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Separation of metalloporphyrins by capillary electrophoresis with UV detection and inductively coupled plasma mass spectrometric detection

K.L. Ackley, J.A. Day, J.A. Caruso*

Department of Chemistry, University of Cincinnati, ML 0172, Cincinnati, OH 45069, USA

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Abstract

Vitamin B_{12}, cobalt protoporphyrin, manganese protoporphyrin, and zinc protoporphyrin were separated using capillary electrophoresis, and a comparison was made between detection with inductively coupled plasma mass spectrometry (ICP-MS) and UV detection. Absolute limits of detection were slightly better with ICP-MS detection than with UV detection, but for both methods absolute detection limits were in the picogram range. The migration times of the analytes decreased by several minutes when ICP MS detection was employed, and this phenomenon was believed to be a result of a ''suction effect'' that developed when the CE capillary was interfaced to the ICP-MS nebulizer. However, the resolution between species containing the same metal atom was not altered significantly, and the separation was completed in much less time relative to separations performed with UV detection. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Metalloporphyrins are of great biological and geological significance. Heme and vitamin B_{12} are examples of two naturally occurring metalloporphyrins that are of biological importance. Zinc protoporphyrin (ZnPP) is an intermediate metabolite of heme biosynthesis [1], and elevated levels of ZnPP are found in the blood in cases of lead poisoning. Metalloporphyrins are also constituents of geological materials such as shale and petroleum [2].

Chromatographic techniques are often employed in the separation of porphyrins and metalloporphyrins. Reversed-phase HPLC has been used to separate free porphyrins [1,3], metalloporphyrins [1,4,5], and porphyrin and porphyrin precursors [6]. Gas chromatography has been used to separate metalloporphyrins of geological significance [7–10].

Capillary electrophoresis (CE) has also proven to be an effective technique for the separation of porphyrin species. CE was used to analyze polyhaematoporphyrin [11] and Photofrin [12], both used in photodynamic therapy. Urinary porphyrins have been separated by CE using absorbance and fluorescence detection [13], and micellar electrokinetic capillary chromatography (MEKC) has been utilized to separate a variety of porphyrin species including metalloporphyrins [14,15].

The fast equilibration times, rapid separations, and

*Corresponding author. Fax: +1-513-556-0142.

E-mail address: joseph.caruso@uc.edu (J.A. Caruso).

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reduced sample and solvent consumption associated with CE make it an attractive technique for the separation of metalloporphyrins. Inductively coupled plasma mass spectrometry (ICP-MS) is an excellent detector for this type of separation due to its excellent sensitivity and element specificity. Unlike universal detectors, full resolution between species containing different metal atoms is not necessary with ICP-MS since multiple mass to charge ratios may be scanned. However, the interface between CE and ICP-MS is not trivial. A ground connection must be made to the exit end of the capillary since it must be connected with the nebulizer and no longer rests in a buffer reservoir. Furthermore, the low liquid flow-rates associated with CE are an additional consideration. The coupling of CE with ICP-MS has been the subject of many papers and reviews [16–22].

In this work, the CE capillary was interfaced to the ICP using a four way union which accommodated the ground connection, the CE capillary, the buffer sheath flow, and nebulizer connection. The CE interface is similar to those reported by Prange and Schaumlöffel [23] as well as Majidi and Miller–Ihli [20]. The goal of this work was to separate a variety of metalloporphyrin species using capillary electrophoresis and to compare the use of UV detection with ICP-MS detection for this separation.

2. Experimental

2.1. Instrumentation

All CE work was performed on a Waters Quanta 4000 CE system (Millipore, Milford, MA, USA) with a positive power supply, and UV detection was performed at a wavelength of 214 nm. Fused silica capillary with an I.D. of 75 μm (Polymicro Technologies, Phoenix, AZ, USA) was used for all CE separations. The effective capillary length was 78 cm for UV work and 80 cm for ICP-MS work. Samples were injected hydrostatically for 30 s, and the run voltage was 20 kV. ICP-MS work was performed using a PQ2 STE (VG Elemental, Winsford, UK) and a microconcentric nebulizer (Cetac Technologies, Omaha, NE, USA). Table 1 shows the ICP-MS operating conditions. A 40 ml single pass, glass spray chamber was used.

