

Cadmium Analysis of Dried Milk by Pulse Polarographic Techniques

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Abstract

The applicability of differential pulse anodic stripping voltammetry for the determination of ~~little~~ cadmium in milk has been demonstrated. Sample preparation included dry ashing and dissolving the ash in hydrochloric acid. Highest recovery of cadmium was with ashing temperatures between 500 and 540 C. Cadmium recovery was 94% on milk powder spiked at 10 parts per billion (ppb) and 86% on powder spiked at 2 ppb. Samples of foam spray-dried skim milk and freeze-dried whole milk contained less than 3 ppb cadmium on a dry weight basis.

Introduction

We are concerned with the natural amounts of toxic metals in milk products and the effect of manufacturing processes on these levels. Cadmium is very toxic to man (5, 20) and is in the air (1). Until recently, analysis for this element, much below parts per million (ppm), has been difficult. With dithizone extraction-colorimetric analysis, Klein and Wichman (13) reported an accuracy of $\pm 2 \mu\text{g}$ over a range of 0 to 25 μg , and Saltzman (21) reported a sensitivity of .05 μg (in 15 ml). Elwell and Gidley (8) quoted a sensitivity of .03 ppm by atomic absorption spectrophotometry with aqueous aspiration into an air/coal gas flame. Murthy and Rhea (16, 17) determined cadmium in market milk, evaporated milk, infant products, and human milk at .02 to .04 ppm by atomic absorption with hydrogen flame. Schroeder et al. (22) found .0015 to .0036 ppm cadmium in fresh milk with Saltzman's technique and .10 to .49 ppm cadmium in whole fluid and powdered skim milk with atomic absorption (23). Hundley and Warren (12) detected no cadmium in dairy products by anodic stripping voltammetry, although they reported a minimum sensitivity of .01 ppm limited by the reagent blank. Recent developments in atomic-absorption spectropho-

tometry and polarography have lowered these detection limits considerably. Flameless atomic-absorption techniques are capable of analyzing for cadmium below ppb (25) but are expensive. A relatively new technique known as differential pulse anodic stripping (DPAS) combines the best features of anodic stripping voltammetry and differential pulse voltammetry (4, 6, 24). Since this technique held promise for analysis of ppb, we have investigated its applicability to milk powder.

DPAS involves electro-deposition (reduction) of the metal ions of interest onto a suitable electrode followed by a stripping (oxidation) step. The voltage at which stripping occurs is characteristic of the metal. The current flow during stripping is proportional to the concentration of metal at the electrode surface, thus effecting a large increase in concentration over that in the bulk solution. Modern electronic methods employing current sampling and pulse techniques further increase sensitivity over classical D. C. polarography. Details of anodic stripping (4, 6) and pulse polarography (19, 24) have been described elsewhere. Meites (15) also discussed both techniques briefly.

Gorsuch (9) presented results from the wet and dry ashing of cocoa spiked with 14 elements utilizing radio tracer techniques. A number of ashing procedures were used. Cadmium recoveries (10 ppm spiked cocoa) were quantitative with wet digestion, with some loss upon dry ashing. Recovery of 91% was obtained upon dry ashing with no ashing aid. Recovery was less than 80% with nitric acid or magnesium nitrate as an ashing aid and 92% with sulfuric acid. The powders were heated in a silica vessel with bunsen burners until a white residue was obtained. Gorsuch (10) later alluded to the successful use of dry ashing in cadmium analyses by other workers. Success with the chosen preparative method undoubtedly depends on the commodity analyzed. Differences in composition (salts, carbohydrates, proteins, etc.) as well as in physical form might be expected to affect the results. We concluded that while Gorsuch's results were indicative, actual recovery studies with milk would be desirable. Our results are reported here.

