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USING CHEMICAL MODIFIERS AND INCREASING THE PYROLYSIS TEMPERATURE FOR HIGH-SENSITIVITY SPECTROMETRIC DETERMINATION OF CADMIUM IN DAIRY PRODUCTS [**]

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A direct and in situ digestion technique is reported for electrothermal atomic absorption spectrometric analysis of Cd in dairy products. In situ digestion methods offered high sensitivity due to the absence of sample dilution and minimum risk of contamination or analyte loss. Under optimized conditions, the calibration graph was linear in the range of 0–5 ng/mL, with a limit of detection of 0.012 ng/mL. The method was successfully applied in dairy product samples including milks, yogurt, and milk beverages, with spiked recoveries of 91 to 111%. The accuracy of the proposed method was also validated by wet digestion-based method.

Keywords: *cadmium, dairy products, graphite furnace, atomic absorption spectrometry.*

ИСПОЛЬЗОВАНИЕ ХИМИЧЕСКИХ МОДИФИКАТОРОВ И ПОВЫШЕНИЕ ТЕМПЕРАТУРЫ ПИРОЛИЗА ДЛЯ ВЫСОКОЧУВСТВИТЕЛЬНОГО СПЕКТРОМЕТРИЧЕСКОГО ОПРЕДЕЛЕНИЯ КАДМИЯ В МОЛОЧНЫХ ПРОДУКТАХ

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Представлен метод прямого и in situ расщепления для электротермического атомно-абсорбционного спектрометрического анализа Cd в молочных продуктах. Методы расщепления in situ отличаются высокой чувствительностью, так как не требуют разбавления образца и характеризуются минимальным риском загрязнения или потери аналита. В оптимизированных условиях калибровочный график линеен в диапазоне 0—5 нг/мл с пределом обнаружения 0.012 нг/мл. Метод успешно применен к образцам молочных продуктов, включая молоко, йогурты и молочные напитки, с результатами восстановления 91—111% от истинных значений. Точность предлагаемого метода подтверждена методом влажного расщепления.

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Ключевые слова: *кадмий, молочные продукты, графитовая печь, атомно-абсорбционная спектрометрия.*

Introduction. Dairy products are among the most important nutritional foods in daily life [1–3]. Due to the high consumption, the heavy metals contamination of dairy products is considered a significant issue in recent years. Among the heavy metals, cadmium is a critical heavy metal in human health [4, 5]. It enters the organism mainly via the alimentary and respiratory tract and accumulates in the human body due to its low excretion rate. Cadmium may cause renal toxicity, pancreatic cancer, and enhanced tumor growth. A 3 μg/L concentration was established as the maximum allowed limit of cadmium in drinking water by the World Health Organization (WHO). Cadmium intake is now specified according to tolerable monthly intake levels (25 μg/kg bw/month) by the Joint Food and Agriculture Organization (FAO)/WHO Expert Committee on Food Additives.

Atomic spectroscopic techniques [6–8], including atomic absorption spectroscopy (AAS) [9, 10], atomic emission spectrometry (AES) [11, 12], atomic fluorescence spectrometry (AFS) [13, 14], and inductively coupled plasma-mass spectrometry (ICP-MS) [15–19], are the prevalent methods for cadmium determination. High sensitivity, precision, and accuracy are realized by the fast development of atomic spectroscopic method as well as instruments [20–24]. However, the time- and labor-consuming sample wet digestion procedures often bring trouble for the determination of cadmium. Besides, the extraneous reagent associated contamination can result in low accuracy of analytical results.

In this work, a direct and in-situ digestion technique is reported for the electro-thermal atomic absorption spectrometric analysis of Cd in dairy products. In comparison with the wet digestion method, the *in situ* digestion method offered very high sensitivity due to the absence of sample dilution and minimum risk of contamination or analyte loss [25–29]. The experimental conditions, including the chemical modifiers, electrothermal heating program, sample preparation conditions, analytical figures of merit, and real sample analysis, were studied in detail.

Experimental. Determination of cadmium was carried out with an electro-thermal atomic absorption equipment, model Analyst™ 800 from Perkin Elmer with Zeeman background correction and an autosampler for sample introduction. The instrumental conditions are shown in Table 1. A hollow cathode lamp (HCL) of cadmium was used as the radiation source. The signal measurements were performed using the peak area (integrated absorbance) mode.

