

Elimination of Sodium Chloride Interference on Heavy Metal Analysis in High-Salt Food

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Abstract

A simple method using Chelex-100 chelating resin and Atomic Absorption Spectroscopy determination of lead, cadmium and copper in high-salt food was established. This method could solve the problem of interference on the determination of lead and cadmium in a salt matrix. K_d values of sodium (Na), lead (Pb), cadmium (Cd) and copper (Cu) on chelating resin were measured. The influence of pH values of solution on absorption and behavior of ions on resin was investigated. Elution curve for Na and Cd was demonstrated. Absorption capacities of resin to Pb/Cd/Cu/Na were determined. The developed method was applied to the analysis of high-salt samples. The recoveries were in the range of 91.6%~98.6% with relative standard deviations (RSDs) in the range of 2.0%~6.7%.

Keywords: Chelex-100, chelating resin, lead, cadmium, copper, sodium, high-salt food

1. Introduction

The contamination of heavy metals, such as lead, cadmium or copper (II), in food is one of the major concerns in food safety regulation [1]. Analytical methods based on Atomic Absorption Spectroscopy (AAS) or Induced Coupled Plasma-Mass Spectrometry (ICP-MS) are usually used to determine the amount of heavy metals in food [2]. However, due to the interference of sodium chloride, it is very difficult to accurately quantitate trace heavy metals in high-sodium foods. Sodium chloride in the matrix can not only impact the arc temperature and generate high background noise in AAS analysis, but can also cause contamination in torch, sample & skimmer cones or ion lens, thereby suppressing sign intensities in the ICP-MS analysis [3, 4]. To minimize the matrix effects and enhance the sensitivity and accuracy of quantitation, it is necessary to separate the heavy metals from salty matrices.

Commercial Chelex-100 resin has been used widely for sample purification and concentration as a commercial chelating resin. The function group of the resin is $R-CH_2N+(CH_2COO^-)_2$. Two carbonyl groups can form ionic bonds with heavy metals, and the nitrogen in the amine group can also form a metal-ligand bond with heavy metals.

If the pH is greater than 6.5, heavy metals can be retained on the resin; if the pH is greater than 12, calcium and magnesium can be retained on the resin; and if the pH is less than 1.0, heavy metals, calcium and magnesium can be flushed off from the resin [5]. Therefore, Chelex-100 can be applied to selectively separate trace heavy metals from the matrix of alkali metal and alkaline-earth metal in near neutral conditions. Concentrated nitric or hydrochloric acid solution greater than 0.1 M can be used to wash heavy metals from the resin. Under the same acidity conditions, nitric acid solution has better elution effect than hydrochloric acid solution [6].

Bowles *et al.* applied a rapid Chelex column method for the quantitation of metal species in natural waters [7]. The method was optimized by pumping a water sample (pH 6-8.2) through a small plug of the Ca-form of Chelex 100 at a flow rate of 48 ± 4 mL/min to give the shortest possible contact time (0.25 s), but allow the inorganic ions to be retained on the resin. The method has been applied for the detection of cadmium, copper, lead, nickel and zinc in solution.

Leinonen and Lehto studied the behavior of trace nickel on Chelex-100 resin [8]. When the pH of the sample solution was adjusted to 5, the distribution coefficient K_d of nickel is 2,000,000 mL/gram. With the increase of pH, K_d will decrease to 5000 mL/gram, and the capacity of the chelate resin to Nickel is 2.15 mmol/g.

Manouchchri and Bermond investigated the partitioning of

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Figure 1: Small ion exchange column.

Cu, Cd and Pb in EDTA extracts from soil samples in Chelex-100 resin [9]. In his study, thirteen unpolluted soil samples from Burgundy, France were used for the trace element sorption on Chelex-100. The data of the experiments showed that the extraction efficiency of Chelex-100 varied depending on soil carbonate contents. In non-calcareous soil extracts, the largest percentage of trace elements was transferred to the resin. The resin was able to trap up to 98% of Cu, 72% of Cd and 28% of Pb. The fraction of trace elements transferred to the resin in calcareous soil extracts was 40% less than those in non-calcareous samples. The authors also studied the kinetics of cation exchange from soil-EDTA leachates on soil-phase resin with a kinetic model. The results indicated the dissociation rate of Cu in non-calcareous soil extracts was ten times higher than that in calcareous ones.

