Determination of Cadmium and Lead in Raw Milk by Graphite Furnace Atomic Absorption Spectrophotometry

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SUMMARY A simple, pure method was described for graphite furnace atomic absorption spectrophotmetry used for Cd and Pb determination in milk serum prepared from spontaneous souring. Samples of raw milk were collected from the bulk holding tanks of 107 dairy farms in six counties in Taiwan. Raw milk samples were placed at room temperature (28 ℃) for 48 h, allowing the pH to lower below 4.6 and the casein and fat to precipitate. Milk serum was filtered and then directly injected into a graphite tube. The standard addition method was employed, and 0.1 % phosphoric acid was used as a matrix modifier. During ashing, 15 s of oxygen influx was supplemented to prevent charcoal accumulation on graphite tube. The average recoveries of Cd and Pb from three added concentrations were 93.7 % ± 6.9 % and 94.5 ± 10.1 %, respectively. The Cd and Pb concentrations in milk powder (Number A-11) from International Atomic Energy Agency as analyzed using this method were close to the certified values. The average Cd and Pb contents obtained from 107 samples were .044 ng/ml, ranging from .021 to .173, and 2.03 ng/ml, ranging from .98 to 4.45, respectively, without statistical difference among the six counties.

Key words: raw milk, cadmium, lead, heavy metal

INTRODUCTION

Milk and milk products are important components in human food. But lactating cows may possibly be exposed to high quantities of toxic metals such as Cd and Pb in the environment. Therefore, to ensure public health, the concentration of Cd and Pb in milk needs to be monitored.

Different methods have been applied to detect Cd and Pb contents in milk, and wide ranges for these two elements in milk have been given at .2 to 20 ng/g for Cd and 2 to 400 ng/g for Pb. Narres et al. [10] suggested that these wide ranges were most probably due to systematic errors of the methods that were used. Therefore, better methods of sample preparation are needed to avoid contamination and to reduce the interference by the matrix of milk. Conventional sample preparation methods inc-

lude digestion and dry ashing, but both are tedious and susceptible to contamination. Graphite furnace atomic absorption spectrophotometry (GFAAS) has been used for direct determination on Cd and Pb in milk, with added soluene, triton, and ethanol (1, 10, 12). Jenness (4) found that a milk serum prepared by acid precipitation could be analyzed directly for major cations in milk. Trichloroacetic acid has been widely used to prepare milk serum (3, 5, 13, 14). However, regardless of the acids or reagents used, contamination is inevitable; a tedious sub-boiling distillation technique is required to purify these acids. However, when milk is placed at room temperature (28 ℃) for about 48 h, acidity increases to about .55 % and curd is then formed because of lactic acid-forming bacteria in raw milk. Therefore, milk serum can be generated without any foreign acid or reagent and thus, no contamination problem or high reagent blank occurs. The objectives of this study were to use GFAAS to determine directly Cd and Pb in milk serum prepared by spontaneous souring.

MATERIALS AND METHODS

Sample preparation

Samples of raw milk were collected from the bulk holding tanks of 107 dairy farms in six counties in Taiwan and then quickly stored at −20 ℃ until measurement. Clean polyethylene bottles were used to contain the raw milk. when the samples were to be analyzed, the milks were thawed and placed at room temperature (28 ℃) for 48 h to become acid. Before filtration, transferred some milk of each sample to test the pH of the milk by pH meter (HI 8520, Laboratory Micro-processor pH meter, Hanna Instruments, Limena, Italy) to make sure the pH value was below 4.6. Milk serum was then obtained by milk curd filtered through Toyo 5C filter paper (Toyo Roshi Kaisha, Ltd., Tokyo, Japan) and then Cd and Pb concentrations were determined by GFAAS.

Reagent

1 g of Cd standard as $CdCl_2$ (Fixanal 38556; Riedel-deHaen, Hannover, Germany) and 1 g of Pb standard as Pb (NO_3)$_2$ (Titrisol 9969; E. Merck, Darmstadt, Germany) were diluted with deionized water to 1 L to prepare 1000 μ g/ml stock solutions. The standard 1000 μ g/ml stock solutions were further diluted with 3N HCl (Suprapur, E. Merck, Darmstadt, Germany) to obtain 1 μ g/ml of standard solutions. 1 μ g/ml standard solutions were further diluted with deionized water to obtain 100 ng/ml working solutions for recovery test, and .8 ng Cd/ml and 8 ng Pb/ml solutions for standard addition in graphite furnace.

0.1 % H_3PO_4 (Suprapur; E. Merck, Darmstadt, Germany) was used to stabilize the analytes and to reduce background absorption.

