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www.elsevier.com/locate/talanta

PII: S0039-9140(17)30653-7
DOI: http://dx.doi.org/10.1016/j.talanta.2017.06.021
Reference: TAL17640

To appear in: *Talanta*

Received date: 22 March 2017
Revised date: 3 June 2017
Accepted date: 7 June 2017

Cite this article as: Qi Zuo, Shun Xiong, Zeng-Ping Chen, Yao Chen and Ru-Qin Yu, A Novel Calibration Strategy based on Background Correction for Quantitative Circular Dichroism Spectroscopy, *Talanta* http://dx.doi.org/10.1016/j.talanta.2017.06.021

A Novel Calibration Strategy based on Background Correction for Quantitative Circular Dichroism Spectroscopy

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Abstract

When using circular dichroism (CD) spectroscopy for quantitative analysis, the samples to be analyzed must be free of light-absorbing interferences. However, in real-world samples, the presence of background absorbers is practically unavoidable. The difference in the matrices between the real-world samples to be analyzed and the standard samples (on which either univariate or multivariate calibration model was built) would result in systematic errors in the quantification results of CD spectroscopy. In this contribution, a novel calibration strategy for quantitative CD spectroscopic analysis was proposed. The main idea of the proposed calibration strategy is to project the CD spectra of both the standard samples and the real-world sample to be analyzed onto a projection space orthogonal to the space spanned by the background CD spectrum of the real-world sample and then build a multivariate calibration model on the transformed CD spectra of the standard samples. The performance of the proposed calibration strategy was tested and compared with conventional univariate and multivariate calibration methods in the quantification of Pb^{2+} in cosmetic samples using CD spectroscopy in combination with a G-quadruplex DNAzyme (e.g. PS2.M). Experiments results revealed that the proposed calibration strategy could mitigate the influence of the difference in the matrices between the standard samples and cosmetic samples and realized quantitative analysis of Pb^{2+} in cosmetic samples, with precision and accuracy comparable to atomic absorption spectroscopy. The proposed calibration strategy has the features of simplicity and effectiveness, its combination with CD spectroscopic probes can realize accurate and precise quantification of analytes in complex samples using CD spectroscopy.

Keywords: Background correction, Quantitative analysis, Lead ions, Circular dichroism, G-quadruplex DNAzyme

1. Introduction

Circular dichroism (CD) spectroscopy measures differences in the absorption of left-handed polarized light versus right-handed polarized light, which arise due to the presence of chiral molecules such as proteins. It is particularly good for determining the secondary and tertiary structure of proteins [1-3], investigating collagen fibrillogenesis [4] and characterizing conformational isomerizations of DNA [5, 6]. CD spectroscopy has also been applied to the detection of Hg^{2+} and Pb^{2+} through the combination with nucleic acid functionalized gold nanorods or nanoparticles [7, 8]. Pb^{2+} can induce a conformational change of K^{+}-stabilized G-quadruplex DNAzyme and inhibit its peroxidase-like activity [9]. The ability of Pb^{2+} to inhibit the peroxidase-like activity of G-quadruplex DNAzyme has been utilized to design fluorescence, colorimetric or chemiluminescence biosensors for Pb^{2+} [10-14]. The phenomenon of Pb^{2+} induced conformational change of K^{+}-stabilized G-quadruplex DNAzyme can theoretically be exploited to determine Pb^{2+} by CD spectroscopy. However, when using circular dichroism spectroscopy for quantitative analysis, the samples must be free of light-absorbing interferences, the solvent must have no interference in the detection absorption wavelength, and the sample can be completely dissolved in the solvent to form a homogeneous transparent solution. In real-world samples, the presence of background absorbers is practically unavoidable. Generally, either traditional univariate or multivariate calibration models [15-18] adopted for quantitative CD analysis are built on the CD spectra of some standard samples prepared with certain buffer solution. The difference in the matrices between the standard samples and the samples to be analyzed would result in systematic errors in the predictions of the calibration models. So CD spectroscopy is not used as often as other spectroscopic techniques such as fluorescence spectroscopy in quantitative analysis.

In this contribution, an attempt has been made to develop a novel calibration strategy with a view to solving the problem of background interferences and hence improving the applicability of CD spectroscopy in quantitative analysis. The proposed

calibration strategy was combined with G-quadruplex DNAzyme based CD spectroscopy to achieve accurate quantification of Pb^{2+} in cosmetic samples.

