JAAS

Accepted Manuscript

This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/jaas

Determination of binding sites and binding constants between Pb(II) and DNA using capillary electrophoresis combined with electrothermal atomic absorption spectrometry

Yuying Liang, Biyang Deng*, Caiying Shen, Xiangdong Qin, Shaojun Liang

Key Laboratory for the Chemistry and Molecular Engineering of Medicinal Resources (Ministry of Education of China), School of Chemistry and Pharmaceutical Sciences, Guangxi Normal University, Guilin 541004, China

*Corresponding Author:

Telephone: +86-773-5845726; Fax: +86-773-2120958. Email: dengby16@163.com

Abstract: A new method for study the interaction of lead(II) and DNA was developed using capillary electrophoresis online coupled with electrothermal atomic absorption spectrometry. Under optimized experimental conditions, the detection limit (3σ) for Pb(II) was observed as 1.8×10^{-6} mol L^{-1} through running 10 replicates of the reagent blank. The relative standard deviation (RSD, n = 5) of 0.5×10^{-4} mol L^{-1} was calculated as 3.4%. The primary binding number (n_1) and binding constant (K_1) of Pb(II)–DNA were observed as 0.57 and 6.2×10^4 L mol^{-1}, respectively. The non-specific binding number (n_2) and binding constant (K_2) of Pb(II) and DNA were found as 1.1 and 1.9×10^4 L mol^{-1}, respectively. This new method allows rapid analysis of a small amount of sample in a simple way, whereas it prevents long periods of dialysis and eliminates the interferences from the

other metal ions. It provides a reliable and convenient new way to study the interactions between metal ions and biomolecules.

Keywords: Capillary electrophoresis; Atomic absorption spectrometry; Speciation analysis; Lead; DNA

1. Introduction

Lead, a heavy metal element, is a great harm to human health. It can damage various types of human organs, such as hematopoietic, nervous, urinary, digestive, reproductive, endocrine, cardiovascular, immune, and skeletal systems.[1] According to a survey, lead is the most important heavy metal that influences the children's health. In the body, the accumulation of lead species can affect the central nervous system, kidneys, and blood cells.[2] The absorbed lead in body can not only hinder the production of blood, causing anemia, but even make atherosclerosis, ulcers, and other gastrointestinal symptoms. It can also affect the growth and mental development of infant, causing severe dementia.[3,4] When lead invades the brain tissue via the blood flow after entering the human body, it violates supply of sufficient amounts of oxygen and nutrients to the brain and causing damage. Especially, children in the growth stage are more easily influence by lead than adults. Their adsorption of lead is several times more than adults.

DNA is an important biological macromolecule in organism that carries genetic information and material basis of gene expression.[5] A large number of genetic information are stored in DNA that not only influence the growth

and development of normal life activities for organism, but also are closely related to abnormal life activities, such as cancer and mutation. In organism, some metal ions can change the structure of DNA and thus affect the genetic information of DNA; therefore, it is necessary to study the interactions of metal ions with DNA.

The relationships between metals and life activities at the molecular level and the pathogenesis of metals pathogenic at the genetic level have been studied.[6,7] So far, there have been some methods for studying the interactions between metal ions and biomolecules, including electrochemical analysis,[8] Raman spectroscopy,[9] circular dichroism (CD),[10] UV–vis spectroscopy,[11] fluorescence spectroscopy,[12,13] nuclear magnetic resonance spectroscopy (NMR),[14] inductively coupled plasma mass spectrometry (ICP–MS),[15] inductively coupled plasma optical emission spectrometry (ICP–OES),[16] and electrothermal atomic absorption spectrometry (ETAAS)[17,18]. The interaction between lead and DNA was also reported using multi-techniques.[19-23] Li et al. reviewed the interactions of metal ions and biomolecules and illustrated advantages of CE-ETAAS including simplicity and easy operation, low instrumental and operational costs, high sensitivity, minimum sample and reagent consumption, and providing the stoichiometry, thermodynamics and kinetics information of the interaction between metal species and biomolecules.[24] So far, the interaction of Pb(II) and DNA has not been studied using capillary electrophoresis (CE) online

coupled with electrothermal atomic absorption spectroscopy (ETAAS).

In the past decades, the CE technique has been rapidly developed as a new type of microseparation and analytical technique. It was successfully combined with modern microcolumn separation and classical electrophoresis techniques, making it as a good performance separation technique. Capillary electrophoresis coupled with element-selective techniques has been reported, such as ICP–MS,[25] ICP–OES,[26] atomic fluorescence,[27] and atomic absorption spectrometry[28,29]. Element speciation analysis using ICP was reviewed in details by Timerbaev.[30] The high operation costs and interferences of ICP-based techniques, and the need for well-trained analysts are obviously limitations in establishing CE−ICP for routine speciation analysis. The ETAAS technique has some advantages: extremely high sensitivity and selectivity, fast analysis, wide applications, and little reagent consumption. Therefore, CE online coupled with ETAAS can combine their individual advantages to produce an efficient separation and highly sensitive detection analytical method.

In this paper, a new method is reported that uses CE–ETAAS to measure the equilibrium constant for the interaction between Pb(II) and DNA. The method promises substantial improvements in minimizing sample consumption, avoids long periods of dialysis, enhances analysis speed, facilitates the operation, and eliminates the interferences from the other metal ions. The method provides a reliable and convenient new way to study

the interactions between metal ions and DNA.

