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Manganese speciation using capillary electrophoresis–ICP-mass spectrometry

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Abstract

Manganese is a trace element known to activate many enzymes involved in metabolic processes and it shows protective function against oxidative stress. On the other hand, increased Mn levels are known for damaging the central nervous system, resulting in motoric abnormalities and psychic disorder. Such additional Mn exposure can cause an "Mn overflow" in the liver, accompanied by production of specific (labile) Mn transporters (Mn-species). The speciation of these Mn-compounds is still unknown but they are believed targeting the brain. The aim of this paper was to develop a speciation method for manganese species in liver extracts, which allows to speciate the compounds quickly and with minimal risk of species alteration. Capillary electrophoresis (CE)–inductively coupled plasma mass spectrometry (ICP-MS) offers a valuable tool as analytes are not in contact to a stationary phase which probably affects species stability. Separation usually is fast and ICP-MS detection is element specific and sensitive. The paper describes the set-up and optimization of the hyphenated technique, optimization of separation according to pH and finally the Mn speciation of a liver extract. Several Mn species were found, such as arginase, Mn-transferrine, Mn-albumine and some more. The detection limit of the method was determined at 1.1 μg Mn/L independent on the species.

Keywords: Speciation analysis; Element-selective detection; Detection, CE; Inductively coupled plasma mass spectrometry; Manganese

1. Introduction

1.1. Aspects of Mn in biological function

Manganese is a trace element known to activate many enzymes involved in metabolic processes. Mn is needed for protein and fat metabolism, healthy nerves and a healthy immune system as well as it is needed for blood sugar regulation. Mn is one of the key elements for enzymes in energy production and it increases the level of antioxidative protection [1–3]. Therefore, Manganese is also called "cell-protector", because it increases the level of Mn-superoxide dismutase, which results in a decrease of free radicals. Manganese is involved in utilization of Vitamins B_1 and E and it is required for normal bone growth or for avoiding clotting defects.

On the other hand, manganese is used as anti-knock agent in gasoline, resulting in increased Mn-blood levels as monitored for Canadian children [4]. Increased Mn levels are known for damaging the central nervous system, resulting in motoric abnormalities and psychic disorder [1,3,4]. Generally, studies from occupational health with increased Mn exposure demonstrated a limitation of mental ability, proceeding to psychotic phase with reduction of psychomotoric coordination, damage of the extrapyramidal nervous system and finally leading to symptoms similar to Parkinson's disease. [1–3,5].

1.2. Manganese and liver

Liver was found to be that organ having the major Mn-reservoir. In human liver 1.5 mg/kg manganese were determined [1]. In pig liver up to 3 mg/kg were found (e.g. [6]). With additional (excessive or long-term) Mn exposure an overflow of the liver is known. Excessive Mn transport to other organs, predominantly to the brain, was observed in such cases. Specific Mn transporters and Mn-species are suggested to be generated in liver, however, their speciation still is not investigated. Mn transferrine and/or albumine are asumed to be such Mn-carriers. A further low molecular weight (LMW) transporter, independent from transferrin, was sugested as Mn-carrier to brain, too [2,3,7,8].

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1.3. Crossing blood–brain barrier

A critical question remains in the transport of Mn compounds across the blood–brain barrier (BBB). Reference [2] addresses that no data about Mn speciation are available, however, Mn-speciation is required for understanding this mechanism. Informations about Mn-transporters across BBB generally are rare. Transferrin and an up to now non-identified low molecular weight (LMW) carrier are supposed to play an important role for Mn transport from predominantly liver to other organs, especially the brain. The brain occurs to be a target organ as the enzyme glutaminsynthetase (astrocyte-specific) is known as regulatory target of manganese. Probably also the ratio of Mn^{2+}/Mn^{3+} may be relevant, pointing to Mn^{3+}-transferrine, Mn^{3+}-citrate or inorganic Mn^{3+} compounds, stressing the necessity of Mn-speciation.

1.4. Manganese speciation

Michalke and Schramel [9] performed manganese speciation in human milk. Methods were developed for Mn speciation using size exclusion chromatography (SEC) and strong anion exchange chromatography (SAX)–ICP-MS. Mn-citrate was found (which is a LMW compound of Mn) and identified two-dimensionally. Matzepetakis et al. [10] characterized Mn-citrate complexes by NMR and FTIR and found Mn^{2+} and Mn^{3+} citrate complexes as relevant Mn complexes in biological media which are possibly related to beneficial and toxic effects of manganese in humans [10]. Labile complexes, however, such as Mn-albumine or Mn-transferrine [8] get easily destroyed during SAX. Therefore, these methods are not applicable for investigations where these Mn species may be of significant interest.