High-purity deionized water (DIW) was used in all experiments. The working standard solutions of Cd were prepared daily by dilution from 1000 mg/L stock standard solutions (National Center for Reference Material, Beijing, P.R. China). A solution of 0.1% $Pd(NO_3)_2$ and 0.06% $Mg(NO_3)_2$ in 0.2% (v/v) nitric acid and 0.1% (v/v) Triton® X-100 was employed as the chemical modifier. High-purity argon gas (99.99%) was used as the purge gas. All reagents were of at least analytical reagent grade.

The working standard solutions of Cd were prepared by serial dilution of the standard stock solution of Cd in 0.2% (v/v) nitric acid and 0.1% (v/v) Triton X-100. The dairy product samples were diluted 5 times with 0.2% (v/v) nitric acid and 0.1% (v/v) Triton X-100 and sonicated 20 min before direct analysis by ETAAS. For analysis of the samples, 20-μL aliquots of working standard solutions or sample solutions and 5 μL of chemical modifier solution were directly delivered to the graphite furnace atomizer. All measurements of integrated absorbance were made at least in triplicate. The thermal stabilization studies of Cd during the pyrolysis and atomization stages were investigated in detail. The proposed procedure was applied to the Cd determination in dairy products without treatment and after wet digestion. The temperature program of the electrothermal atomizer is listed in Table 2. For wet digestion of dairy products, a volume of 5 mL

TABLE 1. The Experimental Settings of ETAAS Instrument

Parameter	Settings
Hollow cathode lamp wavelength, nm	228.8
Hollow cathode lamp current, mA	10
Slit width, nm	0.7
Read mode	Peak area
Read time, s	5
Sample volume, μL	20
Background correction	Zeeman effect

of sample was transferred to polytetrafluoroethylene (PTFE) beakers, followed by addition of 8 mL of concentrated nitric acid and 2 mL of concentrated perchloric acid. The solution was digested on an electric hot plate at 80°C for 20 min and at 200°C until nearly dry. The digests were transferred to 25 mL volumetric flasks and diluted with deionized water. Recovery tests were also performed in triplicate by adding a Cd standard solution to the dairy product samples.

TABLE 2. Temperature Program of Electrothermal Atomizer

Step	Temperature, °C	Ramp time, s	Hold time, s	Ar gas flow rate, mL/min
1. Drying	110	1	30	250
2. Drying	130	15	30	250
3. Cooling	20	1	10	250
4. Pyrolysis	600	10	20	250
5. Atomization	1600	0	5	0
6. Clean	2450	1	3	250

Results and discussion. *The selection of chemical modifier.* The complicated sample matrix, especially organic matter in dairy products, can cause severe interference in the determination of Cd. Therefore, to improve the accuracy of analytical results, chemical modifiers are often employed. In this study, six kinds of common chemical modifiers, including palladium nitrate, ammonium dihydrogen phosphate, palladium nitrate-magnesium nitrate mixture, ammonium dihydrogen phosphate-magnesium nitrate mixture, ammonium sulfate, and ammonium nitrate, are compared for the determination of Cd in milk samples. The chemical modifier has great impact in the analysis of Cd. A 0.1% palladium nitrate and 0.06% magnesium nitrate mixture provide the best performance, while ammonium nitrate gives the lowest atomic absorption signal. Thus, the mixture of 0.1% palladium nitrate and 0.06% magnesium nitrate mixture was chosen as matrix modifier for the following studies.

The effect of pyrolysis temperature and atomization temperature. During the ETAAS determination of trace element, the pyrolysis temperature is one of the most important experimental conditions. Without chemical modifier, the atomic absorbance signal increased slightly with the increase in pyrolysis temperature below 500°C, and decreased rapidly after then. The absorbance was decreased to almost zero at 800°C due to the analyte loss in the pyrolysis step. With the 0.1% palladium nitrate and 0.06% magnesium nitrate mixture as chemical modifier, the optimal pyrolysis temperature increased by 200°C to around 700°C, thus greatly improved the matrix removal for the accurate determination of Cd. The atomization temperature is another important experimental condition. Without chemical modifier, the signal increased slightly with increase in atomization temperature to 1700°C and then leveled off. In the presence of chemical modifier, the optimal atomization temperature decreased by 100°C to around 1600°C.

