

Differential Pulse Polarographic Determination of Cd(II) and Pb(II) in Milk Samples after Solid-phase Extraction Using Amberlite XAD-2 Resin Modified with 2,2'-DPED₃P

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In the present paper novel column solid phase extraction procedure was developed for the determination of Cd(II) and Pb(II) in cows', goats', ewes', buffalos' and humans' milk samples using newly synthesized reagent 2,2'-DPED₃P (2,2'-{[1,2-diphenylethane-1,2-diylidene]dinitrilo}diphenol) for preconcentration and separation prior to differential pulse polarography using amberlite XAD-2 in the ranges of pH 4.0-5.0. The sorbed elements were subsequently eluted with 10 mL of 2 M HCl elutes were analysed by differential pulse polarography (DPP). The interference of foreign ions has also been studied. Effects of various instrumental parameters are investigated and received conditions are optimized. The total metal concentration of the milk samples in the study area were in the following ranges 0.030-0.090 $\mu\text{g L}^{-1}$ of Cd(II), 0.009-0.026 $\mu\text{g L}^{-1}$ of Pb(II) respectively. The limits of detections were found to be 0.020 and 0.024 $\mu\text{g L}^{-1}$ for Cd(II) and Pb(II) respectively by applying a preconcentration factor ~ 40 . The proposed enrichment method was applied successfully for the determination of metal ions in cows', goats', ewes', buffalos' and humans' milk samples.

Keywords: Differential pulse polarography (DPP); Milk; Cd(II) and Pb(II).

INTRODUCTION

Milk is recognized as an almost complete food product in the human diet because it provides all macronutrients (such as proteins, lipids and carbohydrates) and all micronutrients (elements, vitamins and enzymes). These fact is particularly true in the case of early childhood, because milk (human, Cow or formula) is the only source of nutrients during the first months of a baby's life and the diet of growing children contains a high proportion of milk and milk products. Accurate data on the concentrations of trace elements in human milk throughout lactation are important to formulate nutritional requirements for infants and to understand the physiology of milk secretion.¹ Human milk is also a source of immune agent which can, among other functions, hold intestinal disease in check-almost as important as nutrition itself.^{2,3} Detection of toxic trace metals in the environment is a challenging analytical problem, there are various available techniques, including atomic absorption, atomic emission, and electrochemical techniques. The proposed differential pulse polarography is not time consuming and less expensive technique.

The differential pulse polarography (DPP) is not only

one of the most important electroanalytical tool has capable of chemical component analysis in food stuffs⁴ but also a highly sensitive technique, though its applicability to the determination of multielement at trace to ultratrace concentrations in food area. The most common techniques available for the preconcentration of metals from aqueous samples are solvent extraction, and column methods using various adsorbents such as thiol cotton,⁵ activated carbon,⁶ green tea leaves,⁷ chelating resins,⁸ cellulose,⁹ and polythioether.¹⁰ The direct polarographic estimation of metals after extraction of their metal complexes into organic solvents,¹¹⁻¹⁴ but in recent years, the solid-phase extraction cartridges and columns have been successfully used for the separation and sensitive determination of metal ions, e.g., preconcentration of indium on microcrystalline naphthalene,¹⁵ separation of manganese with 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol,¹⁶ determination of antimony after preconcentration with 2-nitroso-1-naphthol-4-sulphonic acid.¹⁷ The simultaneous determination of zinc, nickel, cobalt, iron, cadmium and manganese by kalman filter polarography and 2-amino-2-methylpropionic acid-thiocyanate mixed system has been studied.^{18,19} Mean

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while solid phase extraction is a simple and convenient technique for separating and concentrating of trace metal ions such as Cd(II) and Pb(II) in cows', goats', ewes', buffalos' and humans' milk samples. The obtained metal concentrations are compared with the corresponding values of different countries available in the literature.