1.5. CE–ICP-MS for Mn speciation

On the other hand, CE offers high separation capability and less interaction with species, thus resulting in less destabilizing of Mn-species according to a lack of stationary phase [11]. Typically worse detection limits compared to chromatographic techniques are observed. However, CE–ICP-MS is most useful for samples with sufficiently high Mn concentrations and where samples are causing separation problems in chromatography or show stability problems for several species [12]. Therefore, a CE–ICP-MS method was developed for the speciation of manganese in liver extract. This method was planned to provide sufficiently low detection limits, no (or a reduced) suction flow and fast analysis. Besides, interfacing should be as simple as possible resulting in a quick set up and robust operation. Thus, the interface was based on a Meinhard nebulizer. No critical capillary positioning was necessary. Detection sensitivity and suction flow were checked with respective experiments. Separation of Mn-species was optimized with respect to pH affecting species stability and separation efficiency. Finally, a liver extract was speciated to show applicability to real samples. Several Mn-compounds were identified and quantified.

2. Experimental

2.1. Chemicals

Standard compounds (arginase, isocitratedehydrogenase, albumine, transferrine, citrate, pyruvatecarboxylase, concanavalin A and $MnCl_2$) were ordered from Sigma–Aldrich, Deisenhofen, Germany. Tris was purchased from Sigma–Aldrich too, whereas NaOH, HNO_3 and HCl (Suprapure) were ordered from Merck, Darmstadt, Germany. HNO_3 was purified by subboiling destillation. For the sheath flow HNO_3 was diluted to 1 + 999 (v:v) HNO_3 with Milli-Q water. $Argon_{liqu}$ was purchased from Messer, München, Germany. An Ar vaporizer at the tank provided Ar gas.

2.2. Samples and sample preparation

Mn-protein stock standards from arginase and concanavalin A (1 mg powder/mL) were prepared by dissolving the powder of each compound in 10 mL Tris–HCl buffer (10 mM, pH 7.4, close to native liver pH). The individual Mn content was determined by ICP-atomic emission spectrometry (AES) ($n = 3$), as no specific Mn concentration was given. Mn concentrations of the protein stock solutions were 8 mg Mn/L (arginase) and 170 μg Mn/L (concanavalin A). Isocitrate-dehydrogenase (iso) and pyruvatecarboxylase (pyr) were provided as solutions from the manufacturer, containing 43 mg Mn/L (iso) or 380 μg Mn/L (pyr). Stock solution of $MnCl_2$ was prepared by dissolving 100 mg/L (related to Mn). Mn-citrate stock solution was prepared by mixing a solution of 1 g/L citrate with a $MnCl_2$ solution (5 mg/L) using a ratio of 4 + 1 (v:v), resulting in a Mn-citrate stock concentration of 1 mg Mn/L. Mn-albumine and Mn-transferrine stock solutions were prepared in analogy by mixing 1 g/L protein solution with 5 mg/L $MnCl_2$ solution (4 + 1, each), resulting in 1 mg Mn/L for each compound. Stock solutions were aliquoted and stored in the dark at −20 °C. No destabilization of standard compounds was observed using these conditions. Working solutions were prepared daily by appropriate dilution with Tris–HCl, 10 mM, pH 7.4.

First, single standards of arginase (containing inorganic Mn for stabilization) were used as samples for pH-related optimization of separation, followed by analysis of standard mixtures. Finally, a liver extract from porcine liver (purchased freshly on a local market) was prepared according to [6] under Ar gas to prevent the species from oxidation. In short terms: liver was cut into ca. 1 g pieces with a ceramic knife and put into a plastic tube. The slices were shock frozen in liquid nitrogen, then covered with 3 mL 10 mM Tris–HCl, pH 7.4, thawed on ice and then pottered on ice with a homogenisation pistill. Each step was carried out under Ar

atmosphere in a glove bag (Atmos Bag, Sigma–Aldrich). The homogenate was centrifuged (10,000 g, 15 min 4 °C, Biofuge 17 RS, Heraeus–Sepatech, Osterode) and the supernatant was used as extract. Additionally, pieces of liver tissue were collected, later digested and analyzed for total manganese by ICP–AES.

2.3. Instrumentation

2.3.1. ICP-MS

An ELAN 5000, Perkin-Elmer (Sciex, Toronto, Canada) was employed for on-line determination of ^{55}Mn in the graphic mode. The RF power was set to 1200 W, the plasma gas was 15 L Ar/min. The nebuliser gas was optimized (see below) and finally set to 930 mL Ar/min. The dwell time was set to 100 ms. These parameters were the optimal conditions for this instrument.

2.3.2. ICP–AES

An ICP–AES "JY 70 plus" system (Jobin Yvon, Long-Jumeau, France) was used for Mn determination in manganese-enzyme standard compounds and in the liver extract. Sample introduction was performed by a peristaltic pump (1.5 mL/min, Abimed, Langenhagen, Germany), connected to a Meinhard nebulizer which was fitting into a cyclon spray chamber. The measured spectral element line was: Mn: 257.610 nm.