2. Experimental

2.1. Instruments

The measurements were performed using atomic absorption spectrometer (model TAS-986, Beijing Purkinje General Instrument, Beijing, China) equipped with a transversely heated graphite atomizer and a lead hollow cathode lamp as the radiation source. CE experiments were carried out with a HV-303P1 instrument (Tianjin Torch Technology, Tianjin, China). A fused-silica capillary (Yongnian Optical Fiber, China) with an inner diameter of 75 μm and length of 70 cm was used for CE separation. The interface of CE–ETAAS was made according to literature.[29,31] A spray interface was designed to allow the real-time ETAAS monitoring of individual species separated by CE technique. The interface used was made of PMMA transparent sheet, steel tube, glass pointed mouth, plastic box, metal platinum silk, bicomponent adhesive, and PTFE thread. In this interface, the miniature steel tube was used to coat capillary. The system reveals two benefits: It can be fixed so that preventing the carrier gas to collide the capillary and the platinum electrode can be attached to the steel pipe for conductivity purpose. The interface fixed the spray chamber with a round plastic bottle. The aerosol was sent into the electrothermal furnace by argon gas flow. The center hole of graphite tube was blocked by a T-shaped graphite plug. A concentric circular hole was drilled on the graphite tube side

with outer and inner diameters of 2.2 and 1.8 mm, respectively. The ceramic tube was closely inserted into the graphite side hole. The waste gas was excluded through two exit ends of the graphite furnace. The sample exited at the cathode. The improved hybrid technique showed some advantages: it did not need the cooling system; it did not need to introduce auxiliary liquid flow as conductive contact and avoided excess solvent into the atomizer; sample retention time in graphite furnace was prolonged, therefore, its atomizing efficiency was increased; it used graphite furnace heating ceramic tube that was found helpful for aerosol gasification and improvement of nebulization efficiency.

Table 1 The experimental conditions of CE– ETAAS operation

CE separating conditions		ETAAS operating conditions	
CE capillary:	70 cm×75 μm id.	Lamp current:	8 mA
Buffer: 50 $mmol^{-1}$ Tris-HCl, pH = 6.8		Spectrum bandwidth:	0.4 nm
Separation voltage:	24 kV	Measure mode:	Peak height
Injection way:	0.04 MPa ×10s	Carrier gas flow rate:	0.7 L min^{-1}
		Analytical line:	283.31 nm

Table 2 Controlling progress for ETAAS

Temperature (°C)	Heating up time (s)	Holding time (s)
100	10	200
120	10	130
400	3	5
1700	3	60
400	5	5
120	3	100
400	3	5
1700	5	90
2000	1	3

2.2. Reagents

All reagents used in this study were of analytical-reagent grade. The stock standard solutions were prepared with double distilled water and stored at 4 °C. Herring sperm DNA was directly dissolved in double distilled water at 1 × 10^{-3} mol L^{-1}, purchased from BioDee Company (Beijing, China). The Tris(hydroxymethyl)aminomethane (Tris) was obtained from Shanghai Sinopharm Chemical Reagent (Shanghai, China). Ammonium dihydrogen phosphate ($NH_4H_2PO_4$) was purchased form Guangzhou Chemical Reagent (Guangzhou, China). The run buffer solution of 50 mmol L^{-1} Tris-HCl (pH 6.8) was prepared through dissolving 1.401 g Tris and 0.145 g $NH_4H_2PO_4$ in 245 mL double distilled water. The lead stock solutions of 0.1 mol L^{-1} were

prepared through dissolving 3.312 g $Pb(NO_3)_2$ (Jinshan Chemical, Jinshan, China) in Tris-HCl (pH 6.8).

All solutions were filtered through a 0.45 μm filter (Shanghai Xinya Purification Material, Shanghai, China) prior to use.

2.3. Analytical procedure

A new capillary was conditioned via flushing sequentially with methanol for 30 min, then with a solution of 0.1 mol L^{-1} NaOH for 30 min, and finally with double distilled water for 5 min. Prior to separation, the capillary was flushed with the running buffer solution for 5 min. The capillary was reconditioned daily through flushing with 0.1 mol L^{-1} NaOH solution, and double distilled water for 10 min, and then with the running electrolyte for 5 min. The electrolyte used was a solution of 50 mmol L^{-1} Tris-HCl buffer at pH 6.8. The carrier gas for CE–ETAAS was argon. The CE–ETAAS operating parameters and detecting conditions are summarized in Table 1 and Table 2.

3. Results and discussion

3.1. Effect of Pb(II) on UV absorption spectrum of DNA

In order to examine if Pb(II) can combine with DNA, the effect of Pb(II) on UV absorption spectrum of DNA was investigated. With a fixed DNA concentration at 1×10^{-4} mol L^{-1}, the concentration of Pb(II) was varied from 0 to 8×10^{-5} mol L^{-1}. The mixture of Pb(II) and DNA solutions was analyzed with UV spectroscopy technique (Fig. 1). The results show a strong

absorption peak at 260 nm for DNA. With increasing the concentrations of Pb(II), it obviously happens to hypochromic effect. When the concentration ratio of Pb(II) and DNA is equal to 1, the absorption curve is not significantly difference to compare with the ratio for 0.8. The hypochromic effect can be occurred after the reaction between DNA and metal ions, as a measure of DNA combined with metal ions.[32] Therefore, the binding reaction was observed between Pb(II) and DNA. The reason may be the electrostatic interaction of phosphate of external DNA and Pb(II). At the same time, insertion of Pb(II) in the base pairs of DNA changes the DNA conformation, leading to decrease in UV absorption of DNA and produce hypochromic effect.

