mn and Cd peaks in the blank runs (for the composition of the blank sample, see the legend to Fig. 7 and Table II). The fluctuations expressed via the standard deviations of the peak heights corresponding to ca. $5 \cdot 10^{-9}$ mol/l of these cations. For a signal-to-noise ratio of 2 this gives detection limits of ca. $10^{-8}$ mol/l under the experimental conditions (30-μl sample volume, operational system as described in Table I and detection at 580 nm). For both metals this is an improvement of approximately three orders of magnitude compared with the detection limits given by a conductivity detector. The isotachopherograms at $2.5 \cdot 10^{-8}$ and $5 \cdot 10^{-8}$ mol/l Cd$^{2+}$ and Mn$^{2+}$ (Fig. 7b and c, respectively) illustrate their detectabilities at concentrations close to the detection limits. At this concentration level the relative standard deviations of the peak heights were in the range 10–35% (higher values being obtained for the lower concentrations).

For Zn$^{2+}$ the detection limit was lower than those for Mn$^{2+}$ and Cd$^{2+}$. However, problems caused by relatively high concentrations of this cation in the chemicals used for the preparation of the leading electrolyte solutions (also after their purification) made this value unreliable.
Fig. 7. Isotachopherograms from the analyses of Mn$^{2+}$ and Cd$^{2+}$ at concentrations close to the detection limits. (a) Obtained with blank solution No. II (Table II) and serves as a reference; (b) blank solution was spiked with $2.5 \cdot 10^{-8}$ mol/l of Mn$^{2+}$ (1.4 ppb) and Cd$^{2+}$ (2.8 ppb); (c) as (b) but with double the amounts spiked.

**TABLE II**

COMPOSITIONS OF THE BLANK SOLUTIONS

<table>
<thead>
<tr>
<th>Component$^a$</th>
<th>Blank solution$^b$</th>
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<tr>
<td>Ba$^{2+}$</td>
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<tr>
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</tbody>
</table>

$^a$ TMA$^+$ = Tetramethylammonium; Tris = tris(hydroxymethyl)aminomethane; EACA = e-aminocaproic acid; BALA = β-alanine.

$^b$ Constituent concentrations are given in mol/l.
The calibration lines for Mn$^{2+}$ and Cd$^{2+}$ were evaluated from their peak heights in the concentration range $5 \times 10^{-8}$ to $50 \times 10^{-8}$ mol/l (see Table III). It must be stressed that the slopes of the calibration lines obtained in this way depended on the spacing (carrying) constituents employed. This is obvious when it is considered that the quantified metal cations migrated in the interzonal boundaries. Here, both the pH at which the chelates were formed and the steady-state concentrations of the chelating agent, and hence the degree of chelate formation, were partially determined by the spacing constituent used.

From the migration behaviours of the metal cations (Figs. 4 and 5) it can be seen that determinations based on the peak height (peak area) measurement are a priori restricted to a certain concentration range of the analyte for a given composition of the leading electrolyte. An increase in the concentration of the chelating agent in the leading electrolyte solution is effective in increasing this range only to a limited extent as the effective mobility of the metal is also influenced in this way. Hence an undesirable change in its migration position can result when the concentration of the chelating agent is higher than a certain critical value. Therefore, once a leading electrolyte of an optimum composition is employed at a certain concentration of the analyte, only the area corresponding to the signal from the photometric detector on the isotachopherogram provides analytical information relevant to the quantitation (the analyte is spread along the zone of the front spacing constituent). We found that in this instance also the length of the zone of the spacer occupied by the metal as detected by the photometric and/or the conductivity detectors (see Fig. 3) is proportional to the concentration of the analyte. Obviously, here it is desirable that the analyte is preferably present only in one spacing (carrying) zone. A detailed investigation of these alternatives in the quantitation is in progress.

**Practical applicability of the proposed detection method**

The photometric detection of metal cations is intended mainly for their ITP trace analysis. In this respect, we carried out preliminary experiments with tap water samples. The isotachopherograms in Fig. 8 were obtained in these experiments. Here, the sample was mixed with the blank solution before the analysis. The roles of the cations present in this solution were explained in the previous discussion. In this particular instance we used Mg$^{2+}$ and Ca$^{2+}$ instead of Ba$^{2+}$ to avoid analytical disturbances due to the precipitation of sulphate.

**TABLE III**

**REGRESSION EQUATIONS AND CORRELATION COEFFICIENTS FOR Mn$^{2+}$ AND Cd$^{2+}$ IN THE $5 \cdot 10^{-8}$-$50 \cdot 10^{-8}$ mol/l CONCENTRATION RANGE**

The metal cations were injected in 30-$\mu$l sample volumes and they were present in blank solution No. II (Table II).

<table>
<thead>
<tr>
<th>Cation</th>
<th>Regression equation</th>
<th>Correlation coefficient</th>
<th>Number of data points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mn$^{2+}$</td>
<td>$y_{H} = 4.66 + 1.64 \cdot 10^8 x$</td>
<td>0.9961</td>
<td>34</td>
</tr>
<tr>
<td>Cd$^{2+}$</td>
<td>$y_{H} = 4.39 + 1.47 \cdot 10^8 x$</td>
<td>0.9950</td>
<td>34</td>
</tr>
</tbody>
</table>

\[ y_{H} = \text{peak height (mm)}; x = \text{concentration (mol/l)}. \]
When the isotachopherogram from the photometric detector obtained for tap water (Fig. 8b) is compared with that from the blank run (Fig. 8a), it is clear that the sample contained Zn$^{2+}$, Mn$^{2+}$ and Cd$^{2+}$ at detectable concentrations. By adding Zr$^{2+}$ to the sample of tap water and to the corresponding blank solution we confirmed experimentally that the difference between the peaks of Cd$^{2+}$ was due to the presence of this metal in the sample and it could not be ascribed to the displacement effect of Zn (see Fig. 6 and the accompanying discussion). These results also show that the loss of Cd$^{2+}$ along the BALA zone was negligible under our experimental conditions (compare the isotachopherograms in Fig. 6b and c).

Although our results for the analyses of the tap water samples were reproducible and, in comparison with the above model experiments, no other disturbing phenomena (e.g., due to the sample matrix) were observed, further experimental work is essential before this promising detection method can be considered for routine use. In this respect, at least comparative studies with analytical methods currently used in the trace analysis of metals are required in order to evaluate its accuracy.

CONCLUSIONS

This work has shown that the photometric detection of metal cations via their kinetically labile, light-absorbing chelates formed during the migration is
a promising detection alternative for the trace determination of this important group of analytes. To exploit its potential fully in the analysis of practical samples, it is necessary to investigate a wider range of chelating agents.

However, problems inherent in the ITP separation itself as studied here for xylene orange will require further investigation. In this respect, the availability of chelating agents of considerably higher purities relative to those produced, e.g., for complexometric titrations seems very important for a better understanding of the problems associated with losses of the analytes at low ppb concentrations.

REFERENCES