Materials¹ and Methods

Preliminary work indicated that the cadmium content of milk samples was well below the part per million range. Dry ashing was selected to facilitate the handling of large samples with a minimum amount of reagent. The temperature which gave the highest recovery of cadmium was determined on milk powders spiked at a high level to eliminate concern over contamination and permit analysis of the dissolved ash by differential pulse polarography. Though lacking the low detection limit of DP-AS, differential pulse polarography is more reproducible. Sample handling and solution preparation for this preliminary study was according to standard procedures.

For anodic stripping, Barendrecht (3) has quoted an average deviation of from 3% for a 10^{-7} M (ca. 10 ppb) solution to 10 to 20% for a 10^{-9} M (ca. .1 ppb) solution. Thus, the classical procedure of making up solutions to volume in a volumetric flask can be bypassed, eliminating sample handling steps, decreasing preparation time, and minimizing the chance of contamination. Milk powders containing 2 and 10 ppb added cadmium were used to test the method developed here.

All milk was from cows maintained by the Agricultural Research Service, U.S. Department of Agriculture at Beltsville, Maryland. The foam spray-dried skim milk was produced by standard procedures (11). Milk of individual cows was obtained by hand milking directly into plastic containers, and samples were frozen for preservation.

All chemicals were reagent grade from Fisher Scientific Co. and were used without further purification. The hydrochloric acid and sodium acetate solutions were 6M and 4M, respectively. Ashing was in a 50-ml quartz or Vycor crucibles. The instrument was a PAR-174 Polarographic Analyzer from Princeton Applied Research Corp., Princeton, New Jersey, which can be operated in several modes including differential pulse and anodic stripping polarography. A Metrohm hanging mercury drop electrode (HMDE) was purchased from the Princeton Applied Research Corp. The polarograph cell was a U-tube type utilizing a mercury pool as the reference electrode. A Teflon encased magnetic stirring bar was placed directly on the mercury pool and rotated by a

magnetic stirrer placed directly under the cell. Two side arms permitted sparging of the solutions with nitrogen before and blanketing the surface of the solutions during the analysis to eliminate interference due to oxygen. Total cell volume was about 10 ml with an internal diameter of about 15 mm.

Cleaning of glassware. All glassware was given successive rinses of warm 7N nitric acid and distilled water before use. Pipettes were kept on a separate rack isolated from normal laboratory work and were rinsed with 7N nitric acid and distilled water after each use. Crucibles were soaked overnight in warm 7N nitric acid after each use. Nitric acid wash solutions were employed only once, then discarded.

Spiking of milk powder. One hundred grams of milk powder were reconstituted with about 200 ml of distilled water. An appropriate amount of cadmium standard was added to a separate 50 ml aliquot of distilled water (acidified with .1 ml of 7N nitric acid) which was then quantitatively added to the reconstituted milk and stirred to assure uniform distribution. Agitation was by hand in Erlenmeyer flasks, and no implements were introduced into the sample. The spiked sample was stored in a refrigerator, agitated periodically, and freeze-dried after about 1 day. For preparation of the 10 ppm spiked samples, 1 ml of 1,000 ppm cadmium standard was added to the 50 ml aliquot. For the 10 and 2 ppb spiked samples, 1 ml and .2 ml, respectively, of 1 ppm cadmium standard were used.

Treatment of unknown samples. Ten grams of milk powder were weighed into 50-ml vycor or quartz crucibles, ashed overnight in a heavy duty muffle furnace with the temperature rising slowly (4 to 5 h) to 500 C. The furnace was cooled (door ajar) to 200 C or below, crucibles were removed one at a time, and 5 ml 6N hydrochloric acid and 15 ml distilled water were pipetted directly into the warm crucible. Occasionally, samples were removed from the hot (500 C) furnace and cooled 2 to 3 min before addition of reagents. The pipettes were rinsed off with distilled water after each addition to remove any contamination and cleaned thoroughly with nitric acid after each batch of samples. The solutions were mixed thoroughly with gentle swirling to recover all ash from the sides of the crucibles and transferred to storage vials (preferably widemouth) with plastic lined caps. Turbid solutions were either allowed to stand several hours or were