2. Calibration Strategy for Quantification of Pb^{2+} by G-quadruplex DNAzyme Based CD Spectroscopy

Lead is a heavy metal found naturally in the environment as well as in many common consumer products such as cosmetics, low cost toys for kids, lead-acid batteries and even popcorn. Its inorganic forms can't be degradated and therefore eventually accumulate in water and food ingested by people [19]. Because of its prevalence in our surroundings, most people have a small amount of it in their bodies. Lead serves no purpose in the human body. Though a low level of lead exposure isn't always considered dangerous for adults, even a small amount of lead in babies and young kids can cause learning disabilities, behavioral problems, anemia, and reproductive system damage, etc [20-22]. The quantitative monitoring of lead in the environment, consumer products as well as human body is therefore rather important to human health.

In the presence of K^+, PS2.M folds into a unimolecular antiparallel G-quadruplex structure. The addition of Pb^{2+} can induce the conformation transition of K^+-stabilized PS2.M to a more compact G-quadruplex structure (Figure 1) [9, 11, 23]. As shown in Figure 2, the K^+-stabilized PS2.M has a strong positive band near 295 nm. Upon the addition of Pb^{2+}, the absorptive peak at 295 nm is suppressed, and a new absorptive peak appears at 312 nm, which is a typical characteristic of Pb^{2+}-stabilized antiparallel G-quadruplex [9]. Moreover, the height of the absorptive peak at 312 nm increases as the concentration of Pb^{2+} increases. Obviously, PS2.M can be used as a ratiometric CD probe for the quantification of Pb^{2+}. However, the conventionally used univariate ratiometrc model based on the intensity ratio between CD peaks at two wavelengths (i.e., 295 and 312 nm) is not suitable for the PS2M-Pb^{2+} system (a detailed discussion can be found in 'Results and Discussion' section). The following novel calibration strategy was therefore deliberately developed.

Suppose row vector $\mathbf{x}_i$ (i=1, 2, ..., N) is the CD spectrum of the i-th standard sample of Pb^{2+}, it can be expressed as a linear combination of the CD contributions of the K^+-stabilized G-quadruplexes, Pb^{2+}-stabilized G-quadruplexes and the background interference(s).

$$\mathbf{x}_i = c_{K-G4,i} \cdot \mathbf{r}_{K-G4} + c_{Pb-G4,i} \cdot \mathbf{r}_{Pb-G4} + \mathbf{b}_{stand}; \quad (i=1, 2, ..., N) \tag{1}$$

Where $c_{K-G4,i}$ and $c_{Pb-G4,i}$ are the concentrations of the K^+-stabilized G-quadruplexes and Pb^{2+}-stabilized G-quadruplexes in the i-th sample, respectively. $\mathbf{r}_{K-G4}$ and $\mathbf{r}_{Pb-G4}$ represent the CD spectral response of the K^+-stabilized G-quadruplexes and Pb^{2+}-stabilized G-quadruplexes of unit concentration, respectively. $\mathbf{b}_{stand}$ denotes the background interference(s) in standard samples, which is constant for all standard samples. The summation of $c_{K-G4,i}$ and $c_{Pb-G4,i}$ equals the total concentration of PS2.M ($c_{PS2.M}$), which is constant across all the samples (either standard samples or the samples to be analyzed) and in large excess with respect to the total concentration of Pb^{2+} ($c_{Pb,i}$) in the i-th sample. Therefore, $c_{Pb-G4,i}$ can be approximately replaced by $c_{Pb,i}$, and Eq. (1) can be rewritten as follows (see Supporting Information for the derivation of the following equation).

$$\mathbf{x}_i = c_{PS2.M} \cdot \mathbf{r}_{K-G4} + c_{Pb,i} \cdot \Delta\mathbf{r} + \mathbf{b}_{stand}; \qquad \Delta\mathbf{r} = \mathbf{r}_{Pb-G4} - \mathbf{r}_{K-G4} \tag{2}$$

Similarly, the CD spectrum ($\mathbf{x}_{test}$) of an unknown Pb^{2+} sample can be expressed as follows.

$$\mathbf{x}_{test} = c_{PS2.M} \cdot \mathbf{r}_{K-G4} + c_{Pb,test} \cdot \Delta\mathbf{r} + \mathbf{b}_{test} \tag{3}$$