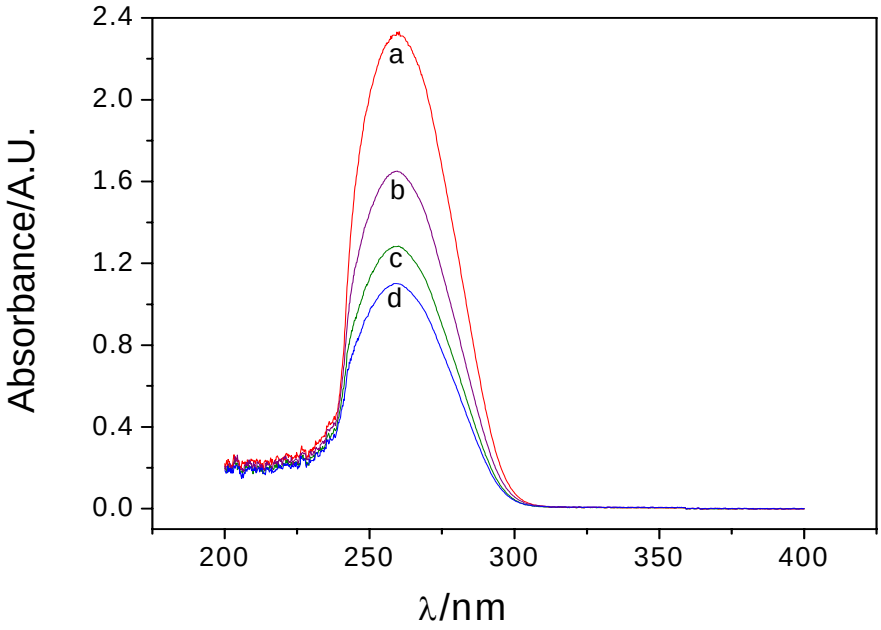


Fig. 1 Effect of Pb(II) on absorption spectra of DNA.

The [Pb(II)] / [DNA] ratio is: (a) 0, (b) 0.4, (c) 0.6, (d) 0.8.

3.2. Effect of incubation time

The UV absorbance spectra were recorded at various incubation times of DNA and lead ions at room temperature (Fig. 2). The Pb(II) was mixed with DNA at a ratio of 1:2 and the changes of DNA absorbance were recorded with time at a wavelength of 260 nm. The time at which the absorbance value is no longer changing is considered as the incubation time. The reaction of lead and DNA reached equilibrium in 10 h (see Fig. 2), thus, it was selected as the incubation time.

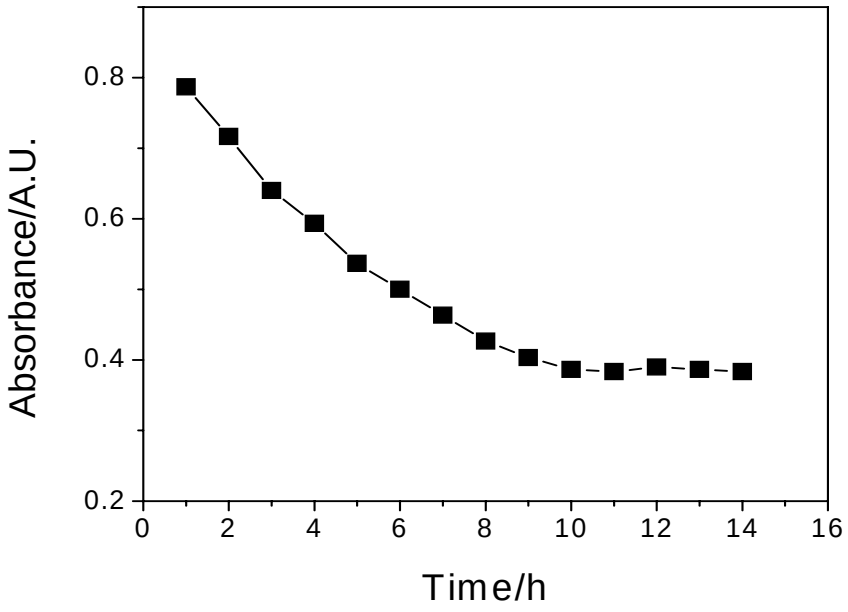


Fig. 2 Determination of incubation time of Pb(II) and DNA. Absorptions were measured at 260 nm, DNA and Pb(II) concentrations were 3.0×10^{-5} and 1.5×10^{-5} mol L^{-1}.