¹ Mention of brand or firm names does not constitute an endorsement by the Department of Agriculture over others of a similar nature not mentioned.

centrifuged at 1,800 rpm ($325 \times g$) in a clinical centrifuge for 15 to 20 min. A 4 ml aliquot of the clear supernatant and 1 ml of 4N sodium acetate were added to the polarograph cell and bubbled with nitrogen for 7 min to remove oxygen. A hanging mercury drop electrode was placed in the cell, a drop metered out, and a potential of $-0.8V$ applied for 2 min with stirring, then for 1 min with no stirring to allow the solution to come to rest. The magnetic stirrer was set at 350 rpm with aid of a stroboscope. Polarographic scans were in the positive (anodic) direction. Typical instrument settings were: amplification $.5 \mu A$ full scale, scan rate 2 mV/s, pulse modulation amplitude 25 mV, current sampling interval .5 s, drop size 7.8 ($\pm .3$) mg (3 large divisions on the HMDE syringe barrel). Reagent blanks were prepared the same way as the unknown solutions but without milk salt ash. The blanks were analyzed as above except a longer deposition time was necessary to develop a measurable cadmium peak.

All samples were treated as in the procedure above except for the powders used in the ashing temperature studies (Table 1 and below). The fluid milks (Table 4) were first concentrated to 25 to 50% total solids in a rotary evaporator, then freeze-dried and treated as above. Two anodic scans were run on each sample.

Treatment of spiked samples. The powders spiked at 2 and 10 ppb cadmium as well as all unspiked powders were treated and analyzed as above. For the powders spiked at 10 ppm Cd, 10-g samples were weighed and ashed as above. The ash was dissolved in 5 ml 7N nitric acid and transferred to 50-ml volumetric flask. Rinsings of distilled water and 7N nitric acid (5 ml) from the crucible were then added to the flask and diluted to the mark. These samples were analyzed by differential pulse volt-

ammetry employing a dropping mercury electrode (4, 19).

Nitration of ash. Where treatment of the ash to remove significant undigested carbon was desired, the samples were removed from the hot furnace and cooled, and the ash was moistened with 1 ml distilled water and 1 ml of 7N nitric acid. The residue was brought to dryness on a hot plate or steam bath and returned to the furnace for 1 h. The ash was then dissolved and made to volume as per the treatment of unknown samples.

Calibration. The instrument was calibrated by the method of standard additions (15). Anodic scans (usually three) were recorded on an unknown solution; then, additional scans (three each) were recorded after each of a series of additions of cadmium to the unknown solution. Microliter and Mohr pipettes (5 to 200 μl) were used to add .5 or 1 ppm cadmium standard (prepared daily from 1,000 ppm stock) to the unknown solutions. Stock cadmium solution was prepared by dissolving 1 g Cd metal in about 10 ml of 6N hydrochloric acid and diluting to 1 liter. The peak height of the unknown was subtracted from the total height obtained after each addition of standard. The results were plotted as peak height vs. concentration of added standard.

Results and Discussion

The sensitivity of the technique is illustrated in Fig. 1. Trace (a) is from a typical analysis of freeze-dried whole milk powder containing 2.1 ppb cadmium. Concentration of cadmium in the polarograph cell was .84 ppb. The dashed lines indicate the method used to determine peak height. The average peak height of two scans per sample was used to calculate Cd concentration. Trace (b) was with a longer deposition time to determine the reagent blank, estimated to be .02 ppb (20 parts per trillion). Some noise can be seen especially in trace (a), but filtering of the output signal was not necessary. A mercury pool in 1 to 2 M chloride ion formed the reference electrode, approximating the normal calomel electrode. The peak potential for scan (a) is $-0.65v$ (HCl/acetate, $pH \approx 2$), the same as for ash dissolved in 1.5 M HCl without acetate ($pH < 1$). The major cations in milk (Na^+ , K^+ , Ca^{++} , Mg^{++}) are not observed polarographically in hydrochloric acid media (14). Trace metals (other than arsenic) that have been found in milk (2) either are not reduced (1M HCl) or exhibit peaks with potentials clearly different from cadmium (14). Of the two elements (As,

TABLE 1. The influence of ashing temperature on cadmium recovery.