Where $c_{Pb,test}$ is the concentration of Pb^{2+} in the test sample. $\mathbf{b}_{test}$ denotes the background interference(s) in the test sample. It should be stressed that $\mathbf{b}_{test}$ is generally different from $\mathbf{b}_{stand}$ due to the difference in the matrices between the test sample and the standard samples. Without being appropriately corrected, $\mathbf{b}_{test}$ would cause significant systematic error in the concentration of Pb^{2+} in the test sample

predicted by the calibration model built on the CD spectra of the standard samples. Fortunately, the CD signal related to the concentration of Pb^{2+} in the test sample is generated from the addition of PS2.M. The background CD spectrum ($\mathbf{b}_{test}$) of the test sample can be measured before the addition of PS2.M. With $\mathbf{b}_{test}$ in hand, the influence of difference in the matrices between the test sample and the standard samples can then be mitigated by projecting both $\mathbf{x}_i$ (i=1, 2, ..., N) and $\mathbf{x}_{test}$ onto a projection space orthogonal to the space spanned by $\mathbf{b}_{test}$.

$$\mathbf{x}_i^* = c_{PS2.M} \cdot \mathbf{r}_{K-G4}^* + c_{Pb,i} \cdot \Delta\mathbf{r}^* + \mathbf{b}_{stand}^*; \qquad \mathbf{x}_{test}^* = c_{PS2.M} \cdot \mathbf{r}_{K-G4}^* + c_{Pb,test} \cdot \Delta\mathbf{r}^* \tag{4}$$

Where $\mathbf{x}_i^* = \mathbf{x}_i\mathbf{P}$, $\mathbf{x}_{test}^* = \mathbf{x}_{test}\mathbf{P}$, $\mathbf{r}_{K-G4}^* = \mathbf{r}_{K-G4}\mathbf{P}$, $\Delta\mathbf{r}^* = \Delta\mathbf{r}\mathbf{P}$, $\mathbf{b}_{stand}^* = \mathbf{b}_{stand}\mathbf{P}$, $\mathbf{P} = \mathbf{I} - \mathbf{b}_{test}^+\mathbf{b}_{test}$. $\mathbf{I}$ is an appropriately sized identity matrix. Superscript '+' denotes Moore-Penrose pseudoinverse. A multivariate calibration model can then be built on $\mathbf{x}_i^*$ (i=1, 2, ..., N) obtained after the above transformation using partial least squares regression (PLS) [18]. Since the transformed CD spectrum ($\mathbf{x}_{test}^*$) of the test sample belongs to the vector space spanned by the transformed CD spectra of the standard samples, the concentration of Pb^{2+} in the test sample can be accurately predicted from the transformed CD spectrum of the test sample by the established multivariate calibration model.

3. Experimental

3.1 Chemicals and Materials

The purified G-rich oligonucleotide (PS2.M: GTGGGTAGGGCGGGTTGG) was obtained from Sangon Biotechnology Co., Ltd. (Shanghai, China). Potassium rhodanate (KSCN, 99.99%), tris (hydroxymethyl) aminomethane (Tris, 99.9%), nitric acid (60%), hydrogen peroxide (30%), acetic acid (99.5%), $Pb(NO_3)_2$ (99.0%) were all purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China). Cosmetic specimen (i.e. face cream) was purchased from local store. All other chemicals were of analytical grade and all reagents were used as received without further purification.

Ultrapure water (18.2 MΩ·cm^{-1}) used throughout this study was produced by an Aquapro water system (Aquapro, Chongqing, China).

3.2 Digestion of Cosmetic Specimen

The cosmetic specimen was digested by the following closed-vessel microwave digestion method. About 1.0 g of cosmetic specimen was accurately weighed and added into a teflon vessel, followed by the addition of 5 mL HNO_3 and 5 mL H_2O_2. The vessel was then placed in a XH-800C microwave digester (XiangHu Science and Technology Development Co. Ltd., Beijing, China) and heated at three stages: at 120°C for 3 min, at 150°C for 5 min, and at 180°C for 5min. After cooling to room temperature, the digested solution was transferred into a clean beaker and then heated while stirring to remove as much acid residue as possible. The pH of the obtained solution was adjusted to a value between 7.0 and 8.0 with ammonium hydroxide.

