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Photometric detection of amino-containing compounds in capillary isotachophoresis based on reaction with copper(II) ions

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ABSTRACT

Some basic aspects of the photometric detection of amines, amino acids and peptides via their complexes (chelates) formed with Cu^{2+} ions in capillary isotachophoresis (ITP) were studied. Experiments performed in this feasibility study were focused on the complexes of separands formed on the addition of Cu^{2+} ions to the sample solution and the cationic mode of migration. The results suggest that only the compounds that form chelates with more than one ring are detectable photometrically as probably only this type of chelate did not bleed during the separation (migration). Detection limits for these ITP separands were in the range 10^{-7}–10^{-6} mol/l (30-μl sample volumes) when detection was carried out at 580 nm. ITP runs with various sample matrices indicate a high analytical selectivity of this mode of detection.

INTRODUCTION

Capillary isotachophoresis (ITP) is a convenient technique for the separation and analysis of amines, amino acids and peptides present in various matrices (see, *e.g.*, refs. 1–6). As many of these constituents do not respond to ITP-selective detectors, their detection by universal conductivity detectors is often the only alternative [1–6]. However, this is a disadvantage when they need to be determined at trace concentrations and/or in complex ionic matrices. In such instances, the use of selective detectors, preferably in the spike mode [7,8], is advantageous. In conjunction with these detectors, pre-column reactions labelling the analyte(s) with appropriate chromophore(s) [9] or fluorophore(s) [10] may also be very useful.

It is well known that many amino-containing compounds form intensely blue complexes (chelates) with Cu^{2+} ions. From the point of view of ITP analysis, it is apparent that these reactions may form a basis for their selective photometric detection. Although complexes of amino acids with Cu^{2+} have been employed to optimize their ITP separation conditions [6,11], no attention was paid to the use of these reactions for detection in ITP or in any of the related capillary electrophoretic techniques. This work was intended to study some basic aspects of the photometric detection of amines, amino acids and peptides via their complexes (chelates) with Cu^{2+} ions in ITP.

EXPERIMENTAL

Instrumentation

A CS isotachophoretic analyser (Labeco, Spišská Nová Ves, Slovak Republic) was used in the column-coupling configuration of the separation unit. The analytical column of the analyser was provided with a laboratory-made photometric detector with an LQ 1411 light-emitting diode

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(Tesla, Rožnov, Czech Republic) as a monochromatic source of light at 580 nm. A KPX 81 phototransistor (Tesla) served as a photosensing element of this detector, described in detail elsewhere [12].

Chemicals

Chemicals were obtained from Serva (Heidelberg, Germany), Sigma (St. Louis, MO, USA), Lachema (Brno, Czech Republic), Reanal (Budapest, Hungary), K & K (Plainview, NY, USA) and Fluka (Buchs, Switzerland). The chemicals used for the preparation of the leading and terminating electrolytes were purified by conventional methods.

Hydroxyethylcellulose 4000 (HEC) (Serva) or methylhydroxyethylcellulose 30 000 (MHEC) (Serva), after purification on a mixed-bed ion exchanger (Amberlite MB-1; BDH, Poole, UK), was added to the leading electrolytes at a 0.1% (w/v) concentration.

Water delivered by a Rodem 1 two-stage demineralization unit (OPP, Tišnov, Czech Republic) was further purified by circulation through laboratory-made PTFE cartridges packed with a mixed-bed ion exchanger (Amberlite MB-1; BDH). Freshly recirculated water was used for the preparation of solutions.