In view of this authors have reported a simple, sensitive and novel analytical method was developed for the separation and preconcentration of metal ions Cd(II) and Pb(II) in cows', goats', ewes', buffalos' and humans' milk samples. The column was loaded with amberlite XAD-2 support impregnated with schiff base 2,2'-DPED₃P. The sorbed elements were sequentially eluted with 10 mL of 2 M HCl. The acid eluates were analysed by differential pulse polarography (DPP). Optimum experimental conditions were investigated with respect to a standard solution of the matrix, in order to examine the possibility of obtaining the maximum extraction efficiency with minor sample treatment and minimal experimental conditions. Under these conditions the preconcentration factor was found to be ~40 for 400 mL of sample solution. The developed method has been checked by analyzing the certified reference materials and the results are found to be in good agreement with the certified values.

EXPERIMENTAL

Apparatus

Analysis were carried out with an Elico Model CL-362, three electrode system consisting of a dropping mercury electrode (DME) as the working electrode, an Ag/AgCl reference electrode and platinum counter electrode. It was outfitted with a Model LX-300⁺ X-Y recorder. A varian Spectra AA55 model atomic absorption spectrometer was used with the following conditions: 10 mm burner height, integration time (5 sec), lamp current (5 mA), acetylene flow rate (0.50 L min⁻¹) with air/acetylene flame and hollow cathode lamps were used for the analysis of cadmium and lead. All instrumental settings were those recommended in the manufacturer's manual book and the instrumental conditions. An Elico Li-129 Model glass calomel combined-electrode was employed for measuring pH values.

Reagents and solutions

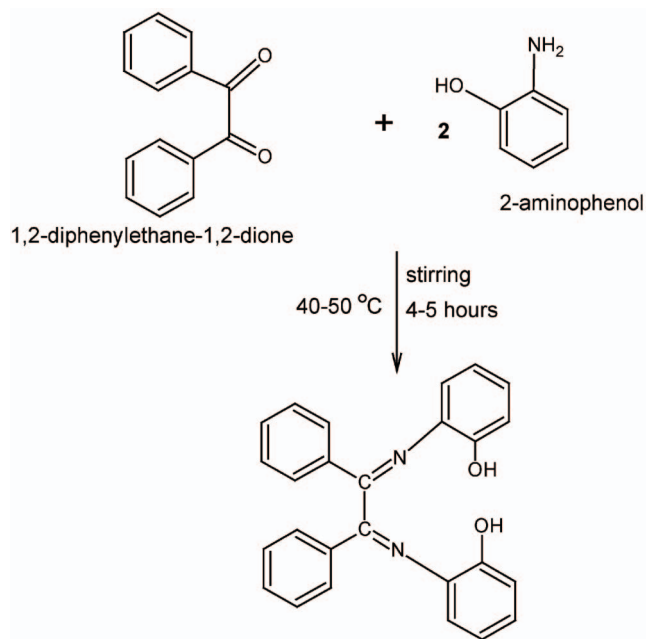
All chemicals used were of analytical reagent grades. All the containers (glassware, polyethylene bottles, etc.) were cleaned, soaked overnight in 10% HNO₃, and were rinsed with distilled water prior to use. Cadmium chloride

and lead nitrate solutions were prepared by dissolving their (Merck Chemicals, Mumbai., India) analytical-grade samples in triply distilled water and standardized by established methods.²⁰ A 0.001% solution of 2,2'-DPED₃P in ethanol was prepared. Buffer solution of pH ± 4.0-5.0 was prepared by mixing 0.1 M HCl and 0.1 M Sodium acetate solutions in appropriate ratio.

Preparation of 2,2'-DPED₃P

The solution of 1,2-diphenylethane-1,2-dione (4 mmol) in 20 mL absolute ethanol, a solution of 2-amino phenol (8 mmol) in 20 mL absolute ethanol were added drop wise at 40-50 °C with continuous stirring for 4-5 h, the reaction mixture was cooled to room temperature and the resulting precipitate was left to stand overnight. The reddish brown solid product was filtered off, washed with H₂O several times, dried in air and recrystallized from aqueous ethanol dried and stored in a desiccator over CaCl₂ and m.p = 205-210 °C, yield 90%, (shown in Scheme I) Anal. Calc. for C₂₂H₂₀N₃O₂: C, 77.47; H, 5.04; N, 7.10; O, 8.10; Found C, 77.57; H, 5.14; N, 7.14; O, 8.15; IR (KBr) ν: 3230, 1591, 1477 cm⁻¹ (Fig. 1).