3.3 Sample Preparation and CD Measurements

Stock solution of Pb^{2+} (48.55 μM) was prepared by dissolving appropriate amount of $Pb(NO_3)_2$ in ultrapure water and then stored in fridge at 4 °C. PS2.M solution (100 μM) prepared with tris-acetate buffer (pH 7.4) was placed in water bath at 88 °C for 10 min to dissociate any intermolecular interaction, and then gradually cooled down to room temperature. 50 μL KSCN (40 mM, serve as a donor of K^+ as well as a masking agent for possible interfering metal ions) was added into 15 μL PS2.M solution. The resulting mixture was incubated for 10 min at room temperature. Subsequently, standard samples with 9 different Pb^{2+} concentration levels (i.e., 0.000, 0.390, 0.970, 1.360, 1.750, 2.920, 4.470, 5.440 and 7.940 μM) were prepared by mixing appropriate volume of Pb^{2+} stock solution with the above mixture and diluting to 200 μL with ultrapure water. For each concentration level of Pb^{2+}, there were three replicate samples, resulting in a total of 27 standard samples.

Cosmetic samples were prepared by adding 40 μL digested solution of the cosmetic specimen to the mixture of 15 μL PS2.M, 50 μL KSCN and appropriate

amount of Pb^{2+} stock solution (for evaluating recovery rate), and then diluted to a final volume of 200 μL with ultrapure water. The cosmetic specimen was spiked with four different concentration levels of Pb^{2+} (i.e.， 0.000, 2.430, 4.860, and 7.280 μM) with three replicates of each concentration.

The concentration of PS2.M plays a significant role on the success of quantification of Pb^{2+}. On the one hand, if the concentration of PS2.M is too low, the CD signal of M^{n+}-stabilized G-quadruplexes will be too weak to be observed. On the other hand, the CD signal of Pb^{2+}-stabilized G-quadruplexes will be overwhelmed by that of K^{+}-stabilized G-quaduplexes, if the concentration of PS2.M is too high relative to that of Pb^{2+}. Considering the concentration range of Pb^{2+} in both the standard samples and cosmetic samples, the concentration of PS2.M was set to be 7.5 μM to get a good balance between the above two aspects.

All the samples were held at room temperature for 50 min, allowing the conformation transition of K^{+}-stabilized antiparallel G-quadruplex PS2.M to more compact Pb^{2+}-stabilized G-quadruplex structures. The CD spectrum of each sample between 270 to 360 nm was then recorded at an interval of 1nm with a MOS-500 circular dichroism (CD) spectrometer (Biologic, France) equipped with an optical chamber of 1 mm path length and 400 μL volume. The background CD spectrum (i.e. $\mathbf{b}_{test}$) of the digested solution of the cosmetic specimen was also measured (Figure S-1, Supporting Information).

3.4 Data Analysis

PLS calibration models with different number of latent variables were built on both the mean-centered raw and transformed CD spectra (Figure S-2, Supporting Information) of the standard samples with Pb^{2+} concentration levels of 0.000, 0.970, 1.750, 4.470, and 7.940 μM (referred to as calibration samples thereafter). The PLS model with the smallest root-mean-square error (RMSE) for the rest standard samples (referred to as validation samples thereafter) was chosen as the optimal model ($\mathrm{RMSE} = (\sum_{i=1}^{N}(c_{Pb,i} - \hat{c}_{Pb,i})^2 / N)^{1/2}$, where $c_{Pb,i}$ and $\hat{c}_{Pb,i}$ are the actual and

predicted total concentrations of Pb^{2+} in the *i*-th validation sample, respectively; *N* is the number of validation samples). For convenience of presentation, the optimal PLS models built on the raw and transformed CD spectra will be referred as PLS_{raw} and PLS_{trans} thereafter, respectively. Both PLS_{raw} and PLS_{trans} were then used to quantify Pb^{2+} in the cosmetic samples from their raw or transformed CD spectra (Figure S-3, Supporting Information), respectively. Their quantitative results were evaluated and compared with those of a AA-700 atomic absorption spectrometry (AAS) equipped with a graphite furnace atomizer (PerkinElmer, UK). The data analysis was performed using Matlab version R2012a (Mathworks, Inc.). All the programs including PLS were written in house.

