Analytical Methods

RSC Publishing

PAPER

View Article Online
View Journal | View Issue

Cite this: *Anal. Methods*, 2013, **5**, 982

A novel method of chemiluminescence detection coupled with high-performance liquid chromatography and its application in direct determination of tartaric, malic and citric acids in fruit juice†

Mao Deng, Fu-nan Chen* and Xing-yu Long

Received 3rd September 2012
Accepted 26th November 2012

DOI: 10.1039/c2ay25974e

www.rsc.org/methods

In the present work, a new method of chemiluminescence detection coupled with high performance liquid chromatography for three organic acids—tartaric, malic and citric acids is described. The detection is based on the enhancement of the organic acids for the chemiluminescence reaction between cerous sulfate ($Ce(SO_4)_2$) and tris (2,2′-bipyridyl)ruthenium(II) ($Ru(bipy)_3^{2+}$). The latter was immobilized on the cationic ion-exchange resin for obtaining high sensitivity and reducing consumption of expensive reagent. Under the optimal conditions, the linear ranges were 7.5×10^{-7} g mL^{-1} to 7×10^{-6} g mL^{-1} for tartaric acid, 4.0×10^{-7} g mL^{-1} to 1.0×10^{-5} g mL^{-1} for malic acid and 1×10^{-7} g mL^{-1} to 1.5×10^{-6} g mL^{-1} for citric acid, and the detection limits were 1.5×10^{-7} g mL^{-1}, 2.0×10^{-7} g mL^{-1}, 4.0×10^{-8} g mL^{-1} for tartaric, malic and citric acid, respectively. The proposed method had been successfully applied to the determination of three organic acids in fruit juice – with only filtration and dilution steps.

1 Introduction

Low molecular weight organic acids that play an important role in organoleptic properties (flavour, colour, and aroma), the stability and microbiologic control of beverages is an important group of compounds in fresh fruit juices and soft drinks. These organic acids come directly from the grapes or from the processes, such as alcoholic fermentation, malolactic fermentation, oxidation of the ethanol, *etc.*[1] In addition, organic acids indirectly affect the phenolic metabolism by altering pH, and act as precursors of phenolics and flavour compounds.[2]

Herein data on organic acids in foods are increasingly required by the food industry for quality control and they can also be used as indicators of deterioration due to the storage, aging, and even to measure the purity and authenticity. To obtain the organic acid profile and determine the most abundant acids, enzymatic[3] or chromatographic[4,5] methods are usually chosen, mainly high performance liquid chromatography on ion-exchange[6] or reversed-phase columns.[7,8] In recent years, the combination of sensitivity of chemiluminescence (CL) with rapidity and selectivity of high-performance liquid chromatography (HPLC) has made the HPLC-CL system extremely attractive,[9–11] especially in food analysis.[12,13] He *et al.* utilized the chemiluminescence of $Ru(phen)_3^{2+}$ with HPLC to the determination of oxalic acid.[14] In the CL system, AuNPs participated in CL reactions as catalyst. Based on this principle, Li *et al.* developed an HPLC-CL method using the triangular AuNPs as post-column CL reagents for the sensitive determination of low-molecular-weight aminothiols, in which the reduced aminothiols could form Au–S bond, leading to a greatly decreased CL intensity of the triangular AuNP-catalyzed luminol system.[15] Although these methods have been successfully applied to analysis of organic acid in a variety of samples, they suffered from tedious procedures, time consumption and high cost. Therefore, to develop a simple rapid inexpensive sensitive analytical method for the determination of organic acids in complex matrices is still significant.

Chemiluminescence (CL) is known as a powerful and important analytical technique because of its extremely high sensitivity along with its other advantages, such as simple instrumentation, wide calibration ranges, and suitability for miniaturization in analytical chemistry.[16–19] The advance of CL detection has catalyzed the growth and popularity of HPLC-CL application, and has made trace analysis possible owing to its

The Key Laboratory of Luminescence and Real-time Analysis, Ministry of Education, School of Chemistry and Chemical Engineering, Southwest University, Chongqing, 400715, China. E-mail: chenfn@swu.edu.cn; Fax: +86-23-68258363; Tel: +86-13752919874