Scheme I Scheme of the preparation of 2,2'-{[(1*E*,2*E*)-1,2-diphenylethane-1,2-diyli-dene]dinitrilo}diphenol



Collection of Samples

Human milk samples were obtained at 2 months post-

partum from 30 healthy volunteer women living in Tirupati, Chandragiri, Pakala and Kanipakam. Samples of raw cow's milk were collected four times in January-2009, from fifteen farms located near Chandragiri, Pakala, Kanipakam surrounding villages. Goats', ewes' and buffalos' were obtained from healthy animals in same places. Humans' milk samples were obtained from both breasts using a manual breast pump. All milk sampling took place between 05:00 AM and 9:00 AM. The sampling places are a low-income, rural area and its population is mainly indigenous. All of the expressed milk is immediately transported in a sterile plastic tube in dry ice and stored at $-20\text{ }^{\circ}\text{C}$ until analysis.

AAS Procedure

An off line column procedure was applied for the preconcentration of analyte ions in different cows', goats', ewes', buffalos' and humans' milk samples. An aliquot of the sample solution was taken and pH was adjusted to the 4.0-5.0 with sodium acetate buffer. The resulting solution was passed through the column at flow rate of 2.5 mL min^{-1} experimentally. The retained metal ions were eluted from the solid phase with a suitable eluent determined experi-

mentally. The concentration of the metal ions was determined by using a calibrated plot and the results obtained were confirmed by atomic absorption spectrometer.

Preparation of the amberlite XAD-2 column loaded with 2,2'-DPED₃P

The glass column, having a stopcock and a porous disk, was 10 cm long and 1.0 cm in diameter. A small amount of glass wool was placed on the disc to prevent loss of the resin beads during sample loading. Then Amberlite XAD-2 was treated with an ethanol-hydrochloric acid-water (2:1:1) solution overnight. Later, the packing of the column must be done using ethanol as eluent water makes resin beads float. The resin was saturated with the reagent by passing 2.0 mL of a 0.001% 2,2'-{benzene-1,2-diylbis-(nitrilomethylidene)}diphenol solution in ethanol at a flow rate of 0.6 mL/min. Further sample loading the column must be preconditioned by passing a buffer solution.

Procedure for the sorption of Cd(II) and Pb(II) on the column

An aliquot of Cd(II) and Pb(II) solution (containing 0.5-150 μg) was placed in a 100 mL beaker and in it was