3.3. Effect of separation variables

The separation efficiency of capillary is associated with a number of influencing factors, especially the type of buffer solution, the buffer concentration, and pH, therefore, the choice of buffer solution is very

important. In consideration of physiological conditions, Pb(II) produces hydrolysis reaction in alkaline. The effect of buffer concentration on the separation was studied using five concentrations of Tris-HCl (20, 40, 50, 60 and 80 mmol L^{-1}) at pH 6.8. The results indicated the most obvious separation resolution with 50 mmol L^{-1} solution concentration. Therefore, a buffer of 50 mmol L^{-1} pH 6.8 Tris-HCl was chosen for further experiments. Because matrix modifier was required for lead ions atomization, the concentration of 0.5 mmol L^{-1} $NH_4H_2PO_4$ was selected as matrix modifier.

The effect of separation voltage on the migration time and electrophoretic resolution was investigated in the range of 18–24 kV. The results for Pb-DNA showed no peak within 10 min from 18 to 23 kV. But, the absorption peak of Pb-DNA was observed in 10 min when the voltage was increased to 24 kV. It was found that higher voltage was favorable to shorten migration time. However, higher voltage leads to worse electrophoretic resolution due to Joule heating effect. Considering the migration time and resolution, 24 kV was selected as the separation voltage.

3.4. Optimization of detection conditions

Atomization temperature affects the sensitivity. At high temperatures, it would provide short lifetime to the graphite tube, whereas low temperatures would cause incomplete atomization. Experimental results show that when the lead atomic temperature is above 1700 °C, the absorbance is essentially the same, thus we selected the atomization temperature of 1700 °C. The

adopted subsection control temperature provides some advantages. It can extend the service lifetime of the graphite tube and avoid high temperature accelerate graphite furnace aging. Subsection control temperature also accord with traditional electrothermal atomic absorption spectrometry characteristics.

The value of lamp current represents the intensity characteristics of radiation source. The greater lamp current provides higher radiation intensity, resulting in a higher sensitivity, but reduce lamp lifetime. In contrary, lower lamp current provides insufficient light energy, leading to lower sensitivity. In this experiments, when the lamp current was 8 mA, the optimal absorbance was obtained, therefore, lamp current of 8 mA used as the following examination.

The variations of lead absorbance were observed as a function of the carrier flow rate. The results are shown in Fig. 3. The lead absorbance increased with increase of the carrier gas flow rate, but too high carrier gas flow rate would dilute aerosol and reduce sensitivity. When the carrier gas flow rate was 0.7 L min^{-1}, the peak intensity reached to the largest and relatively stable value. Therefore, the carrier gas flow rate was selected at 0.7 L min^{-1} in the experiments.

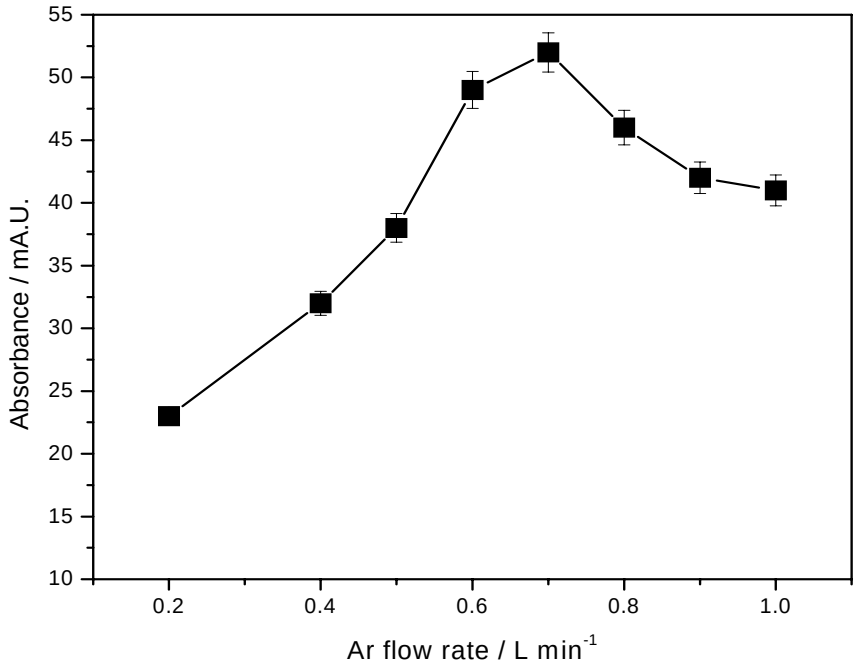


Fig. 3 Effect of Ar flow rate on the absorbance

The concentration of Pb(II) in the sample was determined as 5.0×10^{-5} mol L^{-1}, the examination conditions, except for the Ar flow rate, are listed in Table 1 and Table 2.

3.5. Analytical figures of merits

Under the optimized experimental conditions, the peak height was used for calibration. The detection limit (3σ) for Pb(II) was found as 1.8×10^{-6} mol L^{-1} by running 10 replicates of the reagent blank. The lead concentration of no-atomization and atomization in the reagent blank was 2.5×10^{-6} mol L^{-1} and 8.1×10^{-6} mol L^{-1}, respectively. The relative standard deviation (RSD,

n = 5) of 0.5 × 10^{-4} mol L^{-1} Pb(II) was calculated as 3.4%. When Pb(II) concentration of 5.0 × 10^{-5} mol L^{-1} was used, the recovery with the present CE–ETAAS method was greater than 97.26%. The linear range of Pb(II) concentration was observed between 5.7 μ mol L^{-1} and 0.43 mmol L^{-1}.