4. Results and Discussions

As shown in Figure 3, the relationship between the concentrations of Pb^{2+} and the CD intensities (CD_{312nm}) at 312 nm (the characteristic peak of PS2.M-Pb^{2+}) significantly deviates from a simple linear model, especially for the standard samples with low Pb^{2+} concentrations. So univariate linear calibration model based on CD intensities at the characteristic peak of PS2.M-Pb^{2+} does not seem to be a good choice for the quantification of Pb^{2+}. In the field of bio-chemical sensing technologies, ratiometric models based on intensity ratios between two spectral peaks are generally used for quantitative analysis. However, the relationship between the concentrations of Pb^{2+} and the intensity ratios (CD_{312nm}/CD_{295nm}) between the two CD peaks at 295 and 312 nm for the standard samples is rather complicated (Figure S-4, Supporting Information). Clearly, univariate linear calibration models based on either CD intensities at characteristic peak or intensity ratios between two peaks are incapable of achieving accurate quantitative results for Pb^{2+}. Hence, more sophisticated calibration models are needed to enable the quantitative determination of Pb^{2+} by G-quadruplex DNAzyme based CD spectroscopy.

Figure 4 displayed the concentration predictions of the optimal PLS_{raw} with two latent variables for Pb^{2+} in both the calibration and validation samples. Obviously PLS_{raw} fitted the CD spectral data of the five calibration samples quite well. In general, the concentration predictions of PLS_{raw} for the validation samples were also acceptable, except the prediction for the validation sample with 0.390 μM Pb^{2+} which had a mean relative error of 22.7% (Table 1). Moreover, the variations of the predictions of PLS_{raw} for samples with the same Pb^{2+} concentration levels were quite small. All these results suggested that PLS_{raw} could effectively model the relationship between the concentration of Pb^{2+} and the CD spectra of the standard samples.

The validity and practical applicability of PLS_{raw} was further tested on the cosmetic samples. As shown in Table 2, the recoveries of PLS_{raw} for the concentrations of Pb^{2+} in the spiked cosmetic samples were 97.7%~105%, which seemed rather satisfactory and were comparable to the corresponding values obtained by AAS. However, the prediction of PLS_{raw} for the cosmetic sample without addition of extra Pb^{2+} was significantly smaller than the value determined by AAS. The underestimation of the concentration of Pb^{2+} in the real-world cosmetic sample was obviously caused by the difference in the matrices between the calibration samples and the real-world cosmetic sample, which had not been taken into account in PLS_{raw} model. Therefore, PLS_{raw} model could not accurately determine the concentrations of Pb^{2+} in real-world cosmetic samples from their raw CD spectral measurements.

The optimal PLS_{trans} model with two latent variables built on the transformed CD spectra of the calibration samples (Figure S-2b, Supporting Information) provided rather similar results as the optimal PLS_{raw} model for the standard samples in terms of RMSE values. The RMSE values of PLS_{trans} were 0.11 and 0.17 μM for the calibration and validation samples, respectively. Nevertheless, the predictions of PLS_{trans} for the validation samples with lower concentration of Pb^{2+} were significantly more accurate than the predictions of PLS_{raw} (Table 3). For example, the mean relative error of the prediction of PLS_{trans} for the validation sample with 0.390 μM

Pb^{2+} was 9.1%, much smaller than the corresponding value of PLS_{raw} (i.e., 22.7%), indicating that background correction could improve the quantitative accuracy of PLS_{trans} for samples with low concentrations of Pb^{2+}.

The CD spectral data of the cosmetics samples was used to evaluate the practical applicability of PLS_{trans} in the detection of Pb^{2+} in real-world samples. Table 4 listed the concentration predictions of PLS_{trans} for the cosmetics samples along with the corresponding values determined by AAS. The mean predicted concentration of PLS_{trans} for Pb^{2+} in the cosmetic sample without addition of extra Pb^{2+} was 0.46 μM, which was in good consistence with the corresponding quantification result of AAS. The recoveries of PLS_{trans} for the concentrations of Pb^{2+} in the spiked cosmetic samples were in the range of 98.6%~107%, and had no significant difference from the corresponding values obtained by AAS. In addition, the predictions of PLS_{trans} in combination with CD spectroscopy had better precision compared with the results of AAS, especially for cosmetic samples spiked with higher concentrations of Pb^{2+}. These results suggested that the influence of the difference in the matrices between the standard samples and real-world cosmetic samples had been effectively mitigated through projecting the CD spectra of both the standard samples and cosmetic samples onto a projection space orthogonal to the space spanned by the background CD spectrum (i.e. $\mathbf{b}_{test}$) of the digested solution of the cosmetic specimen; And hence, the PLS_{trans} model based on the transformed CD spectra of the calibration samples could realize accurate and precise quantification of Pb^{2+} in real-world cosmetic samples. The limit of detection (LOD) and limit of quantification (LOQ) of G-quadruplex DNAzyme based CD spectroscopy in combination with PLS_{trans} model for Pb^{2+} in cosmetic face cream samples were estimated to be 0.07 and 0.22 μM, respectively.

