ELSEVIER

Food Chemistry 81 (2003) 607–611

Food Chemistry

www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods Section

Sensitive determination of trace-metal elements in tea with capillary electrophoresis by using chelating agent 4-(2-pyridylazo) resorcinol (PAR)

Huatao Feng, Tianlin Wang, Sam Fong Yau Li*

Department of Chemistry, National University of Singapore, 10 Kent Ridge Crescent, Singapore 119260, Singapore

Received 5 March 2002; received in revised form 15 November 2002; accepted 15 November 2002

Abstract

A capillary electrophoresis method was developed for the determination of trace metals (Cu, Fe, Zn, Co and Ni) in tea based on the conversions of strong complexes between 4-(2-pyridylazo) resorcinol (PAR) and metals. Five metals can be determined in 10 min by a single run. The calibration curves showed a linear range from 50 μg/l to 5 mg/l for these metals with correlation coefficients (r^2) being greater than 0.999. Detection limits (S/N = 3) of Co, Fe, Cu, Zn and Ni varied from 6 to 30 μg/l. Recoveries of these metal ions ranged from 97.9 to 106.2%. The method is simple and separation time is short.

Keywords: Capillary electrophoresis; Tea; Metals; 4-(2-Pyridylazo) resorcinol (PAR)

1. Introduction

Tea is made from the processed leaves of the plant *Camellia sineneis*, which is grown in acidic soil widely from tropical to temperate regions and contains 4–9% inorganic matter (Erdemoglu, Turkdemir, & Gucer, 2000; Yamamoto & Tuneja, 1997). Tea infusion is one of the most popular drinks around the world. The major metal ions in tea infusion are Mg, Al, K, Ca and Mn, but trace metals concentration also plays an important role in the quality of tea. Much higher concentrations of trace metals influence the taste of the tea infusion and do harm to consumers' health. The main sources of trace metals to plant are their growth media, like nutrient solution and soil (Kabata-Pendias & Pendias, 1984). In 1976, the Association of Analytical Communities had stipulated a special Official Method of Analysis for Copper and Nickel in Tea (Helrich, 1990).

Over the last two decades, the growth of the environmental movement combined with concerns about health and quality of diet have led to a rapid growth in the consumption of organic food. In the European Union, the land area under organic production increased from 0.16 million hectares in 1988 to nearly 2.1 million hectares by the end of 1997 (Wright & McCrea, 2000). In the second Draft of Basic Standards for organic products, International Federation of Organic Agriculture Movement made decisions that all relevant measures should be taken to ensure uncontaminated organic food and soil, operators should take reasonable measures to identify and avoid potential contamination.

Atom absorption spectrometry is a widely used technique for the determination of trace-metals, but has limited capability of simultaneously determining multi-elements of metal ions. Inductively coupled plasma–atomic emission spectrometry, inductively coupled plasma–mass spectrometry, X-ray fluorescence spectrometry are sophisticated techniques capable of simultaneous multielement determination, but high cost and low availability are their common disadvantages. Since the 1980s, capillary electrophoresis (CE) has become a powerful modern analytical technique. Several reports have been published on CE separation and detection of trace metal ions using 4-(2-pyridylazo) resorcinol (PAR) as a chromogenic chelating agent (Hardy, Jones, Riviello, & Avdalovic, 1999; Regan, Meaney, & Lunte, 1994; Saitoh, Hoshino, & Yotsuyanagi, 1989; Timerbaev, Semenova, Jandik, & Bonn, 1994). Usefulness of

---

* Corresponding author. Fax: +65-6779-1691.
E-mail address: chmlifys@nus.edu.sg (S.F.Y. Li).

doi:10.1016/S0308-8146(02)00561-7

PAR in the CE separation and detection of trace metal ions lies mainly in its capability of forming intense colour complexes with a number of metal ions under properly controlled experimental conditions and providing highly sensitive detection for these metal ions (Liu, Liu, Chen, & Cheng, 2001; Oszwaldowski & Okada, 1999). But few applications of food analysis have been reported to date. In this paper, we report the development of a CE method for determination of trace metals in tea with use of buffer systems containing PAR.