In a study by Kiptoo *et al.*, a solid-phase extraction procedure was developed by using mini-columns packed with Chelex-100 and two new chelating agents based on polyvinyl chloride, functionalized with 3-ferrocenyl-3-hydroxydithioacrylic acid (PVC-FSSH) and N,N' -[1,1'-dithiobis(ethylene)]-bis(salicylideneimine) (H_2 sales) loaded on microcrystalline naphthalene [5]. The speciation of Cu, Ni, Zn and Pb in various water samples by the three columns was investigated using electrothermal atomic absorption spectrometry (ETAAS). Comparison of the three chelating agents showed that H_2 sales had a weaker metal chelating strength than Chelex-100, but PVC-FSSH had comparable chelating efficiency to Chelex-100.

Although the commercial Chelex-100 resin has been widely used for analysis of trace metals in water samples, there are very few reports about the application of Chelex-100 resin in analysis of trace metals in high-salt food matrices. In this study we developed a simple solid-phase extraction procedure by using a column packed with Chelex-100. Moreover, the distribution coefficients of trace metals on Chelex-100, the impact of pH on distribution, absorption capacity and column elution curve were studied. The method was optimized to successfully separate Pb, Cd and Cu from high-salt matrices for quantitation by Atomic Absorption Spectrophotometry.

2. Material and Methods

2.1. Instruments and Reagents

The Atomic Absorption Spectrophotometer was a Perkin-Elmer AA800 (Perkin Elmer, Waltham, MA). A Multiwave PRO microwave digestion system (AntonPaar, Ashland, VA) was used for digestion of samples. Reagent-grade chemicals were used unless otherwise specified. Deionized water (18 M Ω) from Millipore Milli-Q system was used to prepare the reagents and materials. Nitric acid and hydrochloric acid solutions were all of trace metal grade and obtained from VWR China (Shanghai, China). Lead standard solution (1mg/mL, GBW08619), cadmium standard solution (1mg/mL, GBW080119) and copper standard solution (1mg/mL, GBW08615) were purchased from CRM/RM Information Center of China. Chelex-100 chelating Resin (100~200 mesh size) was purchased from Sigma-Aldrich (St. Louis, MO). The small ion exchange column was prepared in the laboratory.

2.2. Preparation of Small Ion Exchange Column

The small Ion Exchange Column was made with 5-mL glass-stoppered titration burettes with 5-cm long columns and 18 x 50mm wide-mouth as shown in Figure 1.

- **Loaded column:** Polyester yarn was cushioned on the bottom of the column. Then, about 5.0 g Chelex-100 resin was put into a 100-mL beaker, and 20 mL of water was added. After stirring with a glass rod, the resin was transferred into the column with a transfer pipette, followed by the addition of a 0.5-cm thick polyester yarn. A slight compaction was done using a glass rod to make the resin layer 5 cm high and the volume of the resin layer 1 mL.
- **Pretreatment of the column:** The column was eluted with 0.16 mol/L nitric acid solution in order to wash away the metal ions which may be absorbed on the resin, and washed twice with 10 mL of water to remove nitric acid. The pH of the eluent should be greater than 5.

2.3. Sample Preparation

About 0.5 g of homogenized sample was weighed and mixed with 3 mL nitric acid, 2 mL hydrogen peroxide and 2 mL water. The samples were processed by microwave digestion as follows: ramp temperature from ambient to 120 °C over 6 min and hold for 2 min; next, ramp temperature to 160 °C over 5 min and hold for 5 min; then, ramp temperature to 185 °C over 4 min and hold for 20 min. After digestion, the solution was evaporated to near dryness, cooled down to room temperature, diluted with water to 25 mL, and adjusted to pH 6. Then, 20 mL of solution was loaded into the column and the column was eluted at a flow rate of 1 mL/min. The column was washed twice with 10 mL water to reactivate it.

2.4. Experiment for Distribution Coefficient (K_d) of Pb, Cd, Cu and Na

Similar to Strelow's method [10]: In a 100-mL Erlenmeyer flask, 0.5 g of dry resin was mixed with 48 mL of the solution. Then 1.0 mL of Pb/Cd/Cu/Na (1 mg/mL) solution was added, and stirred for 2 min every hour. The stirring step was repeated three times. After standing overnight, the contents of Pb/Cd/Cu/Na were filtrated and measured.