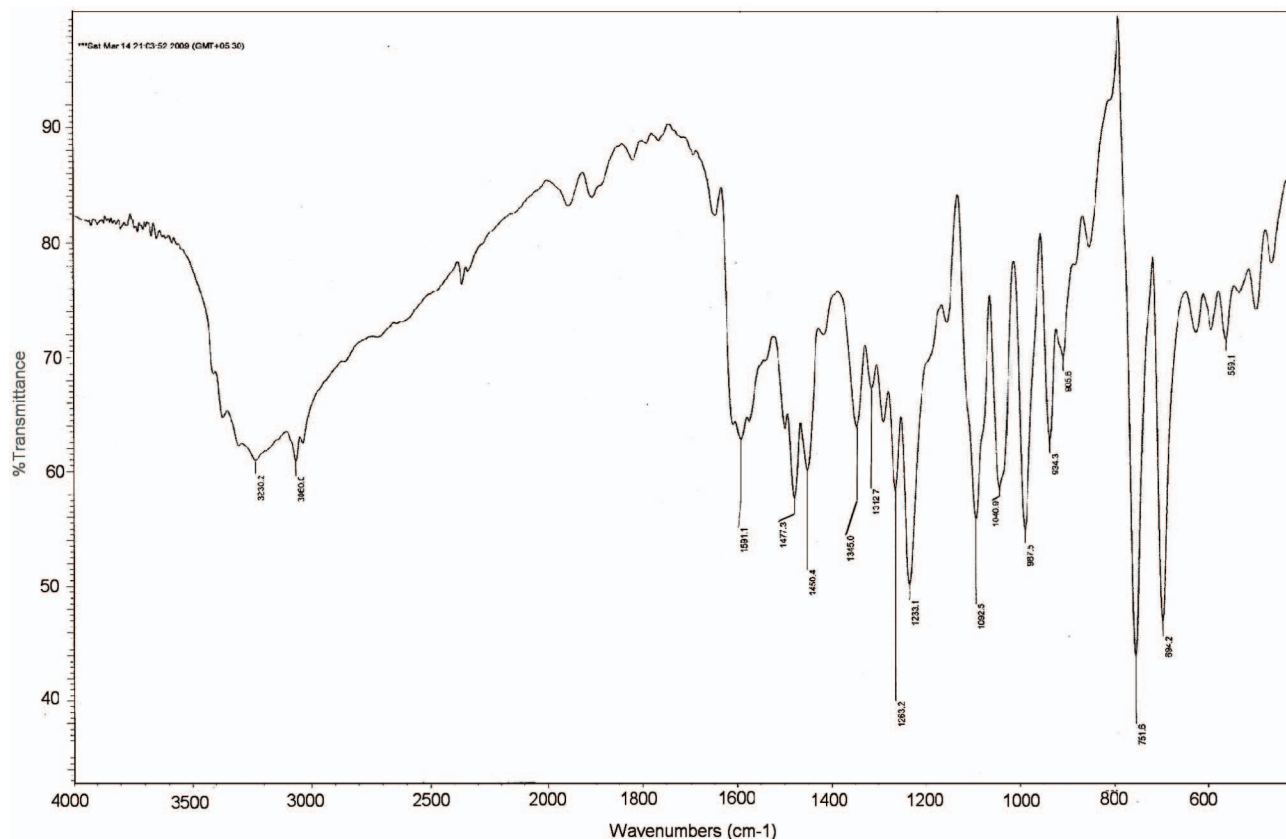


Fig. 1. IR Spectra for 2,2'-{[(1E,2E)-1,2-diphenylethane-1,2-diylidene]dinitrilo}diphenol.

added 4 mL of sodium acetate buffer solution (pH = 4.0-5.0) and then diluted to ~30 mL with distilled water. This solution was passed through the column at a flow rate of 2.5 mL min⁻¹. After passing this solution, the column was eluted with 10 mL of deionized water. The adsorbed Cd(II) and Pb(II) on the column were eluted with 10.0 mL of 2 M hydrochloric acid solution at a flow rate of 1.0 mL min⁻¹. The eluent was collected in a 10.0 mL volumetric flask and Cd(II) and Pb(II) was determined by differential pulse polarography procedure.

Differential pulse polarography

The differential pulse polarogram for Cd(II) and Pb(II) were obtained in the pH 4.0-5.0 was adjusted with sodium acetate buffer medium. The sample solution was transferred into an electrolytic cell and a required amount of supporting electrolyte of sodium acetate was added. The differential pulse polarograms were recorded after oxygen was removed by passing nitrogen gas for 10-15 min. The amalgamate Cd(II) and Pb(II) was differential by pulse polarography by scanning of potential of the electrode between +0.0 V to -1.0 V with a scan rate of 15 mV s⁻¹ and pulse amplitude of 50 mV.

RESULTS AND DISCUSSION

Effect of pH on Differential Pulse Polarogram

The effect of pH on the polarograms was studied in pH range 1-12. The shape of the differential pulse polarograms and peak heights were found to be almost constant when the pH was 4.0-5.0. Meanwhile, the peak potential (E_p) shifted toward the more negative potential as the pH was increased. Meanwhile, a plot of E_p versus pH was not linear, indicating that a protonation reaction did not take part in the overall electrode process. The peak height was considerably decreased above pH 5.0. Therefore, all studies were carried out sodium acetate buffer at pH 4.0-5.0.

Reaction conditions

In the present investigation with 20 µg of Cd(II) and Pb(II) each element respectively. Adsorption was carried out at different pH, and other variables were kept constant. It was found that the Cd(II) and Pb(II) complexes was quantitatively adsorbed on amberlite XAD-2 resin in the pH range of 4.0-5.0 (Fig. 2). In subsequent studies, the pH was maintained at approximately 4.0-5.0 reco. Addition of 2-10 mL of the buffer did not affect the retention of Cd(II) and Pb(II) and the use of 4 mL is recommended. The flow

rate was varied from 0.1 to 5 mL min⁻¹. It was found that a flow rate of 0.1-4.0 mL min⁻¹ did not affect adsorption. A flow rate of 2.5 mL min⁻¹ was recommended in all experiments.