The RF power was set to 1000 W, the plasma gas was 15 L Ar/min, whereas the nebulizer gas was 600 mL Ar/min. Every 10 measurements three blank determinations and a control determination of a certified Mn standard was performed. These parameters were the optimal conditions for this instrument.

2.3.3. Capillary zone electrophoresis

A "Biofocus 3000" capillary electrophoresis system (Bio-Rad, Munich, Germany) was used as the CE device. The temperature was set to 20 °C for sample/ buffer carousels by air cooling and also 20 °C for the total capillary by liquid cooling.

The capillary (120 cm × 50 μm i.d., non coated) was bought from CS-Chromatographie Service GmbH (Langerwehe, Germany).

2.3.4. Operation

Before each run, the capillary was purged with Milli-Q H_2O (180 s, 8 bar) and running electrolyte (180 s, 8 bar).

The separation method applied for interfacing experiments used Tris–HCl buffers as the electrolyte (10 mM, pH varied between 7.0 and 8.3, finally set to pH 8.3) and 10 mM NaOH. The applied voltage was set to +15 kV.

2.4. Hyphenation

Fig. 1 shows the hyphenation interface schematically. The interface was based on a Meinhard nebulizer linked to a T-piece manufactured of PE (e.g. available from luer-lock articles, e.g. BioRad) which fitted exactly to the back side of the nebulizer. Positioning of the CE capillary was not critical with respect to signal response and stability. The capillary was first moved into the nebulizer at a maximum to the transition from i.d. ca. 1.5 mm to the nebulizer capillary (i.d. ca. 0.2 mm) (position a, Fig. 1B) and then was pulled back approximately 1 mm (position b, Fig. 1B). In this position it was close to the beginning of the nebulizer capillary but still it did not block the coaxial sheath flow. No difference in signal height was seen between 1 and 2 mm pulling back. Using two more T-pieces the outlet electrode and the backflow for capillary cooling were mounted.

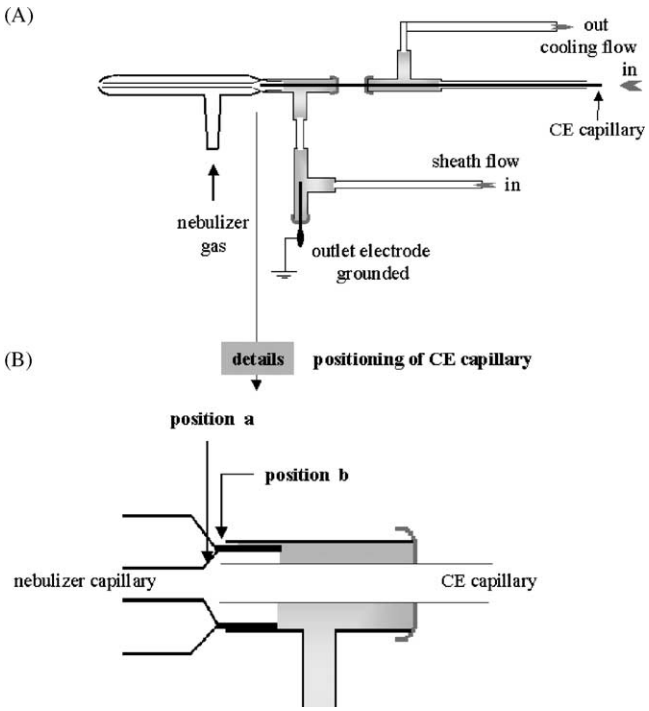

Fig. 1. The hyphenation set-up is shown (A). The coupling is based on a Meinhard nebulizer. The capillary is positioned just in front of the nebulizer capillary: The capillary is first moved at a maximum to the transition from i.d. ca. 1.5 mm to the nebulizer capillary (i.d. ca. 0.2 mm) (position a (B)) and then is pulled back approximately 1 mm (position b (B)). The grounded electrode is mounted via a T-piece. The sheath flow is provided by a peristaltic pump with 0.7 mL/min (see experimental). The capillary is liquid-cooled at 20 °C nearly up to the nebulizer.

HNO_3 (0.1%) was used for providing the electrical connection between capillary outlet and grounded electrode at the nebulizer via sheath flow. Prange and Schaumlöffel [13] investigated effects using HNO_3 or diverse buffers and found that no negative effect was seen using HNO_3 as long as the inlet buffer was renewed regularly. For simplification of the experiments with different pH values in the inlet buffer and because of quantification being nearly independent on species nature HNO_3 was used for sheathflow throughout. Inlet buffers were renewed after each run for avoiding a pH shift. The use of HNO_3 helped also to (partly) decompose

the species (wrap off the Mn, resulting in inorganic Mn reaching the detector) before entering ICP-MS. Thus quantification could be related then only to the calibration curve of inorganic Mn.