2. Experimental

2.1. Apparatus

All CE experiments were carried out using a CE-L1 Capillary Electrophoresis System (CE Resources Pte Ltd, Singapore) with a SPD-10A UV–vis detector of Shimadzu Co. (Kyoto, Japan). Detection was made at 505 nm. Data acquisition and recording of electropherograms were accomplished with a CSW Chromatography Station (CE Resources Pte Ltd, Singapore).

Fused silica capillaries (50 μm i.d.) were the products of Polymicro Technologies (Phoenix, USA). Total length of the capillaries was 80.0 cm, and effective length from the injection end to the detection window was 66.0 cm.

2.2. Reagents

All metal ion stock solutions were prepared from their nitrates (Co^{2+}, Fe^{3+},Cu^{2+}) and sulphates (Ni^{2+}, Zn^{2+}) with pH value 1. The stock PAR (Aldrich, USA) solution was prepared at a pH value of 8.5. Ion additives, tetramethylammonium (TMA) bromide and tetrabutylammonium (TBA) bromide (TCI, Japan) were dissolved in water with a concentration 0.5 M. The separation electrolyte was prepared by dissolving N-tris[Hydroxymethyl]methyl-3-aminopropanesulfonic acid (TAPS) (Sigma, USA) and mixing it with an appropriate quantity of PAR and ion additives to reach the final concentrations of 10 mM for TAPS, 0.1 mM for PAR, 5 mM for TBA and 5 mM for TMA. The pH value was adjusted with NaOH. All reagents were of analytical-reagent grade. Water ($\geqslant$18 MΩ) used throughout the experiments was generated by a NANOpure ultrapure water system (Barnstead, IA, USA).

2.3. Procedures

The separation electrolytes were prepared daily. The precolumn formation of PAR-metal chelates was prepared by mixing an appropriate quantity of metal ions and 1 mM PAR. pH value was adjusted to 9.2 for forming stable chelates. Samples were introduced onto the capillary by applying a pressure, the separation voltage was 30 kV. Before the first daily run the capillary was rinsed with 1 M NaOH for 15 min, followed by water for 15 min and a 15 min rinse with the appropriate electrolyte solution. The rinsing procedure was repeated after every 10 runs.

2.4. Sample preparation

Tea sample (1.0 g) was weighed into a 200-ml beaker and 50 ml HNO_3 was added. After 10 min reaction, the tea sample was placed on a hot plate and evaporated to dryness. The tea sample was then cooled, 25 ml HNO_3 was added, followed by 5 ml $HClO_4$ and heated again to near dryness. The residue was transferred into a 100-ml calibrated flask and diluted to volume with water. A reagent blank, containing same amount of acids, was evaporated as earlier.

The following steps were the same as the procedure of standard curve for the precolumn formation of PAR-metal chelates.

3. Results and discussion

3.1. Composition of background electrolyte

Phosphate, TAPS, 2-(cyclohexylamino)ethanesulfonic (CHES) and N-[2-hydroxyethyl]piperazine-N′-[2-ethanessulfonic acid] (HEPES) were tested as the electrolyte buffer, the buffers of CHES, TAPS and HEPES generated lower currents and showed better separation than phosphate. PAR-metals chelates are stable and soluble in water when the pH value is greater than 8. But a buffer of pH greater than 9 deteriorated the resolution due to high electroosmotic flow (EOF). TAPS was selected as the suitable buffer by taking consideration of its useful pH range (useful pH ranges of three buffers: 6.8–8.2 for HEPES, 7.7–9.1 for TAPS and 8.6–10.0 for CHES), The concentration of TAPS was examined from 5 to 50 mM to be of no obvious influence on separation except that increases in concentration of TAPS reduced slightly the EOF. 10 mM TAPS was applied to the following experiments and it provided satisfying separation efficiency with a low current.

A small amount of PAR (0.1 mM) was added into the electrolyte to prevent the dissociation of relatively unstable chelates of PAR-metals (Cu^{2+} and Zn^{2+}) (Saitoh et al., 1989).

Without adding other modifiers, the peaks of Ni and Zn could not be separated from each other. It was proposed and confirmed that ion-association or ion-pairing reaction can be employed in the separation of metal chelates in CE (Iki, Hoshino, & Yotsuyanagi, 1993; Liu, Liu, & Cheng, 1999). Based on their studies, 5 mM

TMA and 5 mM TBA were added into the electrolyte as ion additives and the peaks of Ni and Zn were separated successfully. A complexing equilibrium by the introduction of another complexing agent may be useful to the resolution too, several complexing agents were tried in this research, including hydroxyisobutyric acid, citric acid, lactic acid, glycolic acid, iminodiacetic acid, urea and nitrilotriacetic acid. As a result of the strong complexing ability of PAR, only nitrilotriacetic acid can improve the separation slightly. However, adding nitrilotriacetic acid is not applicable because the peak heights of Cu and Zn would be reduced seriously.