The volume of the aqueous phase was varied in the range of 10-1000 mL under the optimum conditions, keeping the other variables constant. It was observed that the signal height was almost constant up to 400 mL. However, for convenience, all the experiments were carried out with 30 mL of the aqueous phase. Preliminary observations indicated that Cd(II) and Pb(II) were desorbed completely with 10.0 mL of 2.0 M hydrochloric acid. Therefore, 10.0 mL of 2.0 M hydrochloric acid was used in the present work.

Sorption capacity of resin for ligand and Cd(II), Pb(II)

The sorption capacity of the amberlite XAD-2 resin for ligand and Cd(II) and Pb(II) was also evaluated. The resin has a sorption capacity of 0.8 and 0.2 mg g⁻¹ of XAD-2 resin for ligand and Cd(II) and Pb(II) respectively.

Effect of foreign ions

Various salts and metal ions were added individually to a solution containing 0.5-150 µg of Cd(II) and Pb(II) and the general procedure was applied. The tolerance limit was set as the concentration of the diverse ion required to cause ±2% error in the determination of Cd(II) and Pb(II). The tolerance limit (error < 2%) at the following ions were given in Table 1. Among the salts most of them did not in-

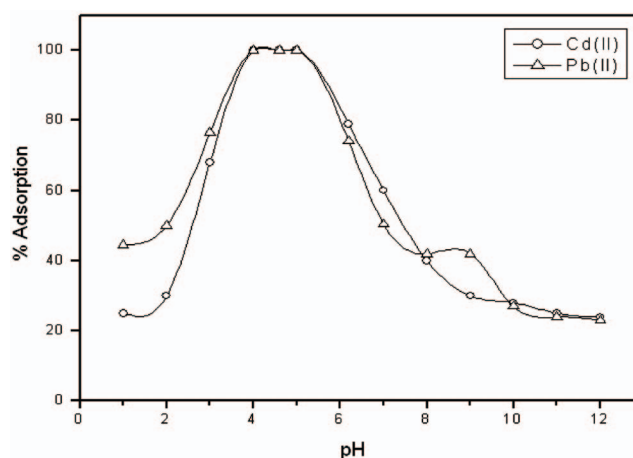


Fig. 2. Effect of pH on metal sorption on amberlite XAD-2. Instrumental settings: scan rate = 12 mV/sec, drop time = 0.5 sec, pulse amplitude = 50 mV.

Table 1. Effect of interfering ions and salts

Interfering ions and salts	Tolerance limit
CH ₃ COONa·3H ₂ O, KNO ₃ , NaCl	1 g
Sodium potassium tartarate	95 mg
Sodium oxalate, trisodium citrate	42 mg
K ₂ SO ₄	180 mg
Na ₂ EDTA	105 µg
Ni(II), Co(II), Fe(II), Cu(II), Zn(II)	500 µg
In(III), Rh(III), Ru(III)	2.5 mg
U(VI), V(V), Te(IV)	5.5 mg
Ga(III), Al(III), Cr(III)	18 mg
Mn(II), Mo(VI), Bi(III), As(III)	50 mg
Sn(II), Sb(III)	0.5 mg
Fe(III)	0.8 mg ^a

^a After masking with 2 mL of 5% triethanolamine solution

terfere at the gram or milligram levels except EDTA which may be due to the high formation constant of the metal-EDTA complex over the Cd(II) and Pb(II) 2,2'-DPED₃P complex. Thus, the method is highly selective and therefore, it has been applied successfully to the trace levels determination of Cd(II) and Pb(II) in determination of various milk samples.