3.6. Separation of free Pb(II) and Pb(II)-DNA

Under the optimized experimental conditions, to eliminate the influence of lead species, a solution containing 1 × 10^{-4} mol L^{-1} DNA was analyzed using CE–ETAAS. The electropherogram (Fig. 4) shows that the DNA does not contain any lead species.

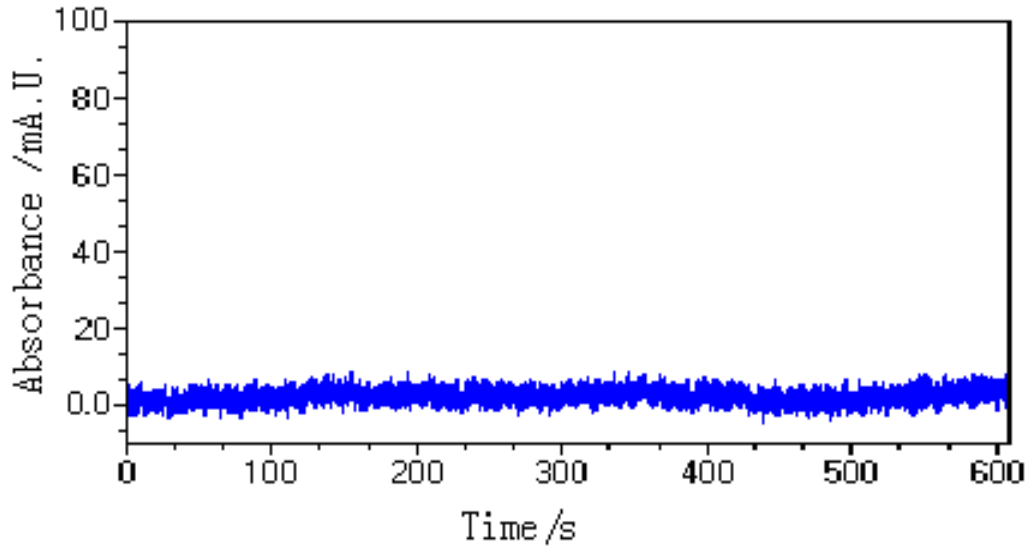


Fig. 4 Electropherogram of 1 × 10^{-4} mol L^{-1} DNA solution. Detection conditions are described in Table 1 and Table 2.

Under the optimized experimental conditions, 0.5 × 10^{-4} mol L^{-1} free lead(II) was analyzed and characterized using CE–ETAAS technique. The migration time of Pb(II) is 370 s (Fig. 5). In this work, we kept the DNA concentration constant at 1 × 10^{-4} mol L^{-1} and changed the concentration of Pb(II) from 0.2

× 10^{-4} to 1.4 × 10^{-4} mol L^{-1}. The solutions were mixed for 10 h before examination with CE–ETAAS technique. As shown in Fig. 5, the migration times of Pb(II) and Pb(II)-DNA were 370 s and 550 s, respectively.