Table 1
ICP-MS operating conditions for CE work

Auxiliary gas flow	1 l min^{-1}
Coolant gas flow	16 l min^{-1}
Nebulizer gas flow	Optimized daily
Nebulizer gas pressure	414 kPa
Radio frequency (RF) power	1350 W
Masses monitored	^{59}Co
	^{55}Mn
	^{66}Zn
	^{64}Zn
	^{115}In

2.2. ICP-MS interface

A schematic of the interface used is shown in Fig. 1. A 4-way polyether ether ketone (PEEK) tee with a 0.0508 cm thru-hole and 4 PEEK finger-tight fittings were used (Upchurch Scientific, Oak Harbor, WA, USA). The center of the tee piece was drilled out with a 1/16 in. (1 in.=2.54 cm) drill bit. The capillary was secured in a length of 0.0508 cm I.D. PEEK tubing and passed through the tee to the nebulizer. PEEK tubing of 0.0762 cm I.D. surrounded the capillary as it passed from the tee piece to the nebulizer. The larger I.D. of the tubing allowed the buffer sheath to flow around the capillary and to the nebulizer. The ground connection was made by exposing to the solution a length of

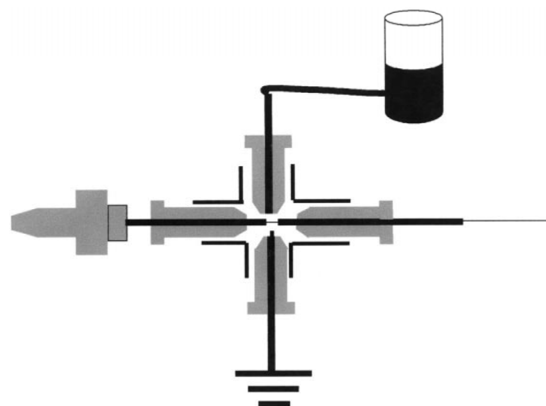

Fig. 1. CE–ICP-MS interface.

platinum wire sealed with a piece of 0.0508 cm I.D. PEEK tubing.

3. Reagents and standards

A variety of CE running buffers were investigated, and the best separation was achieved with a 3-cyclohexylamino-1-propanesulfonic acid (CAPS) buffer (Aldrich, Milwaukee, NI, USA). A 15 m*M* CAPS solution was prepared in distilled, deionized water, adjusted to pH 10 with 14.8 *M* ammonium hydroxide (Fisher Scientific, Fair Lawn, NJ, USA). The buffer sheath flow used in the ICP-MS work was made of a 0.1 m*M* CAPS solution containing 1 ng g^{-1} In. The In (prepared with serial dilutions from a 10 μg ml^{-1} standard, SPEX CertiPrep, Metuchen, NJ, USA) was added to monitor the sample introduction and instrument performance during the CE separations. Protoporphyrin species were obtained from Porphyrin Products (Logan, UT, USA), and vitamin B_{12} was obtained from Sigma (St. Louis, MO, USA). All reagents were used without further purification. Stock solutions were prepared in 2% (v/v) ammonium hydroxide solution, and serial dilutions were performed with the appropriate solvent. Acetone and methanol (Fisher Scientific) were HPLC grade.

4. Results and discussion

UV detection is often utilized with CE separations because it is simple to use, rugged, and inexpensive. Thus, UV detectors are frequently included as part of commercially available CE systems. Fig. 2 shows the separation of vitamin B_{12}, CoPP, MnPP, and ZnPP using standard on-column UV detection. A peak from an unidentified UV absorbing species is observed between the peaks for vitamin B_{12} and CoPP, and another unidentified peak is observed at the beginning of the MnPP peak. With UV detection, one cannot ascertain if the unknown peaks contain metal species and what those metal species are. This illustrates a limitation of UV detection, which is that only limited information is available about the peaks observed in the electropherogram.