2.5. Suction flow

The possible occurrence of a suction flow through the capillary was checked, too, although it was considered to be not very likely due to extended experience with 1.2 m capillaries and a Meinhard-based interface in former studies (e.g. [14]). This possible interference was checked using the following experiments.

The capillary was filled completely with 2 mg/L $MnCl_2$. When the solution reached the nebulizer the signal increased from approximately 250–64,000 cps. Then the inlet pressure was shut down whilst the signal was still monitored. It dropped down again to baseline level within few seconds, showing that no Mn was reaching the ICP-MS. The occuring of a suction flow would have transported at least some Mn still to the nebulizer resulting in a signal higher than baseline. The second experiment was performed in analogy to [15]. The capillary inlet was removed from buffer solution into air for 30 min to enable air intruding into the capillary if a suction flow occured. Then the inlet was dipped into buffer again, high voltage was turned on and the current was measured. It showed 18 μA proving that there was no air bubble in the capillary (which would have been a result of a suction flow). Both experiments proved the absence of a suction flow.

3. Results and discussion

3.1. Optimization of operation parameter

The interface setup was designed to be set up simply, fast and its operation was planned to be robust even with samples like liver extract. According to reference [12] CE–ICP-MS was used for samples with sufficiently high Mn concentrations (as known from liver extracts, [16]) on the one hand and showing separation problems in chromatography or stability problems on the other hand (Mn-transferrine, Mn-albumine [8]).

For signal optimization the nebulizer gas flow as well as the sheath flow were optimized. For this set-up it turned out that a nebulizer gas flow of 930 mL Ar/min was optimal. The flow rate of the sheath flow was optimized with respect to signal height and reduction of noise. When purging the capillary with a 2 mg/L $MnCl_2$ solution the sheath flow rate was varied between 0.2 and 1.0 mL/min. Fig. 2 demonstrates the noise and signal response (and calculated *S*/*N*) depending on the sheath flow rate. No reliable nebulization was achieved at 0.2–0.4 mL/min, resulting in high noise (instable signal). This is not surprising for a regular Meinhard interface at these low flow rates. Useful operation started around 0.4–0.5 mL/min, reaching an optimized signal/noise ratio at 0.7 mL/min. Further increase of sheath flow rate promotes dilution of analytes resulting in signal decrease.

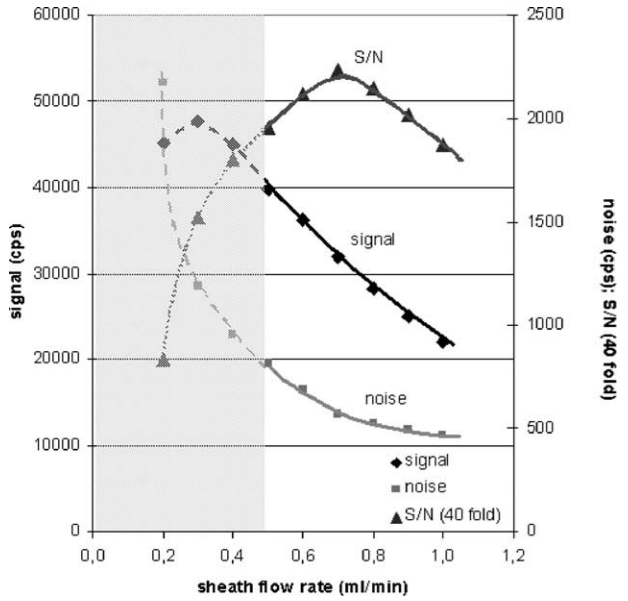

Fig. 2. Demonstration of the noise and signal response depending on the sheath flow rate. Useful operation started around 0.4–0.5 mL/min, reaching an optimized signal/noise ratio at 0.7 mL/min. Further increase of sheath flow rate promotes dilution of analytes and signal decrease.

3.2. Separation improvement at different buffer pH

Separation should be achieved around the physiological pH (which is 7.4 for liver [17]) for maintaining Mn species stable during separation. Therefore, investigations on separation improvement were varying pH between 7.0 and 8.3. A preliminary check using $MnCl_2$ as a sample showed good performance also with 10 mM NaOH. This electrolyte was checked therefore, in addition as an “endpoint”.