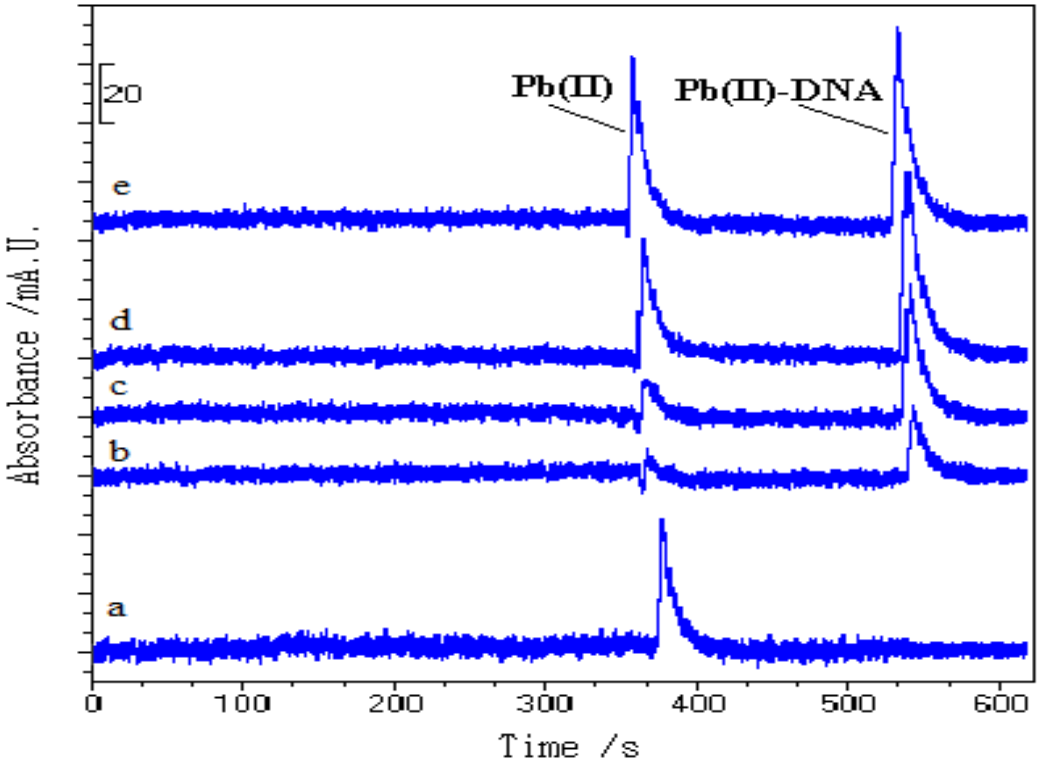


Fig. 5 Electropherogram of Pb(II) and Pb(II)-DNA for: (a) 0.5 × 10^{-4} mol L^{-1} Pb(II), (b) a standard mixture of 1 × 10^{-4} mol L^{-1} DNA and 0.2 × 10^{-4} mol L^{-1} Pb(II) solutions, (c) a standard mixture of 1 × 10^{-4} mol L^{-1} DNA and 0.5 × 10^{-4} mol L^{-1} Pb(II) solutions, (d) a standard mixture of 1 × 10^{-4} mol L^{-1} DNA and 1.2 × 10^{-4} mol L^{-1} Pb(II) solutions, (e) a standard mixture of 1 × 10^{-4} mol L^{-1} DNA and 1.4 × 10^{-4} mol L^{-1} Pb(II) solutions. Separation conditions are described in Table 1.

With increasing the concentration of Pb(II), the peak height of Pb(II)-DNA increased (Fig. 5). When the Pb(II) concentration was increased to 1.2 × 10^{-4} mol L^{-1}, the species peak of Pb(II)-DNA was almost constant. It

means that combination of DNA with Pb(II) has reached to equilibrium at this point.

3.7. Binding sites and binding constant of Pb(II) and DNA

In this study, we used the method of Scatchard to calculate the combination of Pb(II) and DNA equilibrium constant and the binding sites.[33] When DNA is combined with Pb(II) ions, some different energies binding sites (m) and same energy sites (n) may exist. In this method, K_i represents the binding constant of the i class of binding sites and n_i represents the number of sites of the i class in a DNA macromolecule. C_b represents the concentration of metal ions combined with the DNA, C_f represents the free lead ion concentration after combination reaches to the steady state conditions and $[C]$ represents the total DNA concentration. Then, the average binding number (n_c) can be calculated using polynomial equilibrium eqn (1):[34]

$$n_c = \frac{C_b}{[C]} = \sum_{i=1}^{m} \frac{n_i K_i C_f}{1 + K_i C_f} \qquad (1)$$

When DNA molecules combine with lead ions at only one binding site ($m = 1$), the polynomial equilibrium eqn (1) can be simplified to eqn (2):

$$n_c = \frac{C_b}{[C]} = \frac{nKC_f}{1 + KC_f} \qquad (2)$$

After reaching binding equilibrium, various n_c values were plotted against the concentration of free lead ions (Fig. 6).

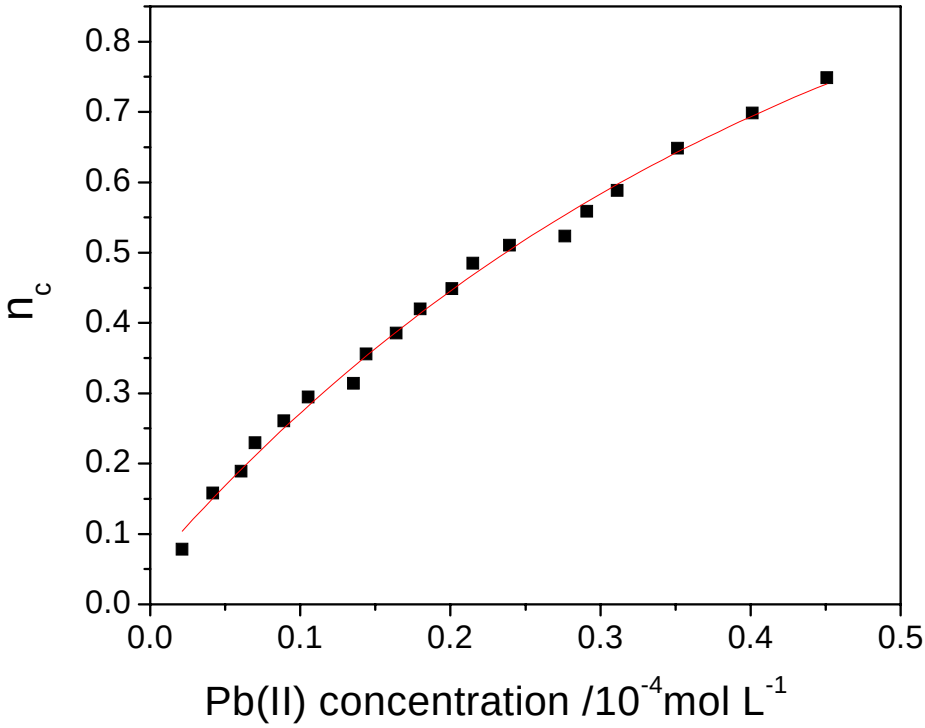


Fig. 6 Effect of concentration of free lead on binding ratio. The concentration of DNA was 1×10^{-4} mol L^{-1}.