The selectivity of PS2.M for Pb^{2+} has been fully investigated by other researchers.[14] To evaluate the selectivity of PS2.M based CD spectroscopy in combination with PLS_{trans} model for the detection of Pb^{2+}, some metal ions commonly found in real-world samples (100 μM Mg^{2+}, 50 μM Zn^{2+}, 50 μM Fe^{3+}, 50 μM Ca^{2+},

100 μM Cu^{2+}, 100 μM Na^{+}, 100 μM Ag^{+}, 100 μM Cr^{3+}, and 10 μM Hg^{2+}) were separately added to the mixture of K^{+}-PS2.M (7.5 μM) and SCN^{-} (10 mM), and the intensity ratio between the CD peaks at 312 and 295 nm were calculated. As shown in Figure 5, with SCN^{-} as the masking agent, only the addition of Pb^{2+} could induce a significant increase in the intensity ratio between the CD peaks at 312 and 295 nm. The addition of other metal ions considered in this study resulted in no obvious changes. Moreover, the masking agent (SCN^{-}) had no significant influence on the CD signals of Pb^{2+}-PS2.M. Such a high selectivity of the G-quadruplex DNAzyme based CD spectroscopy for Pb^{2+} should enable accurate quantification of Pb^{2+} in complex real-world samples.

5. Conclusions

The presence of Pb^{2+} can induce the conformation transition of K^{+}-stabilized PS2.M to a more compact G-quadruplex structure and hence results in a change in the CD spectrum of PS2.M, which can be used for the quantification of Pb^{2+}. However, the practically unavoidable difference in matrices between the standard samples and real-world sample causes conventional calibration methods to fail to provide satisfactory concentration prediction for Pb^{2+} in real-world samples. In this contribution, a novel calibration strategy for quantitative circular dichroism spectroscopic analysis of Pb^{2+} in cosmetic samples was developed as follows: a) Firstly, the CD spectra of both the standard samples and cosmetic sample were projected onto a projection space orthogonal to the space spanned by the background CD spectrum of the real-world cosmetic sample, b) subsequently, a PLS calibration model (PLS_{trans}) was built and optimized based on the transformed CD spectra of the standard samples, c) Finally, PLS_{trans} was utilized to predict the concentration of Pb^{2+} in cosmetic sample from its transformed CD spectral measurements. Experimental results revealed that PLS_{trans} could mitigate the influence of the difference in the matrices between the standard samples and cosmetic sample and realized accurate and precise quantification of Pb^{2+} in cosmetic sample. The concentration prediction of

PLS_{trans} for Pb^{2+} in the real-world cosmetic sample was in good consistence with the corresponding value determined by a AA-700 atomic absorption spectrometry (PerkinElmer, UK). The proposed calibration strategy has the features of simplicity and effectiveness, and has the potential to be applied to improving the accuracy and precision of quantitative CD spectroscopic analysis of other analytes.

Acknowledgment

The authors acknowledge the financial support of the National Natural Science Foundation of China (no. 21475039), the Specialized Research Fund for the Doctoral Program of Higher Education (no. 20130161110027), and the Natural Science Foundation of Hunan Province (no. 2015JJ2028).

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Legends to figures and tables

Figure 1. Schematic illustration of the label-free detection of Pb^{2+} based on the conformation transition of PS2.M

Figure 2. CD spectra of K^+-stabilized PS2.M with the addition of different concentrations of Pb^{2+}

Figure 3. The CD intensities (CD_{312nm}) at 312 nm *vs* the concentrations of Pb^{2+} (c_{Pb}) in the standard samples

Figure 4. The concentration predictions of PLS_{raw} for Pb^{2+} in the standard samples (RMSE values for the calibration and validation samples were 0.10 and 0.18 μM, respectively)

Figure 5. The selectivity of K^+-PS2.M (7.5 μM) for some common metal ions with SCN^-(10 mM) as the masking agent (8 μM Pb^{2+}, 100 μM Mg^{2+}, 50 μM Zn^{2+}, 50 μM Fe^{3+}, 50 μM Ca^{2+}, 100 μM Cu^{2+}, 100 μM Na^+, 100 μM Ag^+, 100 μM Cr^{3+}, and 10 μM Hg^{2+})