† Electronic supplementary information (ESI) available: Fig. S1: immobilized $Ru(bipy)_3^{2+}$ chemiluminescence response *versus* time for the reaction of each $Ce(SO_4)_2$ reagent (8.0×10^{-4} mol L^{-1}) with citric acid (1×10^{-6} g mL^{-1}); Fig. S2: the effect of H_2SO_4 concentration. $Ce(SO_4)_2$: 8.0×10^{-4} mol L^{-1}, 4.5×10^{-6} g mL^{-1} for tartaric acid, 2.5×10^{-6} g mL^{-1} for malic acid, 5×10^{-7} g mL^{-1} for citric acid respectively, flow rate of peristaltic pump: 1.2 mL min^{-1}; Fig. S3: the effect of $Ce(SO_4)_2$ concentration. H_2SO_4: 0.04 mol L^{-1}, 4.5×10^{-6} g mL^{-1} for tartaric acid, 2.5×10^{-6} g mL^{-1} for malic acid, 5×10^{-7} g mL^{-1} for citric acid respectively, flow rate of peristaltic pump: 1.2 mL min^{-1}; Fig. S4: calibration curves for tartaric, malic and citric acids. See DOI: 10.1039/c2ay25974e

capability of measuring pictogram or femtogram quantities of compounds in the column eluate.

Since the first report of tris(2,2′-bipyridyl)ruthenium(II) [$Ru(bipy)_3^{2+}$] as analytical chemiluminescent (CL) reagent by Hercules and Lytle[20] in 1966, the studies and the applications of $Ru(bipy)_3^{2+}$ have been widely carried out in the last 40 years, in which the CL system using $Ru(bipy)_3^{2+}$ has become a powerful tool for determining aliphatic amines, amino acids and oxalates, due to its high sensitivity. Nevertheless, its widespread applications are limited by the requirement to continuously deliver higher concentration of $Ru(bipy)_3^{2+}$ into the reaction zone because $Ru(bipy)_3^{2+}$ is consumed, which implies high cost and pollution.[21] The immobilized $Ru(bipy)_3^{2+}$ has received much attention and appeared in the literature in recent years including Langmuir–Blodgett,[22] self-assembled techniques,[23–25] Nafion films,[26,27] silica nanoparticles,[28] and sol–gel[29] techniques. To our knowledge, once $Ru(bpy)_3^{2+}$ is immobilized, several advantages are achieved.

In this paper, a new HPLC-CL method by immobilizing $Ru(bipy)_3^{2+}$ on cationic ion-exchange resin for the simultaneous determination of tartaric, malic and citric acid is developed. This new method does not need to deliver $Ru(bipy)_3^{2+}$ to CL reaction zone, so it will cut the consumption of expensive reagents, and will not require additional tubing, mixing chamber and pump. The $Ru(bipy)_3^{2+}$ immobilized on cationic ion-exchange resin is stable, and can be used at least for 6 months to react with the dilute $Ce(SO_4)_2$ solution. The reaction of $Ru(bipy)_3^{2+}$ and acidified cerium(IV) produces luminescence, which is enhanced by the presence of tartaric, malic and citric acids. This is because the organic acids can easily react with Ce(IV) to form activated complex and this activated complex will slowly decompose to form the reactive intermediate radical. $Ru(bipy)_3^{2+}$ can be oxidized to $Ru(bipy)_3^{3+}$ by Ce(IV) and this product reacts with the reactive intermediate radical to form $[Ru(bipy)_3^{2+}]^*$, which will change into $Ru(bipy)_3^{2+}$ with CL emission.[30,31] The CL intensity is related linearly to the concentration of tartaric, malic and citric acids. The proposed method has been applied to determine organic acids with satisfactory results.

2 Experimental

2.1 Materials and reagents

Analytical reagent grade chemicals and ultra-pure water were used throughout. $Ru(bipy)_3^{2+}$ and $Ce(SO_4)_2 \cdot 4H_2O$ were purchased from Beijing J & K Chemical Co., Ltd.

Tartaric, malic and citric acid were purchased from the Aladdin Chemistry Co. Ltd (Shanghai, China). Stock standard solutions of tartaric, malic and citric acid were prepared in ultra-pure water at 1 mg mL^{-1}. The stock solutions were diluted with the mobile phase before use.