RESULTS AND DISCUSSION

Migration behaviour of the complexes

There are several ways in which complex equilibria can be employed for the separation [13–16] and detection [17] of ions in ITP. Experiments carried out in this feasibility study were focused on the detection of various amino-containing constituents via their complexes (chelates) formed on addition of Cu^{2+} ions. We preferred this alternative as it provides the possibility of a complete conversion of the constituents of interest into light-absorbing species. On the other hand, it is clear that it cannot be of general use as many complexes will decompose during the migration in an analogous way to that described for the chelates of metals with EDTA [13]. In this respect, considering the behaviour of the studied constituents as ligands in the reactions with Cu^{2+} [18–22], we can classify them as follows:

(a) constituents that can form chelates with more than one ring {diethylenetriamine (DETA); triethylenetetramine (TETA); histidine (HIS); 1,3-bis[tris(hydroxymethyl)methylamino]propane (bis-tris-propane or BTP); spermine; anserine; carnosine};

(b) constituents that can form single-ring chelates (ethylenediamine; 1,2-diaminopropane; 1,3-diaminopropane; histamine);

(c) constituents that probably cannot form chelates [spermidine; 1,4-diaminobutane; 1,5-diaminopentane; 1,6-diaminohexane; 1,8-diaminooctane; 1,10-diaminodecane; tris(hydroxymethyl)aminomethane (Tris); bis(2-hydroxyethyl) iminotris(hydroxymethyl)methane (bis-tris); ethanolamine; imidazole; guanidine; pyridine; 2-aminopyridine; dimethylaniline; *p*-toluidine; quinoline; dimorpholinoethane; creatinine].

We found that under the ITP working conditions employed (Table I), only the constituents which are expected to form with Cu^{2+} ions chelates with more than one ring [group (a)] did not bleed from these chelates during the separation. Consequently, they could be detected by the photometric detector at 580 nm. The stabilities of the complexes (chelates) of the remainder of the constituents were not sufficient

TABLE I

OPERATIONAL SYSTEM

The driving currents were 200 and 35 μA in the preseparation and analytical columns, respectively.

Parameter	Electrolyte[a]	
	Leading	Terminating
Solvent	H_2O	H_2O
Cation	NH_4^+	$EACA^+$
Concentration (m*M*)	10	10
Counter ion	MES^-	AC^-
pH	6.1	*ca.* 5.0
Additive	HEC (MHEC)	–
Concentration (%, w/v)	0.1	–

[a] EACA = ε-Aminocaproic acid; MES = morpholinoethanesulphonic acid; HEC = hydroxyethylcellulose; MHEC = methylhydroxyethylcellulose; AC = acetic acid.

for them to be detected, at least partially. Although separations (migrations) at a higher pH should be favourable from the point of view of complex formation, the values of the stability constants [18–22] and the hydrolytic behaviour of Cu^{2+} [23] suggest that a considerable improvement in this respect can hardly be expected.

Detection limits and competition of the separands for Cu^{2+}

The values of the detection limits (DETA 10^{-6} mol/l, BTP 10^{-7} mol/l and HIS $2 \cdot 10^{-7}$ mol/l for a 30-μl injection volume) were determined using the spike mode of detection [8]. The isotachopherograms in Fig. 1 were obtained from the photometric detector and they show responses of the detector for the constituents forming cationically migrating chelates with Cu^{2+} at concentrations close to their detection limits. From the isotachopherogram in Fig. 1b it can be seen that the complex (chelate) of DETA present in the sample at a concentration of $5 \cdot 10^{-7}$ mol/l was decomposed during the migration. This seems to be the most appropriate explanation for the fact that this amine was not detected via the complex particles. From this we can also deduce that the above value of the detection limit could be lower for separations performed in shorter capillary tubes or in the separation compartment with a lower load capacity [24].

The isotachopherograms in Fig. 2 are intended to illustrate competitions of the separands (ligands) for the central ion. The isotachopherogram in Fig. 2b shows that an excess of BTP added to the sample (relative to the concentration of Cu^{2+} in the sample) is accompanied by the formation of a zone of this constituent (BTP in Fig. 2b) in front of the zone of the chelate (Cu–BTP in Fig. 2b). When we also consider the behaviour of the Cu–DETA complex (see above), it is surprising that the presence of free BTP in the sample did not lead to the decomposition of Cu–DETA. The complete disappearance of the Cu–BTP complex on the addition of histidine (Fig. 2c) is also unexpected.