Application of developed procedure for milk samples

An accurately measured 2 mL of the sample was placed in a silica crucible followed by adding of 1:1 concentrated HNO₃ and HClO₄ and was covered with watch glass. The contents in the beaker were heated on an oil bath at 100 °C. After that, the solution was cooled at 70 °C, 2 mL of hydrogen peroxide was added. The watch glass was then

Table 2. Determination of Cd(II) and Pb(II) in various natural milk samples (mean ± SD)

Samples	Method	Cd (µg L ⁻¹)	Pb (µg L ⁻¹)
Goat milk (n = 22)	DPP	0.088 ± 0.004	0.026 ± 0.005
	AAS	0.085 ± 0.003	0.025 ± 0.007
Cow milk (n = 28)	DPP	0.090 ± 0.002	0.018 ± 0.002
	AAS	0.082 ± 0.006	0.020 ± 0.006
Ewe milk (n = 24)	DPP	0.072 ± 0.008	0.017 ± 0.003
	AAS	0.065 ± 0.009	0.015 ± 0.005
Buffelo milk (n = 30)	DPP	0.047 ± 0.005	0.015 ± 0.005
	AAS	0.045 ± 0.004	0.015 ± 0.005
Human milk (n = 30)	DPP	0.034 ± 0.010	0.009 ± 0.003
	AAS	0.030 ± 0.012	0.010 ± 0.002

n = No. of determinations

removed and the acid was evaporated to dryness at 200 °C. To the white residue obtained, 0.1 mL of HNO₃ and 15 mL of triply distilled water was added to the beaker and the contents were heated at 100 °C for 1 hour. The solution was cooled, filtered if needed, and the volume was made to 100 mL with triply distilled water in a calibrated flask. An aliquot (10-50 mL) of the sample solution was taken Cd(II) and Pb(II) was determined as described in general procedure. The obtained results are given in Table 2.

To investigate the applicability of the recommended procedure to synthetic milk samples, the recoveries of known amounts (0.5 and 1.0 µg L⁻¹) of Cd(II) and Pb(II) added to these sample were examined by differential pulse polarography. The results obtained in (Table 3) indicates the synthetic data for the determination of Cd(II) and Pb(II) in various milk samples using standard addition method

Table 3. Synthetic data for the determination of Cd(II) and Pb(II) in various milk samples (n = 3)

Sample	Added (µg L ⁻¹)	DPP				ASS			
		Cd (µg L ⁻¹)		Pd (µg L ⁻¹)		Cd (µg L ⁻¹)		Pd (µg L ⁻¹)	
		Found	Recovery (%)	Found	Recovery (%)	Found	Recovery (%)	Found	Recovery (%)
Goat milk	0.5	0.486	97.20	0.482	96.40	0.494	98.80	0.484	96.80
	1.0	0.988	98.80	0.988	98.80	0.998	99.80	0.966	96.60
Cow milk	0.5	0.498	99.60	0.490	98.00	0.499	99.80	0.485	97.00
	1.0	0.998	99.80	0.964	96.40	0.997	99.70	0.994	99.40
Ewe milk	0.5	0.497	99.40	0.490	98.00	0.500	100.0	0.490	98.00
	1.0	0.990	99.00	0.960	96.00	0.999	99.99	0.980	98.00
Buffelo milk	0.5	0.479	95.08	0.488	97.60	0.497	99.40	0.492	98.40
	1.0	0.969	96.90	0.992	99.20	0.998	99.80	0.980	98.00
Human milk	0.5	0.460	92.00	0.460	92.00	0.486	97.20	0.490	98.00
	1.0	0.965	96.50	0.944	94.40	0.965	96.50	0.980	98.00

n = No. of determinations

with recovery range of 92.0% to 100.0% and compared with AAS method.

Calibration plot

A calibration plot for the determination of lead and cadmium was prepared according to the general procedure under the optimum conditions developed above from its differential pulse polarogram with different concentrations. The detection of lower limits for any newly developed method shows the degree of sensitivity and selectivity of the analytes extraction. For the purpose were performed by passing the 400 mL sample solution through the pre-conditioned resin bed, containing the each metal ions in the ranges of 0.02-150 ppm was preferred for the proposed method, a detection limit was found to be 0.020 and 0.024 $\mu\text{g L}^{-1}$ for analyte ions (Cd(II) and Pb(II)) (signal-to-noise ratio ± 2) was achieved with a correlation coefficient of 0.9996 (Fig. 4) and relative standard deviations of ± 0.95 and $\pm 0.99\%$ respectively.