Instrumentation

A GBC 904 atomic absorption spectrophotometer and system 3000 automated graphite furnace (GBC Scientific Equipment Pty Ltd., Victoria, Australia) were used. The furnace comprised the GF 3000 graphite furnace and PAL 3000 programmable automatic sampler. The absorbances of Cd and Pb were measured at 6 mA of lamp current and .5 nm of slit width and with the mode of peak height. The wave lengths used were 228.8 nm for Cd and 283.3 nm for Pb. The parameters of automatic sampler and the temperature program of the graphite furnace for analysis of Cd and Pb are shown in Tables 1 and 2, respectively.

Analytical Method

The standard addition method was used to overcome the interference problem. In this method, 7 μ l of milk serum were added by automatic sampler with 3 μ l of modifier and 1, 2, 3, 4 μ l of each standard solution for Cd and Pb (Table 1, parameters of automatic sampler) and appropriate volume of deionized water to bring the total volume to 14 μ l, and then injected into the graphite tube.

To check the accuracy of the analytical method, 10 ml of triplicate samples of raw milk were augmented with 1, 2, 3 ml of 100 ng/ml standards to perform the recovery test. Furthermore, a milk powder sample (Number A-11) from International Atomic Energy Agency was used as reference material and analyzed by the above described method.

All glassware and polyethylene bottles for raw milk containers were washed with detergent, followed by soaking in 10 % (vol/vol) nitric acid overnight, rinsed thoroughly with deionized water, and oven-dried before use.

Statistical Analysis

The data of Cd and Pb concentrations were analyzed by SAS[R] / PC Release 6.03 (SAS[R] Inst, Inc., NC, U.S.A). Duncan's new multiple range test was used to compare the difference of metal contents obtained from raw milk samples of different counties.

RESULTS AND DISCUSSION

Recovery test and Analysis of Reference Material

The recoveries of Cd and Pb from serum of raw milk is listed in Table 3. Mean recovery rates from triplicate samples of the different added concentrations of Cd and Pb were all above 90 %. Average recovery rates from three added concentrations of Cd and Pb were 93.7 ± 6.9 %, and 94.5 ± 10.1 %, respectively. The analytical results of reference material are shown in Table 4. The Cd and Pb contents in milk powder (Number A-11) determined from milk serum were 1.9 ng/g and 59 ng/g, respectively, close to the recommended values and within the confidence intervals of the two elements. These results have proved the accuracy of direct determination of Cd and Pb from milk serum prepared by spontaneous souring which is simple and has little risk of contamination.

Evalution of Applied Method

Splashing during the drying step and formation of charcoal on the graphite tube are the major problems encountered in direct injection of milk into graphite tube. Several workers (1, 10, 12) have used different reagents to overcome splashing. In this experiment, without any foreign reagent, splashing of milk serum was avoided by a carefully controlled drying. A drying temperature of 70°C maintained for 30 s was satisfactory for 14 μ l of milk serum.

To prevent charcoal formation, Oikawa et al. (12) added nitric acid to the sample solution to promote the decomposition of organic matter. Narres et al. (10) reported that nitric acid as a charring aid was not acceptable because its addition to milk resulted coagulated milk protein; on the other hand, the influx of oxygen into graphite furnace during the ashing step was an effective and pure way to diminish background absorption by the complex organic matrix of charcoal. This experiment confirmed the useful role of oxygen during the ashing step. Without oxygen influx during ashing step, charcoal quickly deposited on tube. Because oxygen severely shortened the lifetime of tube in this experiment, the 140 s of oxygen influx during both the drying and ashing steps developed by Narres et al. (10) was modified to 15 s during the ashing only. The resulting tube lifetime and ashing effect proved satisfactory.

Some workers (10, 12) carefully avoided the coagulation of milk protein during sample preparation. Lopez (5), however, used trichloroacetic acid to precipitate protein and then determined Cd, Pb and the essential elements in the serum. In this experiment, protein precipitation by lactic acid was the major method to reduce matrix interference. Although protein precipitated after the thawing of frozen milk at the higher pH, the cations could not be determined from milk serum when acidification of milk was incomplete. Therefore, as long as the acidity of milk was high enough (pH below 4.6) and the curd was formed, most of the Cd and Pb transferred to milk serum (Table 3, recovery

test). Most of the casein and fat were removed from milk serum; hence, interference because of organic matrix of charcoal was reduced. A lot of labor can be saved by direct determination of milk serum.