Detection of complex-forming constituents in various matrices

Ampholytic buffers for isoelectric focusing contain mixtures of polyaminopolycarboxylic, polyaminopolyphosphonic and polyaminopolysulphonic acids or their salts [25,26]. The isotachopherogram in Fig. 3 shows that many of these constituents form strongly visible light-absorbing and cationically migrating complexes

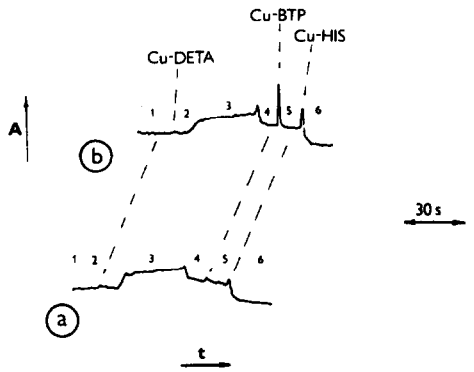

Fig. 1. Migration behaviour of the complexes of DETA, BTP and HIS at concentrations close to their detection limits. (a) Blank run with the sample containing $1 \cdot 10^{-4}$ mol/l Na^+ (2), $5 \cdot 10^{-5}$ mol/l tetraethylammonium (4) and $5 \cdot 10^{-5}$ mol/l bis-tris (5) as discrete spacers and 10^{-4} mol/l Cu^{2+} (3). (b) Run as in (a) except that the sample contained DETA, BTP and HIS at $5 \cdot 10^{-7}$ mol/l (symbols of the corresponding complexes mark the peaks and/or indicate the migration positions). 1,6 = Zones of the leading and terminating constituents, respectively (see Table I). A = Light absorption (580 nm); t = time.

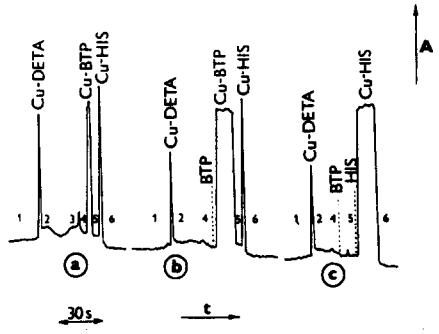

Fig. 2. Competition of the amino-containing separands for Cu^{2+} ions. (a) Same mixture of discrete spacers as in Fig. 1 containing Cu^{2+} (3) at $1 \cdot 10^{-4}$ mol/l and each of the analytes (DETA, BTP and HIS) at $1 \cdot 10^{-5}$ mol/l. (b) Same sample as in (a) except that BTP was present at $4 \cdot 10^{-5}$ mol/l. (c) Same sample as in (a) except that HIS was present at $4 \cdot 10^{-5}$ mol/l. Other symbols as in Fig. 1. For separation conditions, see Table I.

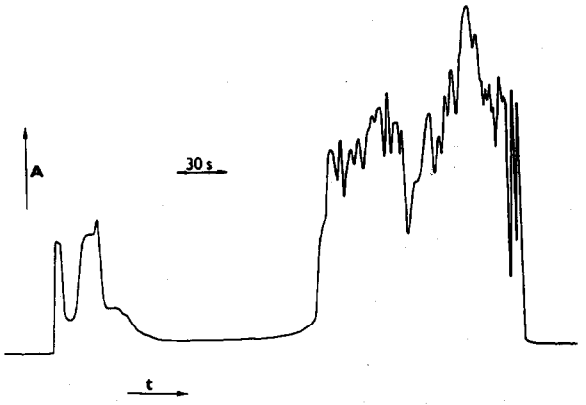

Fig. 3. Isotachophoretic profiling of cationically migrating complexes formed by ampholytic constituents with Cu^{2+} cations. The sample solution was prepared by diluting a Servalyt buffer for isoelectric focusing (pH 2–11; Serva) with water in the ratio 1:1000 (v/v). The concentration of Cu^{2+} ions in the diluted buffer was $5 \cdot 10^{-4}$ mol/l. A 30-μl volume of the sample solution was taken for the separation performed under the conditions described in Table I.