Application of proposed method for the determination of Cd(II) and Pb(II) in milk samples with reported methods

A rapid and facile analytical method was developed for the simultaneous determination of Cd(II) and Pb(II) by differential pulse polarography (DPP) in cows', goats', ewes', buffalos' and humans' milk samples in Tirupati, Chandragiri, Pakala and Kanipakam. The standard addition

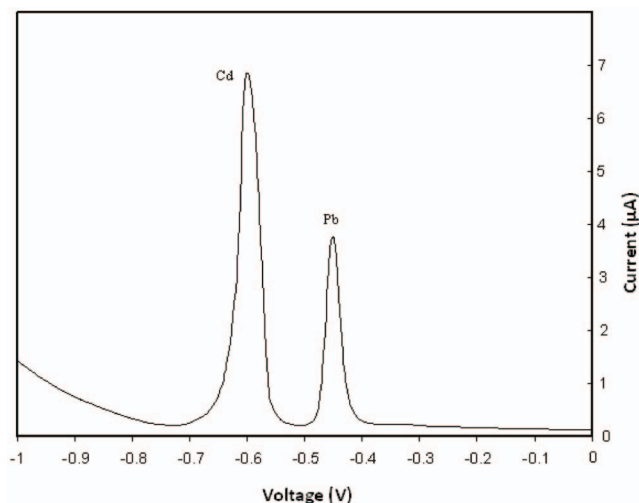


Fig. 3. The differential pulse polarogram of Cd(II) and Pb(II). Instrumental settings: scan rate = 12 mV/sec, drop time = 0.5 sec, pulse amplitude = 50 mV.

procedure was applied for the determination of Cd(II) and Pb(II) in milk samples. The standard solution was scanned from +0.0 to -1.0 volts versus a (Ag/AgCl) electrode by using the differential pulse mode, peak half wave potentials ($E_{1/2}$) were -0.6 V and -0.46 V for cadmium and lead respectively. The polarogram of metal ions for the milk samples solution, which is shown in (Fig. 3).

The Cd(II) level varied from 0.034 to 0.090 $\mu\text{g L}^{-1}$ ($p < 0.01$) ($R^2 = 0.9998$) and Pb(II) concentration varied from 0.009 to 0.026 $\mu\text{g L}^{-1}$ ($p < 0.01$) ($R^2 = 0.9999$) in whole milk samples. The concentrations of each metal are given in Table 3. The results obtained from the analysis of standard reference material which is distributed by International Atomic Energy Agency (IAEA) milk samples for the determination of Cd(II) and Pb(II) by differential pulse polarography method is presented in Table 4. These recovery studies indicate the validity accuracy of the proposed method.

The highest cadmium content was $0.090 \pm 0.002 \mu\text{g L}^{-1}$ in cows' milk, whereas the lowest cadmium content was $0.034 \pm 0.0012 \mu\text{g L}^{-1}$ in humans' milk. The highest Pb(II) content was $0.026 \pm 0.005 \mu\text{g L}^{-1}$ in goats' milk, whereas the lowest content was $0.009 \pm 0.003 \mu\text{g L}^{-1}$ in humans' milk. Cadmium and lead toxicity affects many target tissues such as blood vessels, kidneys, lungs, bones, brain, heart, kidneys, liver, nervous system, and pancreas. It may cause many signs and symptoms such as abdominal pain, anemia, anorexia, anxiety, auto exhaust, bone pain, brain damage, confusion, constipation, convulsions, dizziness,

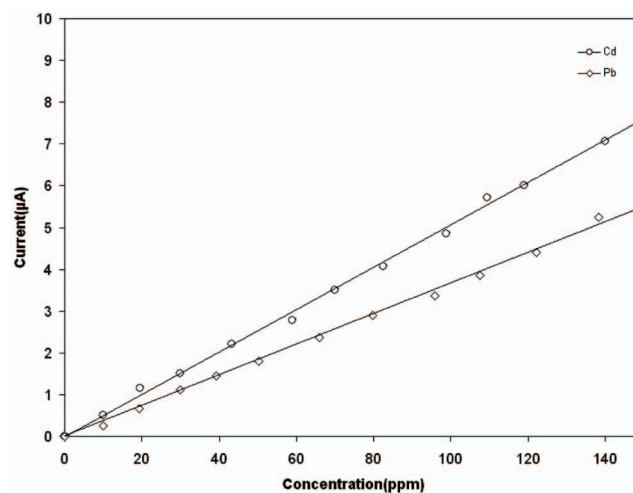


Fig. 4. The calibration curves of Cd(II) and Pb(II). Instrumental settings: scan rate = 12 mV/sec, drop time = 0.5 sec, pulse amplitude = 50 mV.