Detection limits for the analytes were calculated using UV detection and ICP-MS detection, and they are reported in Table 2. Detection limits for ZnPP were not calculated using UV detection since the ZnPP peak was so broad. A 100 μg g^{-1} ZnPP solution was barely distinguishable from the

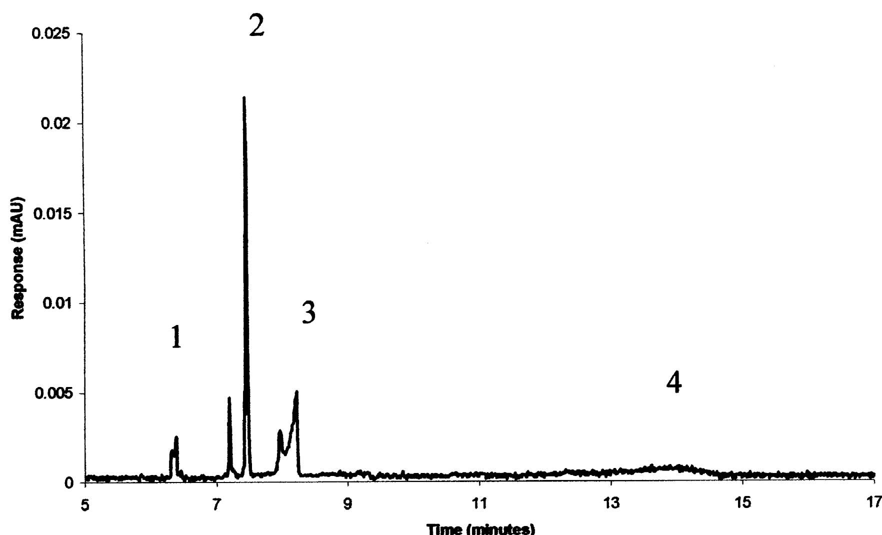

Fig. 2. Electropherogram of a 100 mg kg^{-1} mix of analytes in methanol obtained using UV detection. Peak identification: 1=vitamin B_{12}, 2=cobalt protoporphyrin, 3=manganese protoporphyrin, 4=zinc protoporphyrin.

Table 2
Absolute limits of detection obtained with UV detection and ICP-MS detection

	Absolute detection limit (pg)[a]			
	Vitamin B_{12}	CoPP	MnPP	ZnPP
UV detection	195	10.5	73.0	Not determined
ICP-MS detection	15.2	4.44	88.8	496

[a] Absolute limits of detection were determined by multiplying the sample volume by three times the standard deviation of the peak area for a blank divided by the slope of the calibration curve.

baseline. Absolute detection limits for the analytes were calculated to take into account the slight differences in experimental parameters between the UV work and the ICP-MS work such as the total capillary length and sample solvent viscosity. The detection limit for vitamin B_{12} was large with UV detection because the migration time of vitamin B_{12} corresponds to the migration time of a neutral species. The UV detector was zeroed based on the absorbance of the CAPS buffer. A disturbance in the baseline was consistently observed at this point as neutral solvent molecules passed by the detector window. This disturbance resulted in a high standard deviation in the peak area obtained when the blank was injected resulting in an elevated limit of detection. The detection limit was low for CoPP, which is not surprising since the peak obtained when CoPP was injected was very sharp.

Overall, detection limits obtained with UV detection were slightly higher than those obtained with ICP-MS detection. However, with both methods of detection, the absolute detection limits are in the picogram range. Because the volume of sample injected on the capillary is typically less than 100 nL, the concentration based detection limits for both methods were much higher ranging from the low μg g^{-1} to high ng g^{-1} concentration.

Table 3 shows the migration times of the analytes in a variety of solvents when UV detection was utilized. The migration times were relatively independent of the sample solvent, so the separation was relatively insensitive to matrix effects. However, as mentioned previously, the UV detector was zeroed based on the absorbance of the CAPS buffer. When acetone was used as the sample solvent, a large negative peak was observed at the expected migration time of vitamin B_{12}. The negative peak was believed to be a result of neutral acetone molecules passing through the detector at the same time as the vitamin B_{12}. The absorbance of the acetone is less than the absorbance of the buffer creating a negative peak despite the fact that vitamin B_{12} molecules are present.