Cd and Pb contents in Raw Milk

The standard calibration curves for Cd and Pb are shown in Figures 1 and 2. The Cd and Pb contents in the serum of raw milk samples from six counties are listed in Table 5. The mean concentrations of Cd and Pb were not significantly different ($P > .05$) among six counties. The mean content for Cd were .044 ng/ml of milk with the range of .021 to .173 ng/ml (n = 107) and for Pb were 2.03 ng/ml with the range of .98 to 4.45 ng/ml (n = 107). These concentrations are comparable with the .048 ng Cd/ml (.020 to .190) and 1.76 ng Pb/ml of milk (.85 to 4.08) reported by Narres et al. (10), who added Triton X-100 to whole milk for direct determination of Cd and Pb. These comparable data suggest that direct determination of Cd and Pb from milk serum or from whole milk will produce similar results.

Early work indicated that little Cd was secreted into milk (8, 9, 11). Following a single oral dose, radioactive Cd secreted into cow's milk was only .00008 %/d (11). The quite low concentration of Cd in milk bas been attributed to sequestration of Cd by metallothionein-like proteins that have been isolated in mammary tissue and placenta (6). Raw milk also contains a relatively low amount of Pb. Stanley et al. (15) reported that only .0003 % of the ingested Pb transfers to milk. Milk from cows showing clinical signs of Pb toxicity contained only 9 ppb of Pb (2). Holstein cows given 11 mg of $PbCO_3$/kg of body weight for 2 wk secreted milk containing only 5.9 ppb of Pb (7).

The results suggest that the procedure described here in is simple, of low risk for contamination, and especially suitable for determination of large numbers of raw milk samples. In addition, the quite low concentrations of Cd and Pb in raw milk indicate the negligible exposure to these two elements from consuming milk.

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TABLE 1. Parameters of automatic sampler for the direct determination of Cd and Pb in milk serum.

Solutions	Standard[1]	Sample[2]	Blank[3]	Modifier[4]
		(μl)		
Blank	0	0	11	3
Sample	0	7	4	3
Addition 1	1	7	3	3
Addition 2	2	7	2	3
Addition 3	3	7	1	3
Addition 4	4	7	0	3

[1] .8 ng Cd/ ml and 8 ng Pb/ ml.
[2] Milk serum.
[3] Deionized water
[4] .1 % H_3PO_4

TABLE 2. Temperature program for the direct determination of Cd and Pb in milk serum

Step	Temperature		Ramp time		Hold time		Gas[1]
	Cd	Pb	Cd	Pb	Cd	Pb	
	——— °C ———		—— S ——		—— S ——		
Drying	70	70	10	10	30	30	Ar
	110	110	10	10	5	5	Ar
Ashing	600	650	10	10	5	5	O₂
	600	650	5	5	5	5	Ar
	600	650	1	1	2	2	None
Atomization	1800	2000	6	7	2	2	None
Cleaning	2000	2300	1	1	2	2	Ar

[1]Gas flow rate: Ar 300 ml / min, O₂ 50 ml / min.

TABLE 3. Recovery of added Cd and Pb from raw milk.

Metal	Raw milk (10 ml)		
	Added[1]	Found[2]	Recovery
	——————— (ng) ———————		—— (%) ——
Cd	0	.41	—
	100	94.6 ± 7.2	94.2 ± 7.2
	200	183.0 ± 16.5	91.3 ± 8.2
	300	287.2 ± 10.9	95.6 ± 3.6
Mean recovery	—	—	93.7 ± 6.9
Pb	0	19.8	—
	100	111.2 ± 8.5	91.4 ± 8.5
	200	222.4 ± 20.2	101.3 ± 10.1
	300	292.2 ± 24.3	90.8 ± 8.1
Mean recovery	—	—	94.5 ± 10.1

[1]Added 1, 2, 3 ml of 100 ng / ml Cd or Pb standards into 10 ml of raw milk, respectively.

[2]Mean ± SD of triplicate samples.

TABLE 4. Accuracy checks for the direct determination of Cd and Pb in milk powder (Number A-11) from International Atomic Energy Agency

Elements	Cd	Pb
	——— (ng / g) ———	
Recommended value	1.7	54
Confidence interval[1]	1.2 − 2.2	29 − 79
Direct determination[2]	1.9 ± 0.2	59 ± 5
of milk serum		

[1]Confidence intervals are for P = .05

[2]Mean ± SD of three determinations.

TABLE 5. The mean Cd and Pb contents in serum of raw milk samples from six counties.