2.5. Experiment for Measuring Absorption Capacity of Chelex-100 Resin to Pb/Cd/Cu

In a 100-mL Erlenmeyer flask, 0.1 g of dry resin was mixed with 100 mL of deionized water and 1 mL of Pb/Cd/Cu solution (1 mg/mL), which is concentrated enough to saturate the resin. The pH was adjusted to 6-7 and shaken one time per hour overnight. The concentration of metals in the final solution was then measured.

3. Results and Discussion

3.1. Impact of pH on the Distribution Coefficient (K_d) of Pb, Cd, Cu and Na on Chelex-100 Resin

The distribution coefficient (K_d) is a very important index in ion exchange separation. It usually represents the absorption capacity of the ion exchange resin. The method has been described in section 2.4.

Calculate the distribution coefficient, K_d , of Pb/Cd/Cu/Na by using the formula:

$$K_d = \frac{m \times v}{m' \times m_{\text{resin}}}$$

m = Mass weight of lead/cadmium absorbed on the resin (mg).

m' = Mass weight of lead/cadmium in the solution (mg).

v = Volume of solution (mL). In the experiment, v is 50 mL.

m_{resin} = Mass weight of resin (g). In the experiment, m_{resin} is 0.5 g.

If K_d is high, the absorption ability of the resin for the ion is strong. Otherwise, it is weak. If K_d is higher than 40, the ion can be strongly absorbed by the resin. If K_d is lower than 10, the ion cannot be retained by the resin [4, 5]. According

to the difference of metals and distribution coefficients, parameters were optimized to effectively separate the metals and the matrix. As shown in Table 1, if the pH is higher than 4, the resin can strongly absorb lead and chromium, but not sodium.

3.2. Effect of pH on the Absorbance of Pb/Cd/Cu by Chelex-100 Resin

The pH value has significant impact on the absorption ability of the resin for lead, cadmium and copper because of the chelation reaction between resin and the metals. A plot of the data in Table 1 is shown in Figure 2.

The absorption coefficient, K_d , increases as the pH value increases. Considering that metals may form unsolvable compounds in basic condition, the pH of solution was adjusted to around 6 (weak acidic condition) to ensure an effective absorption.

3.3. Measurement of Absorption Capacity of Chelex-100 Resin to Pb/Cd/Cu

To avoid the absorption saturation of heavy metals on Chelex-100 resin, it is necessary to measure the absorption capacity of the resin. The method has been described in section 2.5.

Calculate the absorption capacity of resin to each metal by using the formula:

$$Ac = \frac{m_{\text{addition}} - (c \times v)}{m_{\text{resin}}}$$

Ac = Absorption capacity ($\mu\text{g/g}$)

M_{addition} = Mass weight of metals added (μg)

c = Concentration of metals in solution ($\mu\text{g/mL}$)

v = Volume of solution (mL)

m_{resin} = mass weight of resin (g)

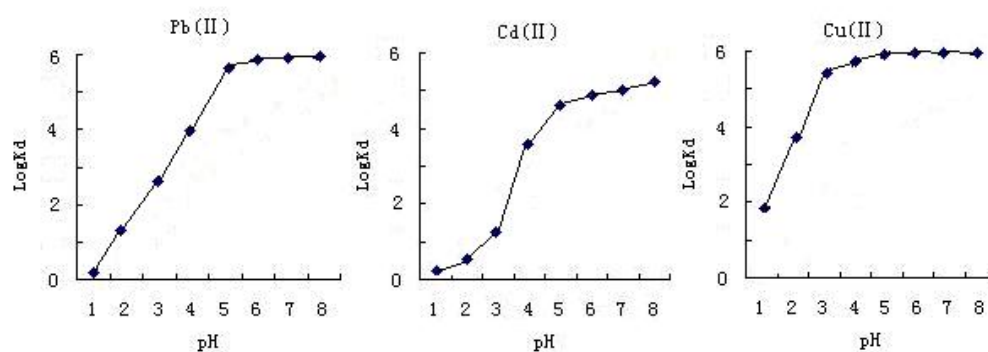
In this study, the mass weight of Pb/Cd/Cu added is $\sim 1000 \mu\text{g}$, the mass weight of resin is 0.1 g and the volume of solution is 100 mL. The absorption capacities of chelex-100 resin to Pb/Cd/Cu are calculated and listed in Table 2.