3.2. Effects of the pH of the separation electrolytes

The maximal absorbance wavelengths of different PAR-metals chelates range from 500 to 510 nm. Thus 505 nm was set for detection. The optimum pH value was investigated in order to get both good peak height and resolution. Fig. 1 shows that the peak heights indicating detection sensitivities of PAR-metals (Cu $^{2+}$and Zn^{2+}) increase with increase of pH value. It may be due to the increase of the stability of PAR-metals ($Cu^{2+}$and Zn^{2+}) at higher pH value. However, a pH value above 9 caused such a high EOF that separation of all the PAR-metals chelates could not be achieved. Therefore, pH 8.75 was chosen as the optimal condition.

Compared with a previous study (Liu et al., 1999), we found that the use of separation electrolyte of pH 8.75 could result in not only more sensitive detection, but also a simpler rinsing procedure for the capillaries. High pH value favours the water solubilities of PAR and PAR-metals chelates. Thus, it becomes unnecessary to rinse capillaries often because capillaries would not be polluted quickly. A procedure for mildly rinsing capillaries with 1 M NaOH and water was employed at room temperature after each 10 runs in our experiments instead of heating the capillaries to 60 °C and performing the rinsing procedure after each 5 runs (Liu et al., 1999).

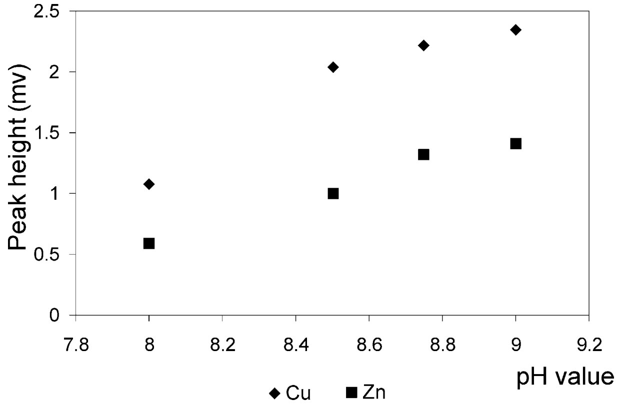

Fig. 1. The effect of pH value on peak heights. The separation electrolyte, 10 mM TAPS, 0.1 mM PAR. Applied voltage, 30 kV. Metal concentrations, 1 mg/l each. Sample introduction, pressure 10 s at 0.29 psi.

3.3. Separation of metal ions

As expected, the peak heights of PAR-metal chelates increased with the sample size introduced into the capillaries in our test. Baseline separation of all the chelates could still be accomplished with a sample loading of 10 s at 0.29 psi, which was set as the optimal sample loading time. When Fe^{3+} and Fe^{2+} ions were added into the standard sample at the same time, only one peak was observed. It might indicate that the Fe^{3+} ion was reduced to Fe^{2+} form under the above experimental conditions (Saitoh et al., 1989). A typical electropherogram for the separation of five metal ions (Co^{2+}, Fe^{3+},Cu^{2+}, Zn^{2+} and Ni^{2+}) was shown in Fig. 2. All of the metal ions were separated in less than 10 min run time. 10 mg/l each of Mn^{2+}, Cr^{3+} or Al^{3+}, which can form colour complexes with PAR normally, did not cause any observable effect on this separation, indicating that the present method was not prone to interference by these ions.

3.4. Quantification

Quantification was performed on the basis of measured peak area. Standard mixtures of the metal ions at six concentration levels were measured and peak areas were calculated. Calibration curves of all the metal ions exhibited linear dynamic ranges from 50 μg/l to 5 mg/l with correlation coefficients (r^2) being higher than 0.999 (Table 1). Relative standard deviations (RSD) were provided by six injections of a solution containing 200 mg/l of each metal ion. Detection limits ($S/N = 3$) of the five metal ions varied from 6 to 30 μg/l.