Sulfuric acid was obtained from Kelong Chemical Reagent Factory (Chengdu, China). Stock solutions of $Ru(bipy)_3^{2+}$ (1.0×10^{-3} g mL^{-1}) dissolved in ultra-pure water and $Ce(SO_4)_2$ (0.1 mol L^{-1}) dissolved in 0.5 mol L^{-1} H_2SO_4 were also prepared. Standard solutions of $Ru(bipy)_3^{2+}$ and $Ce(SO_4)_2$ were prepared before using by dilution of the stock solutions with the ultra-pure water and 0.03 mol L^{-1} H_2SO_4, respectively. 732-type cationic ion-exchange resin was supplied by Kelong Chemical Reagent Factory (Chengdu, China). The HPLC mobile phases consisted of 0.002 mol L^{-1} sulphuric acid and 0.001 mol L^{-1} ammonium acetate. They were prepared fresh daily, filtered through a 0.45 μm membrane filter (Xinya, Shanghai, China), and then degassed prior to use.

2.2 Apparatus

The experimental setup for the HPLC-CL is shown in Fig. 1. High performance liquid chromatography was a Hitachi D-7000 (Japan) liquid chromatography equipped with a Rheodyne 7725i syringe-loading sample injector valve (20 μL-loop Cotati, CA, USA) and a Kromasil TM RP-C18 column (i.d. 150 mm × 4.6 mm, particle size: 5 μm, pore size: 100 Å, DaLian Elite Analytical Instrument Co. Ltd, China). Batch model BPCL ultra weak chemiluminescence analyzer (Institute of Biophysics, Chinese Academy of Sciences, Beijing, China) was employed to study the characteristics of the CL reaction. The CL detection was conducted on a flow injection chemiluminescence system composed of a peristaltic pump (Ruimai Company Xi'an, China), PTFE tubing (0.8 mm i.d.) which was used as connection material in the flow system and cationic ion-exchange column. The change of CL signal in the cationic ion-exchange column was detected and recorded with a computerized BPCL ultra weak chemiluminescence analyzer. Data acquisition and treatment were performed with BPCL software running under Windows XP.

2.3 Sample preparation procedures

Sample treatment was performed as follows. Organic acids were extracted as described by Tomás Pérez-Ruiz and co-workers with some modifications.[32] Briefly, the freshly pressed fruit juice samples were centrifuged for 5 min at 3000 rpm and filtered through a 0.45 μm filter. An amount of 10 mL of the filtrate was diluted to 200 mL with ultrapure water.

2.4 Cationic ion-exchange column preparation

Cationic ion-exchange column were prepared, as was previously reported.[33,34] 732-type resin was regenerated with 2 mol L^{-1} HCl, then washed with NaCl and H_2O. A 2.0 mL of resin was added into a 5 mL of 1.0 mmol L^{-1} $Ru(bpy)_3^{2+}$ solution. After 6 h, a 0.3 mL of resin was filled into the glass tube (15 mm × 3.0 mm i.d.),

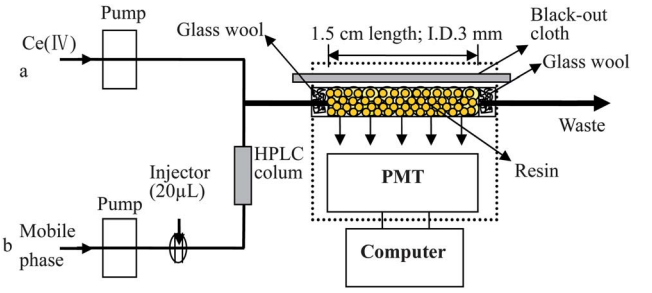

Fig. 1 Schematic diagram of HPLC-CL detection system.