ICP-MS can be a useful detector for CE separations involving metallic species. With ICP-MS detection, full resolution between species containing different metal atoms is not necessary. Furthermore, only species containing the same mass to charge ratio as the element(s) of interest interfere with the separation and detection of the analytes. With UV detection, any species that absorbs in the UV may hinder detection of the analytes of interest. Fig. 3 shows the separation of vitamin B_{12}, CoPP, MnPP, and ZnPP with ICP-MS detection. The interfering species that overlapped with the MnPP peak in the electropherogram obtained with UV detection was a

Table 3
Migration times for analytes with UV detection

Solvent	Average analyte migration time (min)[a]			
	Vitamin B_{12}	CoPP	MnPP	ZnPP[b]
Water	6.9±0.0	8.3±0.1	9.3±0.0	17.0±0.1
Acetone[c]	6.9±0.1	8.2±0.1	9.0±0.1	16.4±0.2
Methanol	7.0±0.1	8.3±0.0	9.0±0.2	16.6±0.0
2% NH_4OH	6.9±0.2	8.2±0.2	9.4±0.3	15.1±0.5
CAPS buffer	7.1±0.3	8.4±0.3	9.3±0.4	16.8±0.6

[a] $n=3$. Results are reported as the mean±2 times the standard deviation of the migration time.

[b] ZnPP peaks were broad, so determining the peak maximum was often difficult.

[c] When acetone was the solvent, a large negative peak corresponding to the expected migration time of vitamin B_{12} was observed. The baseline signal was established with the run buffer, not the injection solvent, so the negative peak may be a result of the plug of acetone passing the detector window. Since acetone does not absorb strongly at 254 nm, a negative peak would be observed. The vitamin B_{12} migration time was taken to be the same as that of the negative peak.