Ashing temperature (C)	Percent recovery ^a	
	Whole milk	Skim milk
470	92.3(.7) ^b	87.1(.7) ^b
500	94.2(.4)	96.9(.5)
540	95.8(1.2)	95.5(1.2)
600	87.7(1.5)	95.2(.8)

^a Samples spiked with 10 μg Cd/g powder (10 ppm). Natural Cd < 10 ppb.

^b Numbers in parentheses are standard errors (standard deviation of the mean) of four samples.

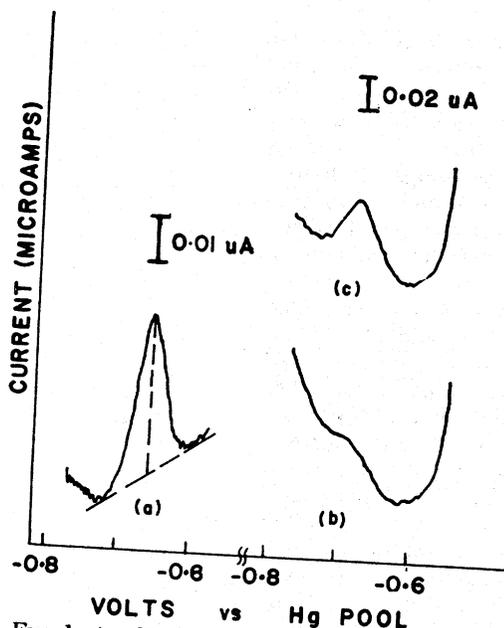


FIG. 1. Anodic stripping polarograms of cadmium. (a) milk powder containing 2.1 ppb Cd. Three minute deposition, $.2 \mu\text{A}$ full-scale amplification, 25 mV modulation amplitude, 2 mV/s scan, current sampling interval, .5 s (b) + (c) reagent blank (b) and reagent blank + .05 ppb Cd (c). Eleven minute deposition, $.5 \mu\text{A}$ full-scale amplification 50 mV modulation amplitude, 2 mV/s scan, current sampling interval .5 s.

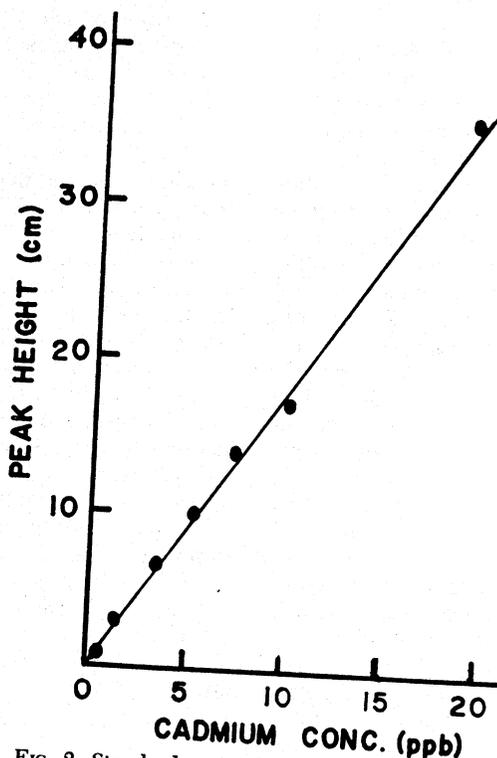


FIG. 2. Standard curve for cadmium. Three minute deposition 25 mV modulation amplitude, 2 mV/s scan, current sampling interval .5 s. Peak height is height measured from polarogram times amplification factor (various). 25.4 cm equals one microamp.