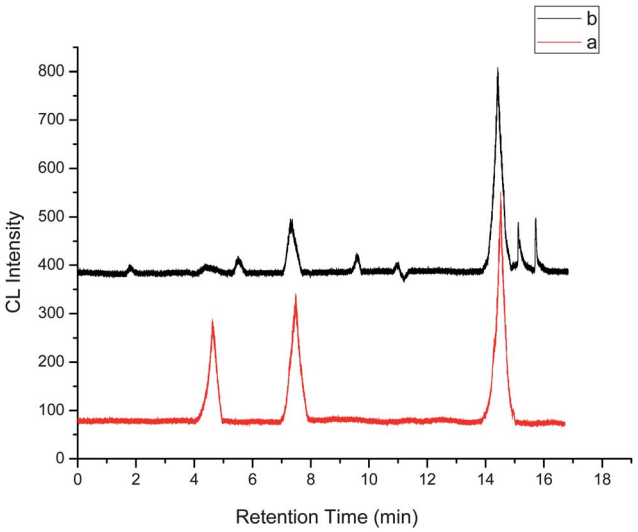

Fig. 2 Chromatogram of organic acids. Typical chromatograms obtained with (a) a standard mixture solution of organic acids; (b) organic acids in fruit juice. Peaks: tartaric (4.6 min), malic (7.4 min) and citric (14.4 min).

and some glass wool was inserted at both ends to prevent loss of resin.

2.5 Procedures

Flow lines were inserted into the $Ce(SO_4)_2$ solution and mobile phase respectively. The mobile phase was pumped through the column at a flow rate of 1.0 mL min^{-1}. The 20 μL standard solution or sample was injected into the mobile phase. This stream mixed with $Ce(SO_4)_2$ at a mixing-tee and then flowed into cationic ion-exchange column Fig. 1. Light emission was monitored by the photomultiplier tube. The quantitative determination was based on the net CL intensity $\Delta I = I_S - I_0$, where was the CL intensity in the presence of sample and I_0 is the CL intensity of blank signal.

3 Results and discussion

3.1 Cationic ion-exchange column stability

The stability of the cationic ion-exchange column was assessed in terms of their chemiluminescence intensity upon reaction with a citric acid standard using flow injection analysis (FIA). The relative CL intensity decreased only from 100–96.5% during 6 months, the $Ru(bipy)_3^{2+}$ immobilized on cationic ion-exchange resin was very stable (Fig. S1 in ESI†). A successfully prepared sensor can be used for at least 6 months, during which time it reacts with dilute $Ce(SO_4)_2$ solution.

3.2 Optimization of CL conditions

To obtain the maximum relative CL intensity and the highest sensitivity signal-to-noise ratio, the concentration of H_2SO_4, $Ce(SO_4)_2$ and the flow rate of peristaltic pump were investigated.

The effect of the type and concentration of the acidic medium, such as HCl, HNO_3, PPA (polyphosphoric acid), H_3PO_4 and H_2SO_4 on CL intensity was investigated. The results showed that sulfuric acid was a more suitable medium for all analytics since it gave the strongest light intensity and the highest signal-to-noise ratio (S/N). The effect of sulfuric acid concentration in the range 0.01–0.07 mol L^{-1} was further studied (Fig. S2 in ESI†). Sulfuric acid solution 0.04 mol L^{-1} was chosen as the optimum sulfuric concentration in $Ce(SO_4)_2$ solution.

As an oxidant, $Ce(SO_4)_2$ had a good effect on oxygenating $Ru(bipy)_3^{2+}$ and organic acids, respectively. The middle oxidation products interacted with each other and emitted luminescence owing to energy transfer. The effect of Ce(IV) concentration on the relative CL intensities was investigated from 8.0×10^{-5} to 2.0×10^{-2} mol L^{-1} (Fig. S3 in ESI†). At a concentration of 8.0×10^{-4} mol L^{-1}, we detected the highest CL signal. Above that, the CL intensity decreased and then gradually became stable. The was perhaps due to the decrease of the amount of Ce(IV) reactive species. So 8.0×10^{-4} mol L^{-1} $Ce(SO_4)_2$ was selected as optimum for the present system.

The flow rates of solutions are very important to CL reactions and should be regulated. Under the optimum conditions described above and at a constant flow rate of high-pressure pump 0.8 mL min^{-1}, the experimental results showed that maximum CL signal was achieved at its greatest when the flow rate of peristaltic pump was 1.2 mL min^{-1}.