Fig. 3 shows the consecutive electropherograms using arginase standard solution (stabilized with inorganic Mn, totally 2 mg/L determined by ICP–AES) as a sample at different pH values: At pH 7.0 arginase is detected at 3.53 min in a broad distorted peak. It is detected earlier than inorganic Mn, monitored at 6.3 min. The single peak areas were determined and compared to the total peak area (Mn-arginase peak + peak of inorganic Mn) at pH 8.3, which was set to 100%. Generally, approximately 1/3 of Mn was found at arginase, two-third of Mn were derived from the inorganic Mn for stabilization throughout the pH-related experiments. At pH 7.0, the peak area of arginase accounted for 27.8%, whereas that of inorganic Mn showed 71.2%, both together reached 99.0%. However, the peak height of Mn-arginase was low reaching only 4250 cps. Separation at pH 7.4 resulted in a co-migration of inorganic Mn and arginase. The elution sequence of both compounds is changing between pH 7.0 and 7.8 (see below), resulting in the same migration

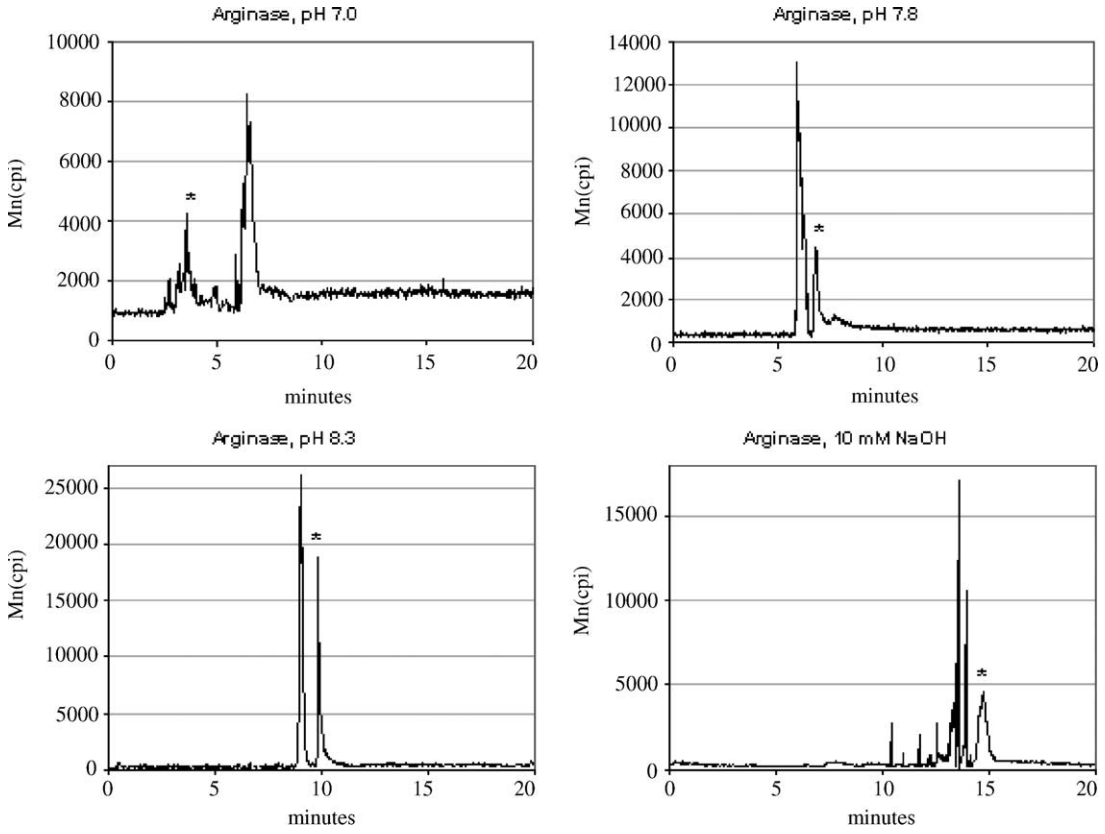

Fig. 3. Optimization of separation and stability conditions. Consecutive electropherograms of a Mn-arginase standard solution (stabilized with inorganic Mn, 2 mg/L total Manganese) as a sample at different pH values (a) 10 mM Tris–HCl, pH 7.0 (b) 10 mM Tris–HCl, pH 7.8 (c) 10 mM Tris–HCl, pH 8.3 (d) 10 mM NaOH. Separation at pH 7.4 resulted in a co-migration of inorganic Mn and arginase. Voltage: +15 kV. The recovery was 2.1 mg/L (105%) when summing up peak concentrations determined by CE–ICP-MS at pH 8.3. ✱: Arginase.

time at pH 7.4. At pH 7.8 the migration order changed and inorganic Mn was detected at 6.5 min, which is earlier than arginase at 7.45 min. Peak focusing is significantly improved, but still a tailed hump detected directly after arginase at 8.43 min points to some destabilization. Peak area percentages were 61.4% for inorganic Mn, 27.6% for arginase and 7.7% at the hump, all together 96.7%. The peak height in Mn-arginase was determined at 4500 cps. The comparatively low amount of Mn found in arginase also points to a destabilization of this enzyme with a loss of Mn to the inorganic fraction.