County code	Farms	C d			P b		
		mean	S D	Range	mean	S D	Range
	(No.)	——————— (ng / ml) ———————					
1	9	.044	.005	.037 − .053	2.05	.52	1.60 − 3.30
2	21	.044	.021	.023 − .117	2.13	.69	1.11− 3.63
3	19	.045	.035	.021 −.173	2.00	.68	1.22 − 3.35
4	11	.042	.013	.024 − .072	1.75	.67	1.24 − 3.70
5	19	.037	.007	.026 − .053	1.82	.41	1.09 − 2.54
6	28	.050	.022	.030 − .114	2.22	.78	.98 − 4.45
Total	107	.044	.022	.021 − .173	2.03	.66	.98 − 4.45

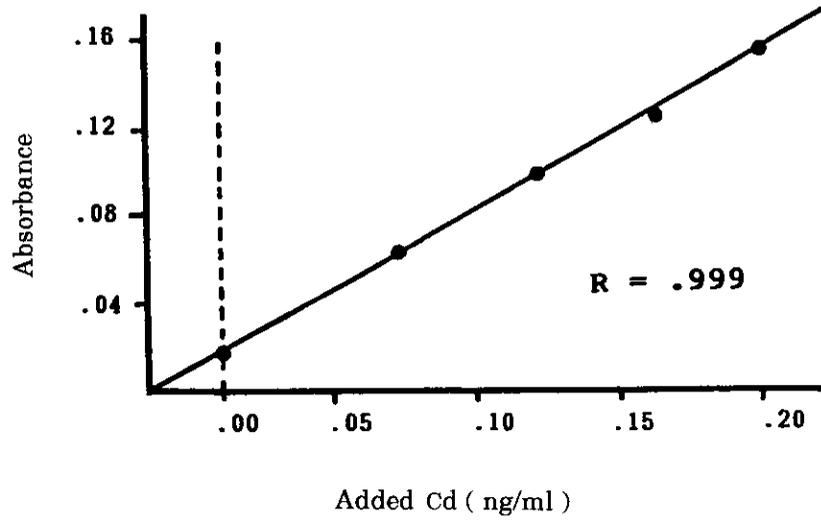[1]No significant difference occurred (P >.05) among counties.

Figure 1. Calibration curve of Cd by standard addition method.
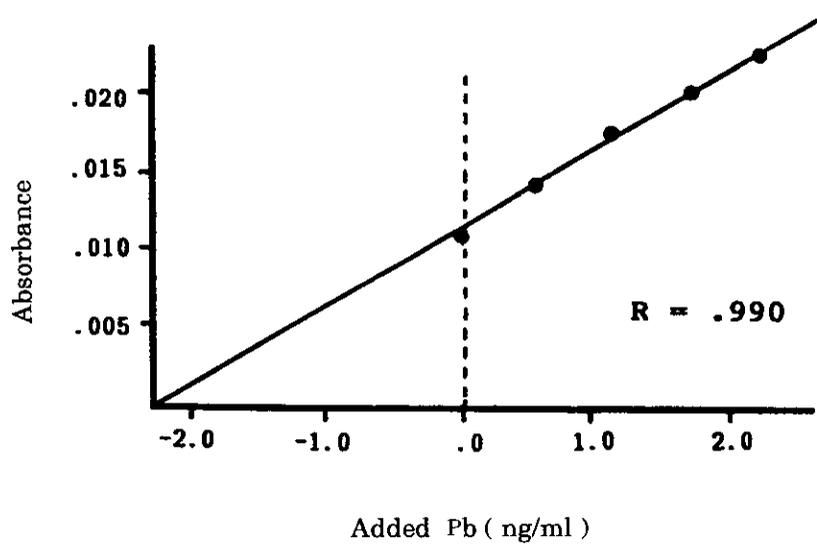

Figure 2. Calibration curve of Pb by standard addition method.

以石墨爐原子吸光儀測定生乳中鎘與鉛之含量

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摘要　自本省 6 縣收集到 107 戶酪農場之生乳總乳樣品供鎘與鉛含量之檢測。將待測生乳置於室溫下（ 28 ℃ ）48 小時，任其解凍及酸敗。酸敗後之生乳以濾紙過濾，取乳清以石墨爐原子吸收光譜儀直接測定鎘與鉛之含量。測定時以標準品添加法來製備檢量線，並以 0.1 ％ 磷酸為基質修飾劑。灰化過程中，通入 15 秒的氧氣以加強有機物質之分解，避免碳粒堆積於石墨管上。以本法測定鎘和鉛在三種不同添加濃度下之回收率均在 90 ％以上，平均回收率為鎘 93.7 ％ 和鉛 94.5 ％ 。測定購自國際原子能總署所製之乳粉重金屬標準品（編號 A-11），所得鎘與鉛之含量與標示值相近：分別為鎘 1.9 ng/g （標示值 1.7 ）和鉛 59 ng/g （標示值 54 ）。107 個生乳總乳樣品平均含鎘 0.044 ng/ml（範圍：0.021 至 0.173 ），含鉛 2.03 ng/ml（範圍 0.98 至 4.45 ）。不同縣間之鎘與鉛平均含量均無顯著差異（ P > 0.05 ）。

關鍵語：生乳、鎘、鉛、重金屬