As shown in Fig. 6, the binding ratio increases with increasing free lead ion concentration. Transformation of eqn (2) results in the following linear equation:

$$\frac{n_c}{C_f} = -Kn_c + nK \qquad (3)$$

In this equation, known as Scatchard equation,[33] there are primary binding constant (K) and primary binding sites (n). If DNA combines with Pb(II) at one binding, plotting n_c/C_f variations with n_c gives a straight line. The number of average binding sites was 0.96 and the average binding constant

was 3.21×10^4 L mol^{-1} by Origin 8.3. The RSDs of binding sites and binding constant for five replication determinations were less than 3.1%. When there are two classes of binding for metal ions, m value will be two and eqn (1) will be in the following equation:

$$n_c = \frac{n_1 K_1 C_f}{1 + K_1 C_f} + \frac{n_2 K_2 C_f}{1 + K_2 C_f} \qquad (4)$$

In eqn (4), plotting n_c / C_f changes against n_c does not give a straight line. However, the chart is a similar inverse function of the curve, which can be analyzed as two straight lines, shown in Fig. 7. The lines are two asymptotic lines of curve belong to two separable activities. The straight line closer to the Y-axis presents the primary category, with the slope, K_1, denoting the primary binding constant and the intercept of line with the X-axis represents the primary binding sites (n_1). The other line closer to X-axis is related to the non-specific binding that its slope gives K_2, denoted as the non-specific binding constant, and the intercept with the X-axis gives the non-specific binding sites (n_2). The concentrations of free lead ions in various Pb(II)-DNA mixed solutions were determined by CE-ETAAS and treated according to the Scatchard equation. It can be seen that the Scatchard plot shows two sites binding for Pb(II)-DNA. The values of n_1 and K_1 for primary binding of Pb(II) to DNA are 0.57 and 6.2×10^4 L mol^{-1}, respectively, by the Scatchard analysis and the values of n_2 and K_2 for the non-specific binding of pb(II) to DNA are estimated by the Scatchard analysis to be 1.1 and 1.9×10^4 L mol^{-1}, respectively. The RSDs of binding sites (n_1, n_2) and binding

constants (K_1, K_2) for five replication determinations were less than 3.2%.

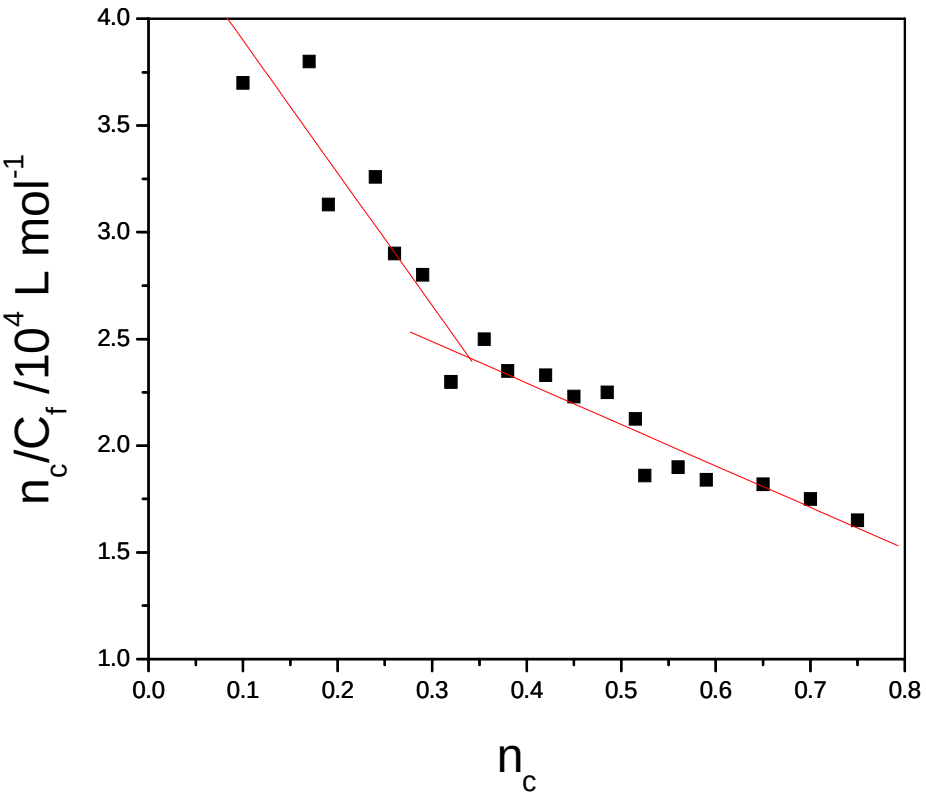


Fig. 7 Scatchard plot of Pb(II) and DNA.

4. Conclusions

A new method to study the interaction of Pb(II) and DNA was developed using CE–ETAAS hybrid technique. Under the optimized experimental conditions, the primary binding number (n_1) and binding constant (K_1) of Pb(II)-DNA were observed as 0.57 and 6.2 × 10^4 L mol^{-1}, respectively. The non-specific binding number (n_2) and binding constant (K_2) of Pb(II) and DNA were found as 1.1 and 1.9 × 10^4 L mol^{-1}, respectively. This method is rapid, simple, and accurate for studying the interactions between metal ions

and DNA.

Acknowledgments

This work was financially supported by the National Natural Science Foundation of China (Grant no. 21365006) and by the Guangxi Science Foundation of China (Grant no. 2014GXNSFDA118004).