3.5. Sample analysis

The method was used for the determination of metal concentrations in three tea samples. No Co^{2+} was found in those samples. A typical electropherogram obtained was shown in Fig. 3. Since metals in the large amount of acids used for the digestion procedure were finally dissolved into the sample solution, low concentrations of Cu^{2+}; Fe^{3+}; Ni^{2+} were found in the reagent blank, the concentrations of those three metals were below 50 μg/l and very close to the detection limits. After subtracting the concentrations of metals in reagent blank, the concentrations of metals in the original tea samples were shown in Table 2. To obtain data for the calculation of recoveries, the sample solution was spiked with 400 μg/l each of the metal ions. Recoveries of the metal ions ranged from 97.9 to 106.2%.

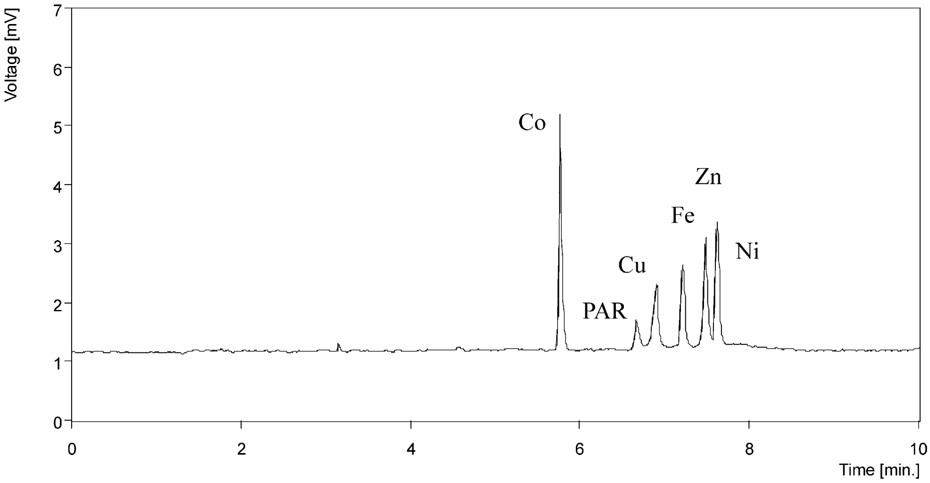

Fig. 2. Electropherogram of five metal ions under optimal conditions. The separation electrolyte, 10 mM TAPS, 0.1 mM PAR, 5 mM TBA, 5 mM TMA, pH value 8.75. Applied voltage, 30 kV. Sample introduction, pressure 10 s at 0.29 psi.

Table 1
The experimental quantification data under optimised condition

Metal ions	Calibration curves	Correlation coefficients	Detection limits (μg/l)	RSD (%) ($N=6$)
Co	$Y=0.1902X+0.0387$[a]	0.9998	17	0.7
Cu	$Y=0.2333X-0.114$	0.9992	6.0	1.2
Fe	$Y=0.3465X-0.0412$	0.9990	30	1.0
Zn	$Y=0.2489X-0.0031$	0.9996	24	1.5
Ni	$Y=0.2025X+0.0725$	0.9990	22	1.2

[a] X, Peak area (mVs); Y, Conc. of metal ions (mg/l).

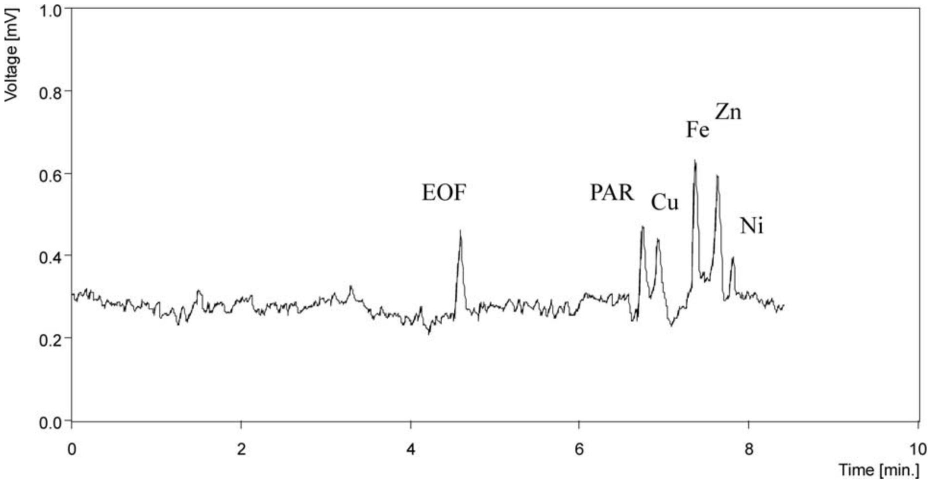

Fig. 3. Determination of metal ions in *Cui Ming* green tea. Running conditions were the same as Fig. 2.