As shown in Table 2, the Chelex-100 resin can strongly absorb Pb, Cd and Cu from solution. It means that a small ion exchange column containing only 0.1 gram Chelex-100 resin can absorb at least $800 \mu\text{g}$ from a sample. If the weight of the sample was set as 1 gram, with optimized conditions, even if the concentration of metals in a sample is 800 mg/kg , the column will not be saturated. In fact, the concentration of Pb/Cd/Cu in most food samples is at trace amount. Moreover, the actual amount of resin for preparation of an ion exchange column in the developed method is 0.5 gram. Therefore, the developed method can ensure that the column will not be saturated when testing Pb, Cd and Cu in food samples.

3.4. Performance of Pb, Cd, Cu and Na on Chelex-100 Resin and Elution Curve

To determine the performance of Pb, Cd, Cu on Chelex-100, the following experiment was designed: About 2 g of

Elements	pH of solution	After absorption equilibrium (- μg)		K_d
		Solution	Resin	
Pb	1	1000	0	<3
	2	869	131	15
	3	189	811	429
	4	11	989	8990
	5	0.8	999.2	$>10^4$
	6	0.2	999.8	$>10^4$
	7	0.2	999.8	$>10^4$
	8	0.1	999.9	$>10^4$
Cd	1	994	6	<3
	2	979	21	<3
	3	803	197	24
	4	15	985	6566
	5	5.2	994.8	$>10^4$
	6	3.7	996.3	$>10^4$
	7	2.2	997.8	$>10^4$
	8	0.7	999.3	$>10^4$
Cu	1	557	443	80
	2	13	987	7592
	3	0.4	999.6	$>10^4$
	4	0.3	999.7	$>10^4$
	5	0.4	999.6	$>10^4$
	6	0.4	999.6	$>10^4$
	7	0.4	999.6	$>10^4$
	8	0.4	999.6	$>10^4$
Na	1	999	1	<3
	2	999	1	<3
	3	999	1	<3
	4	999	1	<3
	5	999	1	<3
	6	999	1	<3
	7	999	1	<3
	8	999	1	<3

Table 1: K_d of Pb/Cd/Cu/Na in different acidic conditions.Figure 2: Effect of pH on $\text{Log}K_d$.

Metal	M_{addition} (μg)	M_{resin} (g)	Volume of solution v (mL)	After absorption		Absorption capacity ($\mu\text{g/g}$)
				Concentration of metals in solution c ($\mu\text{g/mL}$)	Weight of metals in resin (μg)	
Pb	1000	0.1064	100	0.66	934	8778
Cd	1000	0.1005	100	1.47	853	8487
Cu	1000	0.1021	100	0.64	936	9167

Table 2: Absorption capacity of Chelex-100 resin.

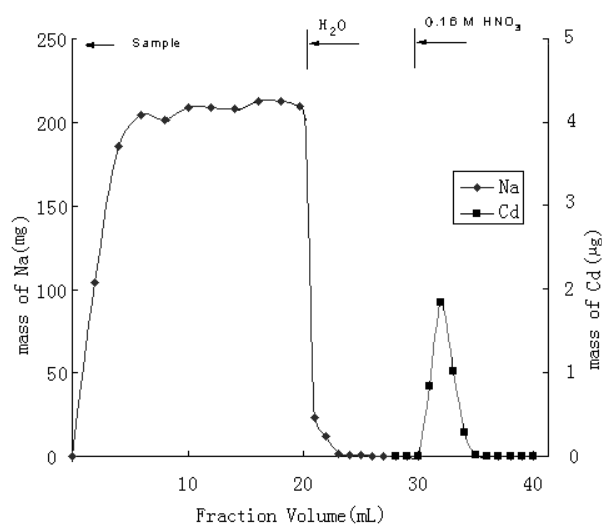


Figure 3: Elution curve for Na and Cd.