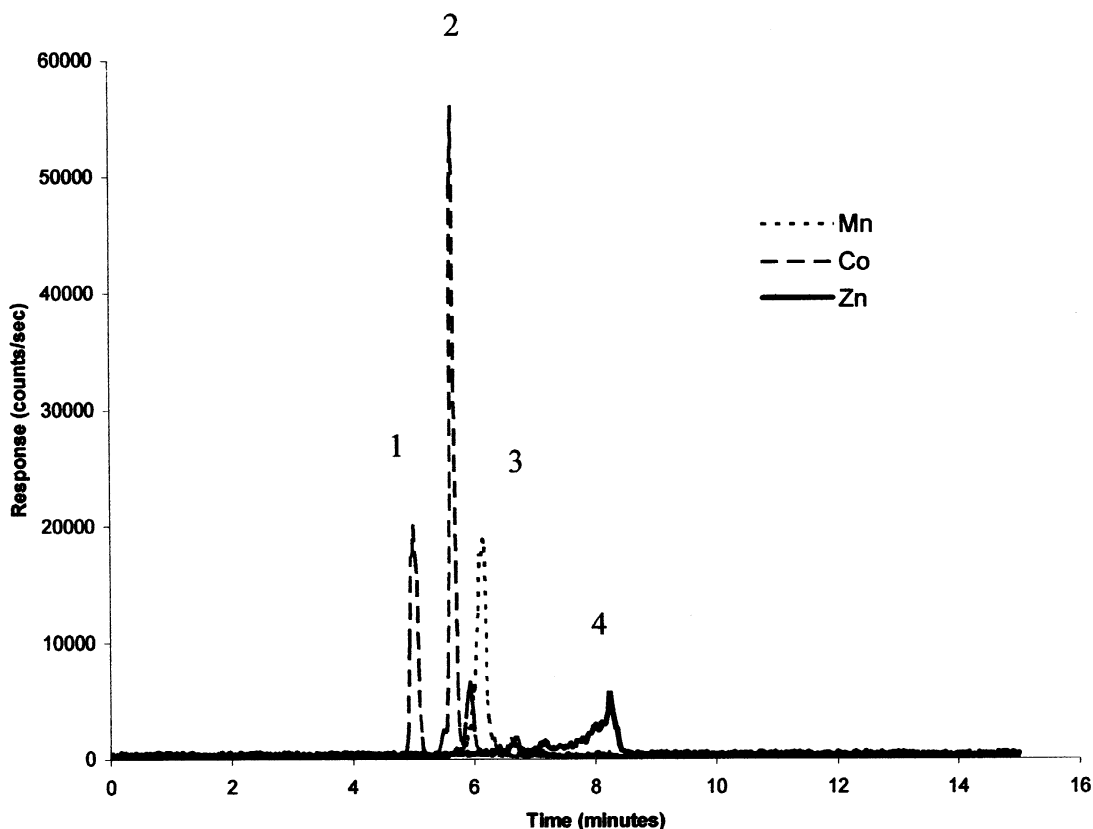

Fig. 3. Electropherogram of a 100 mg kg^{-1} mix of analytes in methanol obtained using ICP-MS detection. Peak identification: 1=vitamin B_{12}, 2=cobalt protoporphyrin, 3=manganese protoporphyrin, 4=zinc protoporphyrin.

cobalt containing species, and thus did not interfere with the determination of MnPP using ICP-MS detection.

Table 4 shows the migration times of the analytes in a variety of solvents when ICP-MS detection was utilized. The migration times were shorter with ICP-MS detection than with UV detection. The shorter migration times may be a result of several factors. The migration time of an analyte is directly proportional to the product of the effective capillary length and the total capillary length. The effective length of the capillary is equal to the total length of the capillary when ICP-MS detection is used. The length of the capillary for ICP-MS work was 80 cm, so the product is 6400 cm^2. The effective capillary length for the UV work was 78 cm, and the total length was 85.7 cm. Thus, the product was 6685 cm^2, and slightly longer migration times would be expected with the UV detection conditions than with the ICP-MS conditions. Also, suction effects can be a problem when ICP-MS detection is used. The "suction effect" is caused by the flowing nebulizer gas that generates a region of reduced pressure at the tip

Table 4
Migration times for analytes with ICP-MS detection

Solvent	Average analyte migration time (min)[a]			
	Vitamin B_{12}	CoPP	MnPP	ZnPP[b]
Water	4.6±0.1	5.4±0.2	6.0±0.2	7.9±0.2
Acetone	5.1±0.5	5.9±0.6	6.6±0.9	8.7±0.8
Methanol	4.9±0.2	5.6±0.3	6.2±0.3	8.3±0.6
2% NH_4OH	4.7±0.1	5.6±0.1	6.3±0.1	8.3±0.2
CAPS buffer	4.9±0.5	5.8±0.6	6.6±0.8	9.1±1.3

[a] $n=3$.
[b] Results are reported as the mean±2 times the standard deviation of the migration time.

of the nebulizer that may pull the analytes through the capillary causing the migration times to be a result of the suction effect and the analytes' electrophoretic mobilities rather than the electrophoretic mobilities alone. The buffer sheath flow that is introduced through the tee piece helps to reduce the suction effect by applying a slight pressure at the end of the CE capillary. Elevating the reservoir supplying the dilute buffer for the sheath flow has also been reported to reduce the suction effect and increase the migration time of the analytes [16,23,24]. The effect of elevating the buffer reservoir was investigated in this work, and dramatic changes in the migration times were not observed when the reservoir was raised several centimeters above the CE–ICP-MS interface. Further attempts were not made to reduce suction effects since with this separation, suction effects were advantageous since migration times were shortened without the loss of resolution between species containing the same metal atom. This illustrates that the suction effect does not always have a negative impact on the CE separation.

5. Conclusions

ICP-MS detection and UV detection are both effective methods for detecting metalloporphyrin species separated by capillary electrophoresis. ICP-MS offers absolute detection limits that are slightly lower than those obtained with UV detection, and complete resolution between species containing different metal atoms is not necessary. However, UV detection is simpler to perform than ICP-MS detection making it a desirable detector despite its slightly higher detection limits.

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