Cd) exhibiting a polarographic peak near $-.65$ volts in 1M HCl, only cadmium exhibits a peak at the same potential in pH 5 acetate buffer (14). Osteryoung and Myers (18) found the arsenic peak at $-.64$ V (1M HCl) to be a polarographic maximum, disappearing below about 300 ppb As. We have observed a (DPAS) peak at $-.65$ V in milk salt ash dissolved in 1M and 1.5 M HCl, acetate/1.5M HCl ($\text{pH} \approx 2$), and acetate/1.5M HCl ($\text{pH} \approx 5$). This behavior can only be attributed to cadmium. Acetate buffer suppressed the evolution of hydrogen, giving a more clearly defined cadmium peak. The data reported here were with acetate/1.5 M HCl (treatment of unknown samples) with $\text{pH} \approx 2$.

The linearity of peak height with cadmium concentration is shown in Fig. 2. The instrument response was identical for identical solutions (unknown sample, above) provided stirring conditions were held constant. For this we set a magnetic stirrer at a speed sufficient for good mixing of the solution without disturbing the hanging mercury drop. The same polarographic cell was used throughout this work

with an identical amount (.8 ml) of mercury forming the pool. The use of the standard addition method insured us that the instrument response for the media being analyzed was obtained. Periodic checks showed that the standard curve was good for several days.

The results of the preliminary studies on ashing spiked milk powder at several temperatures (Table 1) indicated that reproducibility was good and recovery highest between 500 and 540 C. Milk solids ashed at 600 C gave perfectly clear solutions in hydrochloric acid media. Solutions from milk solids ashed at 500 and 540 C were clear and apparently completely digested. A trace of residue appeared in the solutions upon standing a few hours from the 500 C ash and occasionally from the 540 C ash. The residues from six 10-g samples ashed at 500 C were collected and weighed .48 mg, an average of .08 mg. The ash weight from these samples was 800 mg; hence, loss due to inclusion should be insignificant. Carbon residue was readily apparent in solutions from samples ashed at 470 C.

TABLE 2. The recovery of cadmium from dry skim milk.

	Cadmium added	Cadmium found		Cadmium recovered	Percent recovered
		Total	Natural		
ppb Cd	10.00	11.80(.065) ^a	2.42(.15) ^a	9.38(.17) ^d	93.8(1.7) ^d
Relative deviation		.6%	6.6%	1.8%	1.7%
ppb Cd	2.00	3.76(.37) ^b	2.04(.054) ^c	1.72(.37) ^d	86(19) ^d
Relative deviation		9.8%	2.6%	22%	19%

^{a,b,c} Numbers in parentheses are standard errors of (a) four samples, (b) three samples, (c) five samples.

^d Numbers in parentheses are standard errors calculated from a,b,c by propagation of error techniques (7, 27).

To determine the extent of absorption (both to the few carbon particles and to the glassware) we spiked milk powder at much lower cadmium levels (Table 2). We selected 500 C as the most feasible temperature for further study, desiring to minimize possible losses in naturally occurring cadmium. We routinely centrifuged the dissolved ash (in the storage vials) to spin out the few carbon particles which would attach occasionally to the mercury drop during stripping analysis. The results in Tables 2 and 3 constitute the main test of the technique. The procedure for treatment of unknown samples (above) was followed. Quantitative addition of hydrochloric acid and distilled water to the ash followed by transfers to widemouth vials facilitated the handling of small volumes of solution. The total volume of solution thus prepared was 20.3 ml (determined from the weight and density) with 1% reproducibility. Recovery of almost 94% at 10 ppb cadmium (Table 2) indicates the validity of the DPAS technique. The difference between this result and that of Table 1 for skim milk ashed at 500 C (10 ppm added Cd) is small, lending further support to the anodic stripping and sample handling technique. The higher variability at 10 ppb is mainly attributable to the anodic stripping technique (3). Recovery on powder spiked with 2 ppb cadmium was much more variable, a natural consequence of the manner of calculating the relative standard deviation of the recovered cadmium. See reference (7) or (27) for a discussion of propagation of error. Under the circumstances we think the accuracy (86% recovery)