3.3 Optimization of HPLC conditions

The HPLC system equipped with an ODS column was used to separate the organic acids. The choice of chromatographic conditions that ensured resolved peaks for the analytics were based on previous works.[35–38] We tested the situation of retention time and the CL behavior of some mobile phases such as methanol–water, methanol–sulphuric acid–water, methanol–phosphoric acid–water, sulphuric acid–water. Good separation was achieved by using an isocratic mobile phase composed of 0.002 mol L^{-1} sulphuric acid and 0.001 mol L^{-1} ammonium acetate. The flow-rate of the mobile phase was set at 0.8 mL min^{-1} in order to obtain a reasonable analysis time with a window 18 min.

Under these optimum conditions, HPLC was used to separate organic acids in fruit juice. Fig. 2 (curve a) shows the HPLC-CL chromatogram obtained from a standard mixture solution of

Table 1 Calibration curves, detection limits and precisions of organic acids ($n = 11$)

Organic acid	Equation (C: 10^{-3} g mL^{-1})	Correlation coefficient	Linear range (10^{-6} g mL^{-1})	LOD (10^{-6} g mL^{-1})	LOQ (10^{-6} g mL^{-1})	RSD (%)
Malic	$\Delta I = 36\,572C + 79.52$	0.9975	0.75–7	0.2	0.75	3.2
Tartaric	$\Delta I = 69\,554C + 86.21$	0.9995	0.4–10	0.15	0.4	3.4
Citric	$\Delta I = 824\,760C - 4.91$	0.9991	0.1–1.5	0.04	0.1	2.5

Table 2 Determination of tartaric, malic and citric acids in fruit juice ($n = 3$)

Samples	Organic acid	Detected (10^{-6} g mL^{-1})	Added (10^{-6} g mL^{-1})	Found (10^{-6} g mL^{-1})	Recovery (%)	RSD (%)
Apple juice 1	Tartaric	ND[a]	1.0	0.962	96.2	1.2
	Malic	4.822	0.5	5.325	100.0	1.5
	Citric	0.540	0.05	0.572	96.9	4.2
Apple juice 2	Tartaric	ND[a]	2.0	1.948	97.4	0.8
	Malic	4.792	1.0	5.932	102.4	1.4
	Citric	0.551	0.1	0.662	101.8	4.5
Apple juice 3	Tartaric	ND[a]	4.0	3.962	99.0	2.4
	Malic	4.851	2.0	7.087	103.5	2.0
	Citric	0.553	0.2	0.771	102.8	3.2
Orange juice 1	Tartaric	ND[a]	1.0	0.957	95.7	3.6
	Malic	0.621	0.5	1.082	96.4	3.8
	Citric	0.426	0.05	0.481	101.0	4.4
Orange juice 2	Tartaric	ND[a]	2.0	1.962	98.1	1.8
	Malic	0.641	1.0	1.681	102.4	2.5
	Citric	0.434	0.1	0.551	103.1	3.7
Orange juice 3	Tartaric	ND[a]	4.0	3.944	98.6	2.6
	Malic	0.612	2.0	2.594	99.4	2.2
	Citric	0.430	0.2	0.598	103.2	0.7

[a] ND (not detected).

organic acids; Fig. 2 (curve b) shows the chromatogram obtained from organic acids in fruit juice. From Fig. 2, it can be seen that organic acids can be well separated without the interference of other compounds in fruit juice. The retention times of tartaric, malic and citric acids are 4.6 min, 7.4 min and 14.4 min, respectively. Peak identification was carried out by the standard addition method and the retention time of organic acids. These chromatograms revealed that the application of HPLC-CL to the determination of three organic acids in fruit juice was possible.

3.4 Method validation

Validation of this method included the assessment of the regarding linearity, the limit of detection (LOD), limits of quantification (LOQ) and precision. To test the CL response linearity, a series of organic acids standard solutions at concentrations ranging from 7.5×10^{-7} g mL^{-1} to 7×10^{-6} g mL^{-1} for tartaric acid, 4.0×10^{-7} g mL^{-1} to 1.0×10^{-5} g mL^{-1} for malic acid, and 1×10^{-7} g mL^{-1} to 1.5×10^{-6} g mL^{-1} for citric acid, was determined (Fig. S4 in ESI†). Linear regression analysis of the results is summarized in Table 1. From these calibration curves, the limits of detection at a signal-to-noise of three were 0.2×10^{-6} g mL^{-1} for tartaric acid, 1.5×10^{-7} g mL^{-1} for malic acid, 4×10^{-8} g mL^{-1} for citric acid and the limits of quantification at a signal-to-noise of ten were 7.5×10^{-7} g mL^{-1} for tartaric acid, 4×10^{-7} g mL^{-1} for malic acid, 1×10^{-7} g mL^{-1} for citric acid, respectively.