sodium chloride was dissolved with water. Next, $5 \mu\text{g}$ of cadmium standard solution was added and diluted to 20 mL final volume. The solution was then loaded onto an ion exchange column made of Chelex-100 resin. The eluent was collected every 2 mL and tested for sodium content by AAS. Then, the column was washed with 5 mL water, twice. The eluent was collected every 1 mL and tested for sodium content by AAS. The last 3 mL of water eluent were tested for cadmium content by AAS. The column was then eluted with 0.16 mol/L HNO_3 . The eluent was collected every 1 mL and tested for sodium and cadmium content by AAS. The elution curve of cadmium was plotted based on the experimental results, as shown in Figure 3. The experiments were repeated to make the elution curves of lead and copper. The curves for lead and copper were the same as that of cadmium and are therefore not presented here.

The curves shown in Figure 3 indicate the following:

1. The portion of 0-20 mL of the sample eluent contained the highest concentration of sodium, which indicates that

most of the sodium in the sample was not retained by the column.

2. The concentration of sodium in the portion of 0-5 mL (corresponding to 20-25 mL on the x-axis) of water eluent decreased as the fraction volume increased. In the portion of 7-10 mL (corresponding to 27-30 mL on the x-axis) of water eluent, neither sodium or cadmium were detected. This indicated that all sodium, but not cadmium, was washed from the column.
3. In the portion of 0-5 mL (corresponding to 30~35 mL on the x-axis) of 0.16 mol/L HNO_3 eluent, all of the cadmium was eluted from the column; therefore, the column can effectively separate cadmium from sodium in the sample.

3.5. Application to Commercial Samples

The developed method has been applied to a variety of high-salt food matrices. The sample preparation procedure was de-

Sample	Pb	Cd	Cu
	Solid:µg/kg Liquid:µg/L	Solid:µg/kg Liquid:µg/L	Solid:µg/kg Liquid:µg/L
Pickle	25.3	4.5	34.5
Salty Kohlrabi	52.5	13.4	43.1
Salted Fish	14.3	8.3	10.6
Salted Brine	23.6	2.2	10.3
Edible Salt	8.2	0.8	6.3
Soy Sauce	111	11.3	45.1

Table 3: Application to commercial samples.

Sample	Pb			Cd			Cu		
	Fortified Conc.	Average Recoveries	RSD	Fortified Conc.	Average Recoveries	RSD	Fortified Conc.	Average Recoveries	RSD
	Solid:µg/kg Liquid:µg/L	(%,n=6)	(%)	Solid:µg/kg Liquid:µg/L	(%,n=5)	(%)	Solid:µg/kg Liquid:µg/L	(%,n=5)	(%)
Pickle	40	97.0	2.1	10	96.3	4.2	100	97.2	3.1
Salty Kohlrabi	100	94.2	2.3	20	95.5	3.4	100	94.8	3.3
Salted Fish	20	94.5	3.4	20	95.7	4.0	20	96.5	5.7
Salted Brine	40	97.2	1.9	10	97.1	6.9	20	97.5	4.1
Edible Salt	20	96.8	2.2	4	94.8	7.6	20	95.2	5.3
Soy Sauce	200	96.3	3.4	20	95.4	3.8	100	95.5	3.4

Table 4: Assessment of accuracy and precision using commercial salty food samples.

scribed in section 2.3. The soy sauce sample (T0366-2008, concentration of Pb is 910 ± 65 g/L) was used as quality control in the method application.. The results are shown in Table 3.

Moreover, the accuracy and precision of the developed method were evaluated by fortification experiments. As shown in Table 4, the range of average recoveries of fortified samples is 94.2%-97.5%, and the range of RSDs is 1.9%-7.6%.

4. Conclusion

The Chelex-100 chelating resin proved to be able to selectively absorb lead, cadmium and copper, but not sodium, from the extract of high-salt samples. The developed method was applied for analysis of commercial high-salt food samples such as soy sauce and monosodium glutamate. The matrix effect in sample analysis was effectively eliminated and the performance of the method exhibited excellent accuracy and precision. It could be satisfactorily applied as a routine procedure to quantify Pb/Cd/Cu in high-salt foods in laboratories of food quality and safety control.

5. Declaration of Conflicting Interest

The authors declare that there is no conflict of interest.

6. Acknowledgements

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7. Article Information

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