The effect of sample preparation. Most of the nitrate is easy to dissolve in water without precipitation and hydrolysis; thus, nitric acid was applied as the diluent acid medium. With a nitric acid concentration higher than 0.2%, flocculent precipitation was present in the milk samples; thus 0.2% nitric acid was selected for the subsequent studies. Due to the high concentration of organic matter in milk samples, the surfactant Triton X-100 was added to stabilize the samples; 0.05, 0.1, 0.2, 0.3, 0.4, and 0.5% Triton X-100 were investigated as stabilizers; 0.1% Triton X-100 can provide satisfactory precision, while higher concentrations often cause bubbles in the sample solution. Thus 0.1% Triton X-100 was selected for subsequent experiments. To provide satisfactory precision, sonication was also applied to stabilize the sample solutions. With a sonication time longer than 20 min, the absorbance signal remained stable; thus 20 min sonication time was selected for further studies.

Analytical figures of merit. Under optimized conditions, the calibration graph was linear in the range 0–5 ng/mL for Cd with the use of the linear equation $Y = 0.0376X$ (Fig. 1), a correlation coefficient R^2 of 0.9981, and RSDs lower than 2.0%. The limit of detection was 0.012 ng/mL.

To validate the proposed method for trace cadmium determination in dairy products, a series of dairy product samples bought from a local supermarket was analyzed. The dairy product samples were diluted 5 times to form homogeneous sample solutions. As shown in Table 3, the recoveries of the sample solutions spiked with 0.25 to 4.0 ng/mL of Cd ranged from 91 to 111%. To further verify the accuracy and precision of the method, the milk samples were analyzed with the wet digestion-based method, which revealed a good agreement between the results of the two approaches of in-situ digestion and wet digestion.

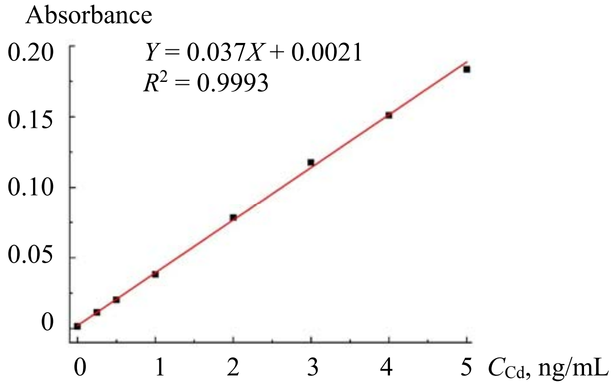

Fig. 1. The calibration curve for cadmium.

TABLE 3. Analytical Results and Recoveries of Dairy Product Sample Solutions

Sample	Added, ng/mL	Detected, ng/mL	Recovery, %
Milk 1	0	0.142	n.d.
	0.25	0.385	97
	0.5	0.657	103
Milk 2	0	0.234	n.d.
	0.25	0.494	104
	0.5	0.759	105
Milk 3	0	0.190	n.d.
	0.25	0.459	108
	0.5	0.678	98
Milk 4	0	0.270	n.d.
	0.25	0.507	95
	0.5	0.785	103
Yogurt 1	0	n.d.	n.d.
	0.25	0.255	102
	0.5	0.527	105
Yogurt 2	0	0.054	n.d.
	0.25	0.301	99
	0.5	0.570	103
Milk beverage 1	0	0.102	n.d.
	0.25	0.372	108
	0.5	0.582	96
Milk beverage 2	0	0.135	n.d.
	0.25	0.413	111
	0.5	0.660	105
Milk beverage 3	0	0.937	
	1	1.849	91
	2	2.875	97
	4	4.882	99
Milk beverage 4	0	0.850	
	1	1.883	103
	2	3.014	108
	4	4.935	102

Note. n.d. – not detectable.

Conclusion. Dairy products possess a complicated sample matrix and especially high organic matter content, which causes high interference for cadmium determination. In this work, after using palladium nitrate and magnesium nitrate as chemical modifier, *in situ* digestion of dairy product samples was realized for cadmium determination under high pyrolysis temperatures for matrix removal. The pyrolysis temperature was increased by 200°C after the addition of chemical modifier. This method does not require a sample predigestion step and minimizes time, cost, and contamination of the samples. This analytical method may be applied for ETAAS analysis of other organic substances similar to dairy products, such as vinegar, alcoholic drinks, soft drinks, liquid cosmetics, etc.

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