The precision was tested with 11 repeated injections of two sample solutions containing organic acids at the concentration level of 1×10^{-6} g mL^{-1}. The relative standard deviations were 3.2% for tartaric, 3.4% for malic and 2.5% for citric respectively.

3.5 Application of the method

In order to validate the applicability of the proposed method in real samples, tartaric, malic and citric acids in fruit juice samples were analyzed by the present HPLC-CL system. The typical chromatograms with CL detection obtained with a standard mixture of tartaric, malic and citric acids are shown in Fig. 2. From Fig. 2 it can be seen that tartaric, malic and citric acids were simultaneously detected in fruit juice. The organic acids content of fruit juice were calculated by the calibration formula. The results of organic acids contents in fruit juice were shown in Table 2.

In order to evaluate the validity of the proposed method for the determination of organic acids in fruit juice, a recovery experiment was carried out by adding the known amounts of

Table 3 Determination of citric, malic and tartaric acids in fruit juice ($n = 3$)

	Citric (10^{-3} g L^{-1})		Malic (10^{-3} g L^{-1})		Tartaric (10^{-3} g L^{-1})	
Sample	HPLC-CL	HPLC-UV	HPLC-CL	HPLC-UV	HPLC-CL	HPLC-UV
Apple juice	0.09 ± 0.01	0.10 ± 0.01	7.80 ± 0.20	7.60 ± 0.20	ND[a]	ND[a]
Orange juice	8.40 ± 0.20	8.30 ± 0.20	0.43 ± 0.01	0.42 ± 0.01	ND[a]	ND[a]

[a] ND (not detected).

organic acids into the extracted samples, and analyzed by using the described method. The mean recovery percentages and the RSDs of the samples are showed in Table 2. The good recoveries (from 95.7% to 103.2%) of the known amount organic acids in fruit juice samples demonstrated the reliability of the present method for determining tartaric, malic and citric acids in fruit juice. The data of Table 3 shows that the content of the organic acids was in excellent agreement with that obtained by HPLC-UV method.

4 Conclusion

This work describes the simultaneous and sensitive detection of tartaric, malic and citric acids in fruit juice using reversed-phase high performance liquid chromatography with immobilized reagent chemiluminescence detection. In this method, luminescence reagent $Ru(bpy)_3^{2+}$ was immobilized on cationic ion-exchange resin, which made this method very simple, quick, reagent-saving and sensitive. It can be used to determine a large number of samples simultaneously.

Acknowledgements

The authors are grateful to Southwest University of China for financial support of Doctoral Project (no. 2120040511).