Table 1: The concentration predictions of PLS_{raw} with two latent variables for Pb^{2+} in the validation samples

True concentration (μM)	Mean predicted concentration (μM)	Mean relative error (%)
0.390	0.30 (0.01)[a]	22.7
1.360	1.20 (0.03)	11.9
2.920	3.16 (0.04)	8.4
5.440	5.60 (0.03)	2.9

a. the numbers in parentheses are standard deviations

Table 2: The concentrations of Pb^{2+} in the cosmetics samples determined by PLS_{raw} with 2 latent variables and atomic absorption spectrometry (AAS), respectively

Sample	Concentration added (μM)	PLS_{raw}		AAS	
		Concentration found	Recovery rate[b]	Concentration found	Recovery rate

		(μM)	(%)	(μM)	(%)
1	0.000	0.29 (0.02)[a]	-	0.45 (0.02)	-
2	2.430	2.84 (0.05)	105	2.74 (0.06)	94.2
3	4.860	5.39 (0.04)	105	5.43 (0.14)	102.5
4	7.280	7.42 (0.08)	97.7	7.38 (0.44)	95.2

a. The numbers in parentheses are standard deviations.

b. Recovery rate $= \frac{(\hat{c}_i - \hat{c}_1)}{c_{add,i}} \times 100\%$ ($\hat{c}_1$ and $\hat{c}_i$ are the predicted concentrations of the first and *i*-th samples respectively; $\hat{c}_{add,i}$ is the concentration added into the *i*-th sample $\hat{c}_{add,i}$)

Table 3: The concentration predictions of PLS_{trans} with 2 latent variables for Pb^{2+} in the validation samples

True concentration (μM)	Mean predicted concentration (μM)	Mean relative error (%)
0.390	0.35 (0.03)[a]	9.1
1.360	1.28 (0.03)	5.8
2.920	3.19 (0.03)	9.5
5.440	5.61 (0.04)	3.1

a. the numbers in parentheses are standard deviations

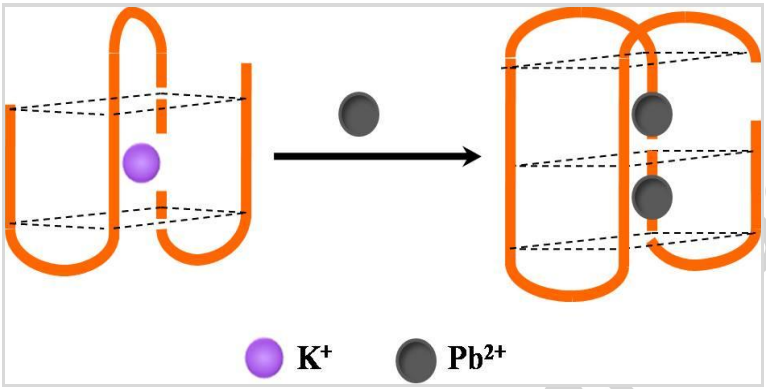
Table 4: The concentrations of Pb^{2+} in cosmetics samples determined by PLS_{trans} with 2 latent variables and atomic absorption spectrometry (AAS), respectively

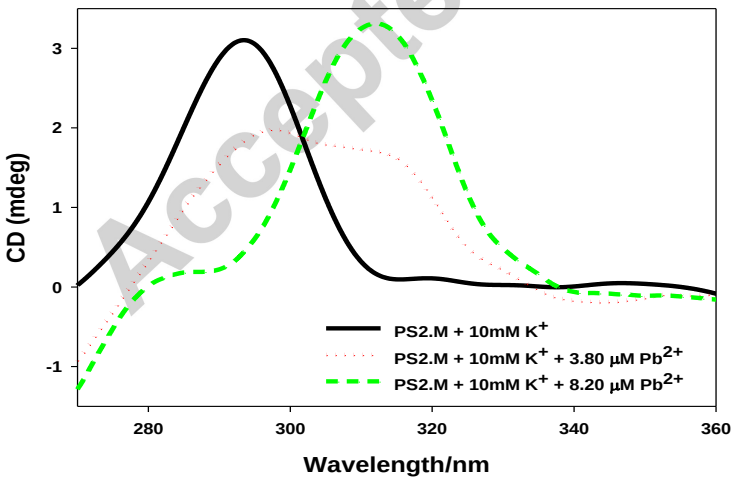
Sample	Concentration added (μM)	PLS_{trans}		AAS	
		Concentration found (μM)	Recovery rate (%)	Concentration found (μM)	Recovery rate (%)
1	0.000	0.46 (0.02)[a]	-	0.45 (0.02)	-
2	2.430	3.04 (0.06)	106	2.74 (0.06)	94.2
3	4.860	5.65 (0.02)	107	5.43 (0.14)	102.5
4	7.280	7.65 (0.08)	98.6	7.38 (0.44)	95.2