is good and the standard error in the total Cd (9.8%) and natural Cd (2.6%) a better measure of the reproducibility of the technique. Spiking of the fluid milk followed by overnight equilibration should allow the cadmium to mix thoroughly and interact with the milk components, especially proteins which are known to bind metal ions (26). The added cadmium would be expected to reflect the behavior of any naturally occurring ionic or bound cadmium. The behavior of naturally occurring cadmium in one sample was studied under a number of ashing conditions (Table 3). The conditions and results are given in columns 2 to 6 with appropriate labels in column 1. Similar results between 20-g and 10-g samples (500 C) indicate no significant pickup of contamination during sample handling (reagent volumes the same for both samples). Differences between the 10-g and 20-g samples (500 C) and nitric acid treated sample (10 g, 460 C) are not significant, suggesting that neither loss due to volatilization nor adsorption due to the few carbon particles is significant in the above (treatment of unknown) procedure. The use of nitric acid here is post-treatment after most of the carbon has been driven off by dry ashing, whereas the cadmium losses incurred when nitric acid was used as an ashing aid involved pre-treatment of the sample before digestion (9). Gorsuch (10) discussed a possible mechanism for the loss of cadmium when nitric acid is used in the presence of carbon. Thus, the procedure of dry ashing (without pre- or post-treatment) of milk powder at 500 C provides a residue suitable for cadmium analysis

TABLE 3. Effect of ashing conditions on cadmium assay of dry skim milk.

	460	460	500	500	600
Ash temp (C)	460	460	500	500	600
Sample weight (g)	10	10	10	20	10
Nitric acid treatment	no	yes	no	no	no
No. samples	4	5	6	6	4
ppb Cd	.67(.06) ^a	.85(.14)	.79(.06)	.75(.12)	.32(.04)

^a Numbers in parentheses are standard errors.

TABLE 4. Cadmium content of dried cows' milk.

Sample description	Cadmium content (ppb) of dry milk ^a
Foam spray-dried skim herd milk	2.4(.2) ^c
Freeze-dried whole milk Cow 1627 ^b	.7(.1)
	1.7(0)
Cow 1673 ^b	1.6(.6)
	2.3(1.3)
	1.9(.1)
Cow 1687 ^b	2.9(.2)
	1.6(.6)

^a For approximate values in single strength fluid milk, divide by 10 (skim milk sample) or 8 (whole milk samples).

^b Periodic samples during 3/1 to 4/2/72.

^c Numbers in parentheses are mean deviations of two to three samples.

with the least chance of contamination. The sample ashed at 460 C but without nitric acid treatment contained over 1 mg of carbon, probably accounting for the slightly lower cadmium value.

The cadmium content of cows' milk (Table 4) illustrates typical results of the DPAS method described. Cadmium content of dried cows' milk ranged from .7 to 2.9 ppb, corresponding to 90 to 360 parts per trillion in single strength fluid milk. This accounts for Hundley and Warren's (12) result of no detectable cadmium at .01 ppm. Omitting one result (cow 1673—2.3 (1.3) ppb) and pooling the remainder (a total of 18 individual determinations) we calculate the relative standard deviation of the individual determinations to be 28%. Relative standard deviation pooled from columns 3 to 5 of Table 3 is 33%. For the minimum analysis that seems desirable to us (two weighed samples per analysis, two scans per sample) this leads to a probable error of 14 to 16% in the results. The results indicate the feasibility of analyzing milk for cadmium by dry ashing followed by differential pulse anodic stripping analysis. In our laboratory a useful lower analytical limit for routine analysis is about .5 ppb cadmium in milk solids, corresponding to about 50 to 60 parts per trillion cadmium in fluid milk. Applying the procedure developed here to commodities other than whole milk or skim milk products should be done with caution, however. Gorsuch (10) discussed the variable results often obtained for cadmium when organic material was dry ashed.

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