References

1 M. Inés, S. Silvia and F. H. José, *Food Res. Int.*, 2005, **38**, 1175–1188.
2 P. Flores, P. Hellin and J. Fenoll, *Food Chem.*, 2012, **132**, 1049–1054.
3 R. Puchades, M. A. Herrero, A. Maquieira and J. Atienza, *Food Chem.*, 1991, **42**, 167–182.
4 V. Dufkova, R. Cabala, D. Maradova and M. Sticha, *J. Chromatogr., A*, 2009, **1216**, 8659–8664.
5 H. Kelebek, S. Selli, A. Canbas and T. Cabaroglu, *Microchem. J.*, 2009, **91**, 187–192.
6 V. Magne, M. Mathlouthi and B. Robilland, *Food Chem.*, 1998, **61**, 449–453.
7 J. Erro, A. M. Zamarreno, J. C. Yvin and J. M. Garcia-Mina, *J. Agric. Food Chem.*, 2009, **57**, 4004–4010.
8 Y. J. Zheng, Y. T. Duan, Y. F. Zhang, Q. H. Pan, J. M. Li and W. D. Huang, *Chromatographia*, 2009, **69**, 1391–1395.
9 R. Li, T. Kamedab, Y. Li, A. Toribab, N. Tang, K. Hayakawa and J. M. Lin, *Talanta*, 2011, **85**, 2711–2714.
10 L. Wu, Q. L. Zhang, X. Y. Zhang, C. Lv, J. Li, Y. Yuan and F. X. Yin, *J. Agric. Food Chem.*, 2012, **60**, 9377–9383.
11 R. Li, T. Kameda, A. Toriba, K. Hayakawa and J. M. Lin, *Anal. Chem.*, 2012, **84**, 3215–3221.
12 E. Wołyniec, J. Karpińska, S. Łosiewska, M. Turkowicz, J. Klimczuk and A. Kojło, *Talanta*, 2012, **96**, 223–229.
13 M. Liu, Z. Lin and J. M. Lin, *Anal. Chim. Acta*, 2010, **670**, 1–10.
14 F. Wu, Z. K. He, Q. Luo and Y. Zeng, *Food Chem.*, 1999, **65**, 543–546.
15 Q. Li, F. Shang, C. Lu, Z. Zheng and J. M. Lin, *J. Chromatogr., A*, 2011, **1218**, 9064–9070.
16 S. Zhao, X. Li and Y. M. Liu, *Anal. Chem.*, 2009, **81**, 3873–3878.
17 Z. Wang, S. Y. Chin, C. D. Chin, J. Sarik, M. Harper, J. Justman and S. K. Sia, *Anal. Chem.*, 2010, **82**, 36–40.
18 X. Wang, N. Na, S. C. Zhang, Y. Y. Wu and X. R. Zhang, *J. Am. Chem. Soc.*, 2007, **129**, 6062–6063.
19 Y. Lv, S. Zhang, G. Liu, M. Huang and X. Zhang, *Anal. Chem.*, 2005, **77**, 1518–1525.
20 D. M. Hercules and F. E. Lytle, *J. Am. Chem. Soc.*, 1966, **88**, 4745–4746.
21 Y. J. Park, D. W. Lee and W. Y. Lee, *Anal. Chim. Acta*, 2002, **47**, 51–59.
22 M. M. Ligia, K. Thiago, B. Denis, P. Paolo, S. Neso and U. Paolo, *J. Electroanal. Chem.*, 2010, **640**, 35–41.
23 Y. S. Obeng and A. J. Bard, *Langmuir*, 1991, **7**, 195–201.
24 Y. Sato and K. Uosaki, *J. Electroanal. Chem.*, 1995, **384**, 57–66.
25 W. Y. Zou, L. Wang, B. Y. Lu, H. Li and H. Chen, *J. Appl. Electrochem.*, 2009, **39**, 2015–2020.
26 L. L. Shultz, J. S. Stoyanoff and T. A. Nieman, *Anal. Chem.*, 1996, **68**, 349–354.
27 T. M. Downey and T. A. Nieman, *Anal. Chem.*, 1992, **64**, 261–268.
28 X. Hun and Z. J. Zhang, *Sens. Actuators, B*, 2008, **131**, 403–410.
29 M. M. Collinson, B. Novak, S. A. Martin and J. S. Taussig, *Anal. Chem.*, 2002, **72**, 2914–2918.
30 F. W. Wu, Z. K. He, Q. Y. Luo and Y. E. Zeng, *Chin. J. Chem.*, 1999, **57**, 1213–1218.
31 J. Xi, X. Ai and Z. He, *Talanta*, 2003, **59**, 1045–1051.
32 P. R. Tomás, M. L. Carmen, T. Virginia and M. Jesús, *J. Chromatogr., A*, 2004, **1026**, 57–64.
33 Z. J. Xi, Z. J. Zhang, Y. H. Sun, Z. Shi and W. Tian, *Talanta*, 2009, **79**, 216–221.
34 J. M. Lin, F. Qu and M. Yamada, *Anal. Bioanal. Chem.*, 2002, **374**, 1159–1164.
35 J. Qiu, *J. Chromatogr., A*, 1999, **859**, 153–158.
36 R. Vonach, B. Lendl and R. Kellner, *J. Chromatogr., A*, 1998, **824**, 159–167.
37 C. Anna, S. Lorenzo, T. Carlo and C. Paolo, *J. Chromatogr., A*, 1994, **669**, 59–64.
38 H. A. Samy and M. J. Knox, *J. Chromatogr., A*, 1984, **299**, 288–292.