The best result is seen at pH 8.3, where inorganic Mn is detected at 8.89 min and arginase at 9.86 min. Peak focusing is further improved, no distortion or a hump are seen anymore. Peak areas are now 65.7% for inorganic Mn and 34.3% for arginase, both together set to 100.0%. The peak height of Mn-arginase reaches nearly 18,000 cps. The peak height obtained for arginase is the highest during this series of experiments, indicating optimized stability of Mn-arginase. Also, the peak shape appears to be otimized, showing maximal peak height and minimal peak width, thus improving detection limits. The use of 10 mM NaOH seemed to be promissing in a preliminary investigation, but this electrolyte cannot be considered to be close to physiologic conditions. Therefore, it was planned just as an “endpoint” in pH-related experiments. Using 10 mM NaOH as electrolyte the increased endoosmotic flow (EOF) moved both compounds to late migration times. However, arginase again gets destabilized. Several Mn signals were monitored, whilst the signal of arginase changed to a broader and lower peak at 15.73 min, showing only a peak height of nearly 5000 cps. Peak areas were similar to those at pH 8.3 and account for 34.3% at Arginase and 51.7% for the rest, all together 86.0%.

Based on the above results subsequent investigations were performed with Tris–HCl, 10 mM, at pH 8.3.

3.3. Calibration

When analyzing calibration standards using optimized conditions it was found that detector response was similar for the different compounds. This can be explained by the fact that only few nanoliters of each compound reach the detector being mixed with the HNO_3 sheath flow, which has surplus amount. The compounds may be decomposed and Mn may be wrapped off the labile complexes [7]. Then Mn is present ionically independent on the native species resulting in calibration functions also independent on the original species. Therefore, a 5 point calibration curve of

$MnCl_2$ was performed between 10 and 500 μg/L and was used for quantification. Good linearity ($r^2 = 0.9998$) was achieved. Detection limits were calculated using 3σ criterion (baseline noise) and the signal height of a 20 μg/L standard, resulting in a LOD of 1.1 μg/L (corresponding to 5.1 μg/kg, however, depending strongly on the preceded extraction procedure and dilution for measurement). This was considered to be sufficient for Mn speciation in the liver extract (391 μg/L total manganese determined by ICP–AES).

3.4. Electropherograms of standard compounds, investigations on a liver extract

Subsequent investigations focused on electropherograms from different Mn-species at optimal conditions. Migration times (MT) of different Mn-species were ruled out. Fig. 4 shows the electropherograms of the compounds.

For Mn compounds, which were produced by mixing inorganic Mn with the ligand (e.g. protein), such as transferrine or citrate, two peaks were monitored. They corresponded to the formed Mn–ligand complex and the rest of $MnCl_2$. The arginase standard produced two peaks, too, as $MnSO_4$ was added to the protein powder by the manufacturer for stabilization, as indicated on the label.

Additionally it turned out that a systematic shift in baseline was seen regularly of ca. 350 cps (from ca. 250–600 cps, see, e.g. Mn-albumine electropherogram), which was usually monitored around MT 8.85 min. Inorganic Manganese was migrating in parallel at this position when it was present in the sample, e.g. seen in the $MnCl_2$/Mn-citrate electropherogram. This helped for an assignment of inorganic Mn and Mn-compound in the samples which had been produced by mixing the respective compounds. The standard electropherograms (Fig. 4) showed that the investigated compounds were detected at clearly different migration times, which are given in Table 1. The analysis of pyruvatecarboxylase, however, was critical. The purchased protein solution had 380 μg/L Mn and a high amount of stabilzing compounds interfering partly with separation. For minimizing interferences from the stabilizing compounds strong dilution would have been necessary. However, with respect to the comparatively low Mn concentration a dilution of only 1:3 was carried out. Pyruvatecarboxylase was monitored very close after the baseline shift. Therefore, resolution from inorganic Mn was critically in samples where inorganic Mn species were present.

As the baseline shift occurred in each of the electropherograms nearly around 8.85 min it could be used as an internal marker when analyzing mixed standard samples or a liver extract. Due to varations in conductivity migration time shifts are well known [18–20] and also were seen when analyzing, e.g. extracts. This is demonstrated in Fig. 5. Here the shift is detected at 10.48 min. Several peaks are seen, too. Due to MT correction using the internal marker some of the peaks can be assigned to standard compounds. Using standard addition methods these assignments were mostly confirmed. Table 2 shows the monitored peaks in the liver extract, migration times and identification using standard addition as well as internal marker assignment. Quantification of the single peaks in liver extract was performed, too. The values are given in Table 2. The recovery (sum of peaks in μg/L) was 84% related to the concentration in the extract, which had been determined before at 391 μg Mn/L by ICP–AES. Considering the low concentration of single Mn-species this recovery was sufficient.