Table 4. Concentration of Cd(II) and Pb(II) in the standard reference milk sample (A-11) from IAEA

Metal	Certified concentration (IAEA values) ($\mu\text{g/g}$)	Average observed (DPP) concentrations ($\mu\text{g/g}$)
Cadmium	1.7 (1.2-2.2)	1.7 (2.0-2.2)
Lead	54 (29-79)	53 (50-70)

drowsiness, fatigue, headaches, hypertension, inability to concentrate, indigestion, irritability, loss of appetite, loss of muscle coordination, memory difficulties, miscarriage, muscle pain, pallor, tremors, vomiting, and weakness lead diminishes IQ in children. Infant nutrition acts at three main levels that are minerals and trace elements are essential for growth, development, and maintenance of healthy tissues. The important factors that can influence the intake and bioavailability of minerals and trace elements include types of food consumed, environmental pollution and extreme climatic conditions. Micronutrients are important for the survival of all living organisms and are available to the body only through diet. The cadmium content of the analyzed milk was within the ranges reported by a previous study for human milk cadmium, which ranged from 0.07 to 1.23 $\mu\text{g L}^{-1}$.²²

For goats' milk, the lead concentration found in the present study is the highest value over ewes', cows', buffalo's' and human milk. However, cows', and human milk lead contents are in agreement with some previous studies.²¹ The accuracy of the proposed method was tested by the analysis of synthetic milk samples to which a known amount of analyte ions, preconcentrated and determined by the standard procedure discussed.

There are also wide variations in the published data for the metal concentrations of human and cow milk of different countries,²¹⁻²⁵ some of the results are shown in Table 5. The Cd(II) and Pb(II) concentrations of the present study are comparable with the published data but the present values of Cd(II) and Pb(II) concentrations are normal level compared with the corresponding values of other countries.

CONCLUSIONS

The proposed method solid-phase extraction method offers a simple, economical and eco-friendly methodology for preconcentration and separation of trace Cd(II) and Pb(II) in various cows', goats', ewes', buffalos' and human milk samples prior to their analysis by DPP. The present method has added advantages over the reported methods:

Table 5. Comparisons of Cd(II) and Pb(II) concentration in human and cow milk samples from literature

Country	Samples	Concentration ($\mu\text{g L}^{-1}$)		Reference
		Cd(II)	Pb(II)	
55 countries (Mean value)	Human	1.3	--	28
	Cow	--	--	
India	Human	0.09	1.9	21
	Cow	0.1 ^a	1.6 ^a	
Saudi Arabia	Human	1.9	3.9	23
	Cow	4.7 ^a	3.5 ^a	
Poland	Human	6.2	5.4	27
	Cow	3-20 ^a	22-25 ^a	
Spain	Human	--	--	25, 26
	Cow	--	9.3 ^a	
India (Andhra pradesh)	Human	0.034	0.009	Present study
	Cow	0.090	0.018	

^a $\mu\text{g/kg}$

i) Synthesis of metal chelating agent 2,2'-DPED₃P at ordinary laboratories was simple and economical.

ii) Synthesized organic reagent is distinct in terms of sensitivity, selectivity towards metal ions.

iii) Optimum volume of the surfactant-rich phase obtained by using the present solid-phase methodology permitted to design an extraction strategy presenting robustness, low cost, good extraction efficiency and lower toxicity than those using organic solvents.

iv) The present method was solved most of these problems and has been successfully tested for determination of Cd(II) and Pb(II) in cows', goats', ewes', buffalos' and human milk samples.

Received June 6, 2009.

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