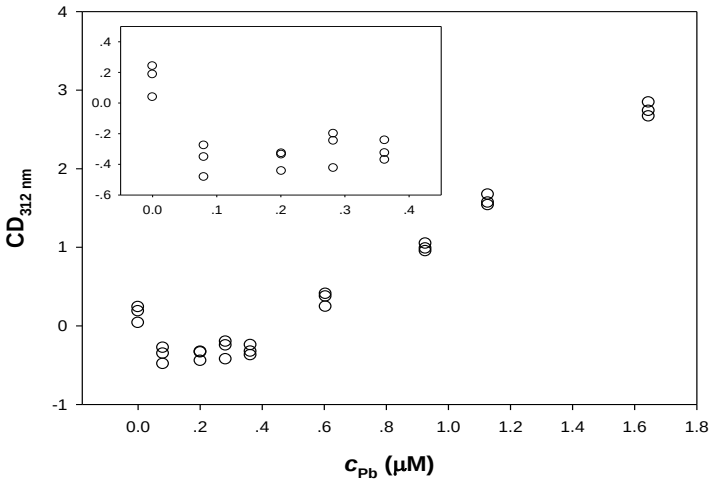
a. The numbers in parentheses are standard deviations.

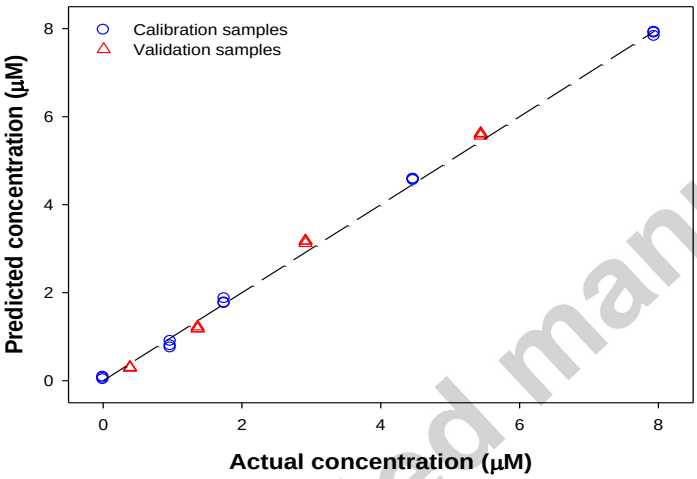
Highlights

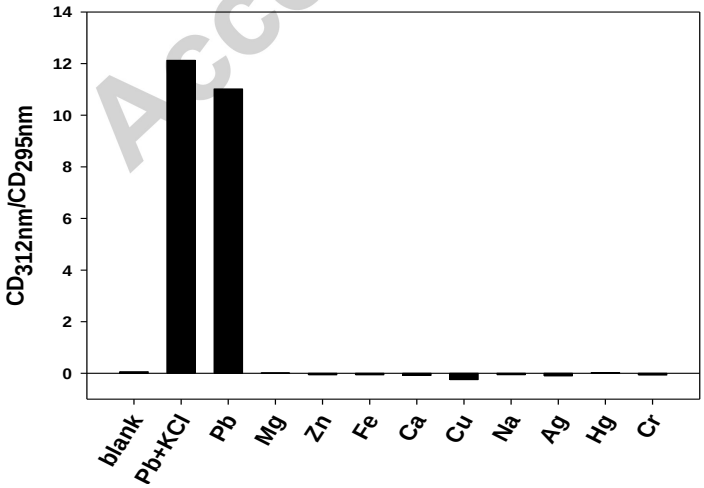
1. A novel calibration strategy was proposed for quantitative CD spectroscopy
2. The proposed method can solve the problem of background absorbers in test samples
3. Accurate detection of Pb^{2+} in cosmetic samples was realized by the proposed method
4. Recovery rates of the proposed method for detecting Pb^{2+} ranged from 98.6% to 107%
5. The quantitative accuracy of the proposed method was comparable to that of AAS











Graphical Abstract