Table 1
Migration times and "normalized" migration times of standard compounds

	MT (min)	$MT_{normalized\ according\ to\ internal\ marker\ at\ arginase}$
Citrate	14.18	13.71
Albumine	7.88	7.42
Transferrine	0.85	0.89
Pyruvatecarboxylase (Pyr)	8.12	9.26
Arginase	9.86	9.86
Concanavalin A (Con A)	22.42	22.58
$MnCl_2$	9.09	8.85
Isocitrate-dehydrogenase (Iso)	20.33	19.02

The equation for "normalization" was: $MT_N = (N/t)MT_1$ with MT_N: migration time normalized, MT_1: migration time of the respective compound, *t*: migration time of the internal marker (baseline shift) in respective electropherogram, *N*: migration time of the internal marker (baseline shift) in arginase electropherogram (set as a reference in this paper).

Actually, the quantification of the single Mn-compounds was partly close to the limit of determination as the peakheight of smallest peaks exceed 10σ of noise just a little. The more prominent peaks, however, were far from the 10σ value.

The electropherogram of the liver extract gives a good example of the advantage of the CE–ICP-MS method. Even the labile Mn-transferrine and Mn-albumine complexes are monitored in a native sample, proving minimized interaction by capillary electrophoresis. This provides a better chance to get more information about the Mn-carrier to the brain and probably also about the Mn species crossing the blood–brain barrier. The finding that Mn-citrate is present in liver, too, is of similar importance. Mn-citrate may be discussed as one of the supposed LMW-manganese transporters to brain. The result of peak assignments via the mathematical MT correction using an internal marker is valuable as it can help for peak assignment as a first step. Final identification, however, should be done by experiments such as standard addition method.

Summarizing, this CE–ICP-MS method provides a simple set-up with sufficient detection power for Mn species in liver extracts. Separation of the Mn compounds was performed quickly. The internal baseline shift offered quick assignment of peaks, however, which still should be verified using standard addition. Several Mn compounds in liver