References

1 W. B. Amos and J. G. White, *Biol. Cell.*, 2003, **95**, 335.

2 Y. C. Tsai and J. Davis, *Electroanalysis*, 2001, **13**, 7.

3 J. H. Wang, *J. Biol. Chem*., 1977, **252**, 4175.

4 C. Sun, *Chem. Life*, 1991, **11**, 34.

5 H. Zhang, Z. Zhu, Z. Zeng and L. Ling, *J. Anal. At. Spectrom.*, 2014, **29**, 1591.

6 S. Sakin. *Int. J. Biol. Macro.*, 2006, **43**, 117.

7 M. Y. Hamed. *J. Mol. Graph. Model.*, 2006, **25**, 234.

8 X. Li, J. Liu and S. Zhang, *Chem. Commun.*, 2010, **46**, 595.

9 A. Torreggiani and A. Tinti, *Metallomics*, 2010, **2**, 246.

10 G. Pescitelli, L. D. Bari and N. Berova, *Chem. Soc. Rev.*, 2014, **43**, 5211.

11 C. Wang, J. Wang, D. Liu and Z. Wang, *Anal. Methods*, 2010, **2**, 1467.

12 N. Bauer, D. R. Fröhlich and P. J. Panak, *Dalton Trans.*, 2014, **43**, 6689.

13 A. Bhogale, N. Patel, P. Sarpotdar, J. Mariam, P. M. Dongre, A. Miotello and D. C. Kothari, *Colloid Surf., B,* 2013, **112**, 257.

14 O. M. N. Dhubhghaill, P. J. Sadler and A. Tucker, *J. Am. Chem. Soc.*,

1992, **114**, 1118.

15 J. Sun, B. He, Q. Liu, T. Ruan and G. Jiang, *Talanta*, 2012, **93**, 239.

16 B. Deng, Y. Wang, P. Zhu, X. Xu and X. Ning, *Anal. Chim. Acta*, 2010, **683**, 58.

17 Y. Li, X. P. Yan, C. Chen, Y. L. Xia and Y. Jiang, *J. Proteome Res.*, 2007, **6**, 2277.

18 Y. Li, X. P. Yan and Y. Jiang, *Angew. Chem. Int. Ed.*, 2005, **44**, 6387.

19 E. A. Popenoe and M. A. Schmaeler, *Arch Biochem. Biophys.,* 1979, **196**, 109.

20 K. Gasiorowski, J. Świątek and H. Kozlowski, *Inorg. Chim. Acta*, 1985, **106**, 1.

21 I. V. Smirnov, F. W. Kotch, I. J. Pickering, J. T. Davis and R. H. Shafer, *Biochemistry-US*, 2002, 41, 12133.

22 F. Hong, C. Wu, C. Liu, L. Wang, F. Gao, F. Yang, J. Xu, T. Liu, Y. Xie and X. Li, *Chemosphere*, 2007, **68**, 1442.

23 H. Zhang, K. Wei, M. Zhang, R. Liu and Y. Chen, *J. Photoch. Photobio. B*, 2014, 136, 46.

24 Y. Li, X. B. Yin and X. P. Yan, *Anal. Chim. Acta*, 2008, **615**, 105.

25 M. F. Giné, C. A. Suárez and A. P. G. Gervasio, *Curr. Top. Anal. Chem.,* 2011, **11**, 1.

26 B. Deng, X. Li, P. Zhu, X. Xu, Q. Xu and Y. Kang, *Electrophoresis*, 2008, **29**, 1534.

27 C. Y. Lu and X. P. Yan, *Electrophoresis*, 2005, **26**, 155.

28 Y. Li, Y. Jiang and X. P. Yan, *Anal. Chem.*, 2006, **78**, 6115.

29 B. Deng, C. Shen, X. Qin, S. Liang and Y. Liang, *J. Anal. At. Spectrom.*, 2014, **29**, 1889.

30 A. R. Timerbaev, *Chem. Reviews*, 2013, **113**, 778.

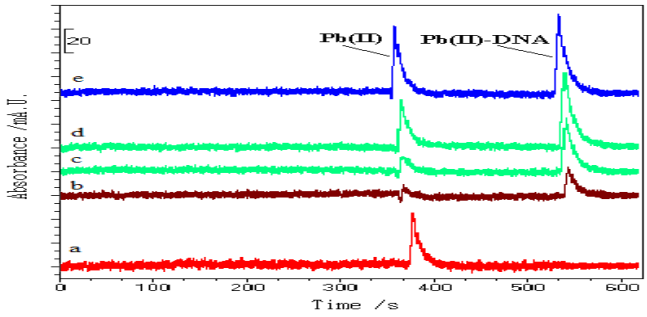
31 B. Deng, P. Zhu, Y. Wang, J. Feng, X. Li, X. Xu, H. Lu and Q. Xu, *Anal. Chem.*, 2008, **80**, 5721.

31 M. E. Reichmanm, *J. Am. Chem. Soc.*, 1954, **76**, 3047.

33 A. M. Pyle, *J. Am. Chem. Soc.*, 1989, **111**, 3051.

34 G. Scatchard, *Ann. N.Y. Acad. Sci.*, 1949, **51**, 660.

*Color graphic：



*Text:

A new method for study the interaction between lead(II) and DNA was developed using capillary electrophoresis electrothermal atomic absorption spectrometry.