Table 2
The metal concentrations in three tea samples

Tea samples	Cu (mg/kg)	Fe (mg/kg)	Zn (mg/kg)	Ni (mg/kg)
Cui Ming (green tea), China	21.83	74.02	48.77	7.51
Sea Dyke (oolong tea), China	14.21	13.97	24.59	3.07
Hill Bird (black tea), Singapore	23.94	135.5	28.42	4.60

4. Conclusion

The capillary electrophoresis method developed upon using PAR as a chromogenic chelating agent can be applied to the determination of trace metals in tea or possibly other foods. Five metals can be determined in a single run. The method showed advantages of simplicity in operation, speediness in separation and high sensitivity in detection.

Acknowledgements

The authors gratefully acknowledge financial support for this research by the National University of Singapore. The authors thank CE Resources Pte Ltd. for the loan of the CE-L1 Capillary Electrophoresis System used in this work.

References

Erdemoglu, S. B., Turkdemir, H., & Gucer, S. (2000). Determination of total and fluoride bound aluminium in tea infusions by ion selective electrode and flame atomic absorption spectrometry. *Analytical Letters*, *33*, 1513–1529.

Hardy, S., Jones, P., Riviello, J. M., & Avdalovic, N. (1999). Construction and investigation of a post-capillary reactor for trace metal analysis by capillary electrophoresis. *Journal of Chromatography A*, *834*, 309–320.

Helrich, K. (1990). *Official methods of analysis of AOAC* (15th ed.). Arlington: AOAC.

Iki, N., Hoshino, H., & Yotsuyanagi, T. (1993). Ion-association capillary electrophoresis, New separation mode for equally and highly charged metal chelates. *Journal of Chromatography A*, *652*, 539–546.

Kabata-Pendias, A., & Pendias, H. (Eds.). (1984). *Trace elements in soils and plants*. New York: CRC Press, Boca Raton.

Liu, B. F., Liu, L. B., & Cheng, J. K. (1999). Analysis of metal complexes in the presence of mixed ion pairing additives in capillary electrophoresis. *Journal of Chromatography A*, *848*, 473–484.

Liu, B. F., Liu, L. B., Chen, H., & Cheng, J. K. (2001). Separation of vanadium, niobium and tantalum as ternary mixed-ligand complexes by capillary electrophoresis using chelation with 4-(2-pyridylazo) resorcinol and tartaric acid. *Fresenius' Journal of Analytical Chemistry*, *369*, 195–197.

Oszwaldowski, S., & Okada, T. (1999). Micellar electrokinetic chromatographic separation of Fe(II)/Fe(III) and Cu(I)/Cu(II) as 2-pyridylazo chelates. *Microchemical Journal*, *62*, 138–146.

Regan, F. B., Meaney, M. P., & Lunte, S. M. (1994). Determination of metal ions by capillary electrophoresis using on-column complexation with 4-(2-pyridylazo)resorcinol following trace enrichment by peak stacking. *Journal of Chromatography B*, *657*, 409–417.

Saitoh, T., Hoshino, H., & Yotsuyanagi, T. (1989). Separation of 4-(2-pyridylazo)resorcinolato metal chelates by micellar electrokinetic capillary chromatography. *Journal of Chromatography*, *469*, 175–181.

Timerbaev, A. R., Semenova, O. P., Jandik, P., & Bonn, G. K. (1994). Metal-ion capillary electrophoresis with direct UV detection effect of a charged surfactant on the migration behaviour of metal-chelates. *Journal of Chromatography A*, *671*, 419–427.

Wright, S., & McCrea, D. (2000). *Handbook of organic food processing and production* (2nd ed.). Oxford: Blackwell Science.

Yamamoto, T., & Tuneja, L. R. (1997). *Chemistry and application of green tea*. New York: CRC Press, Boca Raton.