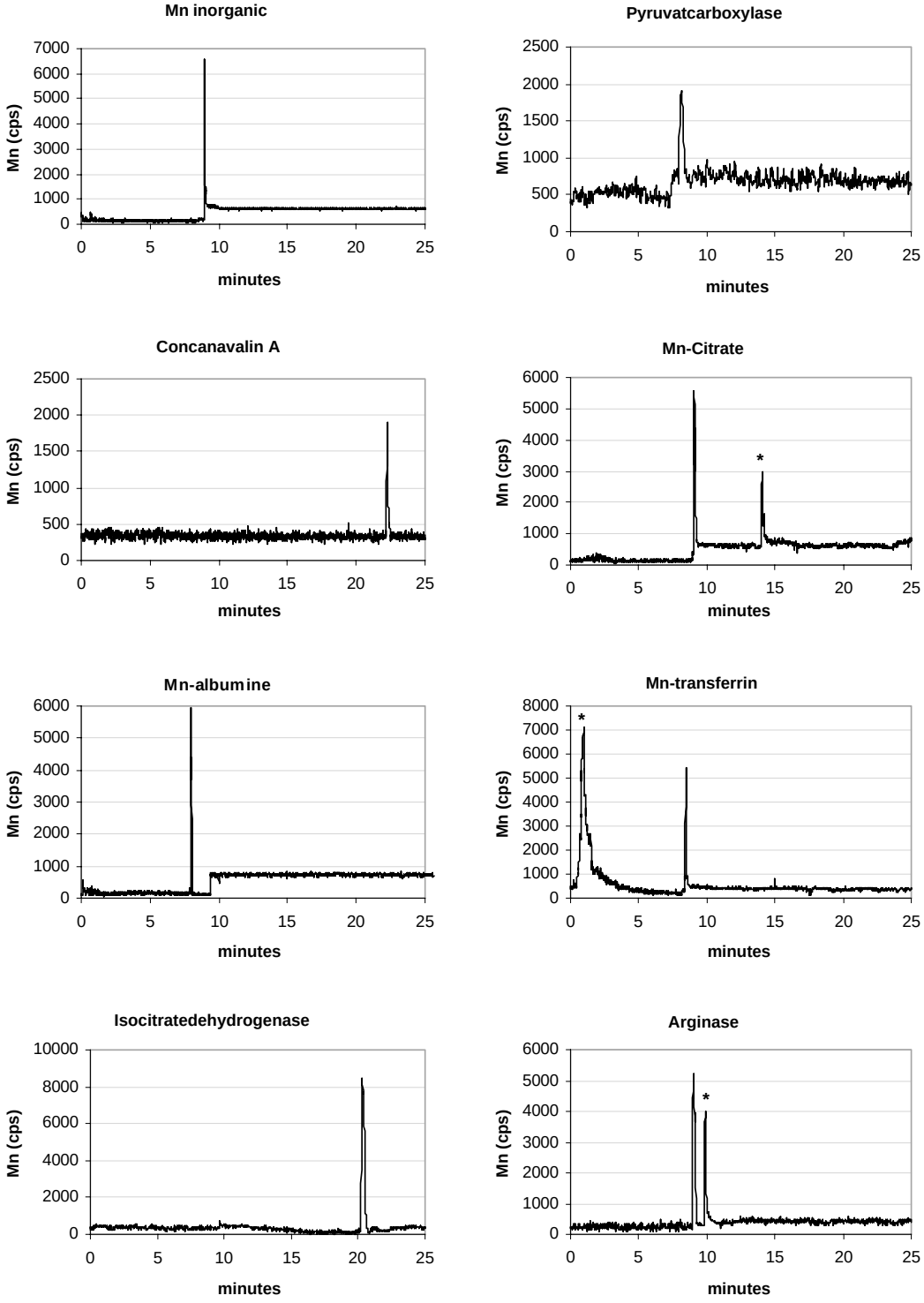

Fig. 4. Standard electropherograms are shown for the investigated compounds. For Mn-compounds produced by mixing inorganic Mn with the ligand, the Mn–ligand complex is marked with an asterisk. A baseline shift occurred in each of the electropherograms nearly around 8.85 min. The shift could also be used as an internal marker when analyzing mixed standard samples or a liver extract. Electropherograms are shown for $MnCl_2$ (100 μg/L, found by CE–ICP-MS: 97 μg/L), pyruvatecarboxylase (130 μg/L Mn, found by CE–ICP-MS: 125 μg/L),), concanavalin A (70 μg/L Mn, found by CE–ICP-MS: 61 μg/L), Mn-citrate (400 μg/L Mn, found by CE–ICP-MS: 396 μg/L in total, 136 μg/L (34%) at Mn-citrate), Mn-albumine (100 μg/L Mn, found by CE–ICP-MS: 107 μg/L), Mn-transferrine (1000 μg/L Mn, found by CE–ICP-MS: 1055 μg/L in total, 941 μg/L (90%) at Mn-transferrine), isocitratedehydrogenase (650 μg/L Mn, found by CE–ICP-MS: 644 μg/L), arginase (400 μg/L Mn, found by CE–ICP-MS: 420 μg/L in total, 143 μg/L at arginase).

Table 2
Migration times of Mn-compounds in a liver extract, quantification and partly identification

Liver extract	Migration time (min)	$MT_{normalized\ corrected\ due\ to\ internal\ marker}$	Assignment according to $MT_{normalized}$ (? = possibly)	Identification according to standard addition	Mn concentration μg/kg liver
Peak 1	0.49	0.41	Probably transferrine ?	Mn-transferrine	68.2
Peak 2	4.47	3.78		Not identified	314.4
Peak 3	7.60	6.41		Not identified	36.1
Peak 4	8.46	7.14		Not identified	at LOD
Peak 5	8.94	7.52	Albumine	Mn-albumine	83.4
Peak 6	10.48	8.85	Inorganic	Inorganic	251.9
Peak 7	11.35	9.58	Pyruvatecarboxylase?	??	78.4
Peak 8	11.83	9.93	Arginase ?	Mn-arginase	90.9
Peak 9	12.88	10.88		Not identified	5.8
Peak 10	13.37	11.29		Not identified	12.9
Peak 11	15.00	12.67	Probably citrate ??	Mn-citrate	48.9
Sum of concentrations					991.4
Total Mn concentration by ICP–AES					1178.9

Due to the problems of resolving pyruvatecarboxylase from inorganic Mn an identification of this compounds could not be achieved clearly. Total recovery (sum/total determination by ICP–AES) is 84%.

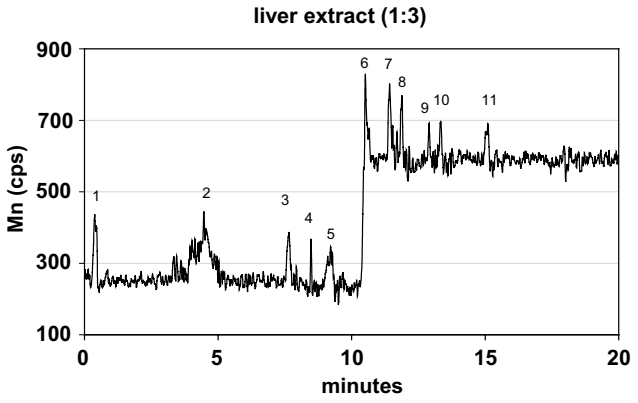

Fig. 5. An electropherogram of a liver extract (1:3 dilued) is shown. Several peaks are monitored, which partly could be assigned to and/or identified as specific Mn compounds given in Table 2. The numbering refers to Table 2.

were identified. Some of them may be discussed as the relevant (labile) species targeting to brain.

Acknowledgements

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