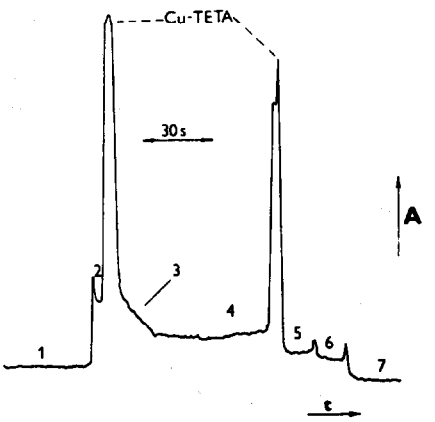

Fig. 4. Isotachophoretic separation and photometric detection of cationically migrating Cu^{2+} complexes of triethylenetetraamine (Cu–TETA) of technical-grade purity. TETA was present in the sample at $5 \cdot 10^{-5}$ mol/l while the final Cu^{2+} concentration was $2 \cdot 10^{-4}$ mol/l. Bis-tris (6), tetraethylammonium (5), Na^{+} (3) and Ca^{2+} (2) served as spacing constituents. 1,7 = Zones of the leading and terminating constituents, respectively.

(chelates) with Cu^{2+} ions. Differences in the absorptivities of the complexes make the resolutions of these contiguous zones possible in spite of the fact that their resolution can hardly be achieved by a high-resolution conductivity detector.

Triethylenetetramine, available in technical-grade purity, under our separation conditions (Table I) forms several zones detectable with a conductivity detector. The isotachopherogram from the photometric detector shown in Fig. 4 indicates that at least two of these constituents form isotachophoretically migrating chelates with Cu^{2+}. In this respect it should also be noted that the sample concentration was close to that corresponding to the detection limit from the response of the conductivity detector. The detection limit with the photometric detector was found to be *ca.* 100 times lower (10^{-7} mol/l), *i.e.*, in the range favourable for some environmental applications (see ref. 1 and references cited therein).

A dilute urine sample mixed with discrete spacers and with Cu^{2+} ions was profiled for constituents that form cationally migrating complexes (chelates) with this metal. The isotachopherograms in Fig. 5 clearly show that with the aid of the spacers used we were able to resolve at least two constituents that form complexes with Cu^{2+} and detectable at 580 nm. In spite of the fact that we did not identify these constituents, it seems reasonable to suggest that one of them could be 3-methylhistidine (a degradation product of actin and myosin proteins) which is excreted in urine and which is a good index of skeletal muscle protein degradation [27,28].

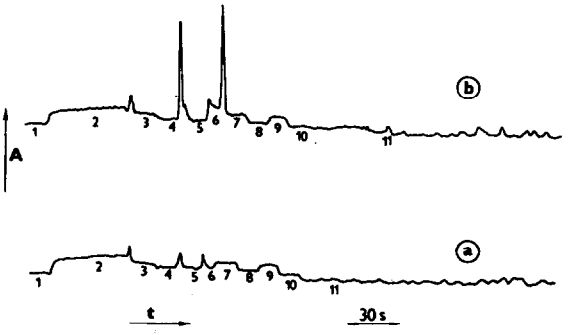

Fig. 5. Profiling of complex forming constituents present in human urine. (a) Blank run with spacing constituents [2-aminopyridine (3), tetraethylammonium (4), bis-tris (5), pyridine (6), dimethylaniline (7), *p*-toluidine (8), quinoline (9), creatinine (10)]. Cu^{2+} was present in the samples at $1 \cdot 10^{-4}$ mol/l. (b) Urine (diluted 1:100 immediately after collection) containing the same constituents as the blank sample (a). 1,11 = Zones of the leading and terminating cations, respectively.

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