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QUALITATIVE DETECTION OF LEAD IN FORTIFIED WATER SAMPLE BY A PAPER CHROMATOGRAPHIC ENZYME INHIBITION TECHNIQUE

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ABSTRACT
Chick heads thrown out as biowaste were employed as a enzyme source for the detection of lead compounds by an enzyme inhibition technique on paper chromatograms. The lead compounds (lead nitrate and lead acetate) appeared as white spots on a pink back ground due to the inhibition of chick brain succinate dehydrogenase employing a chromogenic reagent mixture containing sodium succinate substrate, (2-(4-Iodophenyl)-3-(4-nitrophenyl)-5 phenyl tetrazolium chloride and N-methyl phenazonium metho sulphate. The minimum detectable amounts were about 5 μg of lead nitrate and 5 μg of lead acetate. Many other animal succinate dehydrogenases also gave inhibition spot under the same conditions on chromatograthic paper but they were less sensitive than chick brain succinate dehydrogenase. Water sample was fortified with lead nitrate and lead acetate and were detected by enzyme inhibition technique.

INTRODUCTION

Lead belongs to the group of heavy metals which are extensively used by man. Lead and other heavy metals are toxic and it inhibits many enzyme system (Nathanson *et al*., 1975; Habermann *et al*., 1983; Gabol *et al*.,2006). Lead has great affinity for thiol and phosphate containing ligands, which inhibits the biosynthesis of heme and thereby affects membrane permeability of liver, kidney and brain cells (Bryan, 1976). Lead fulfills no known essential biochemical functions and all the effects appeared to be adverse (WHO Report, 1977). Concentrations of soluble lead in uncontaminated fresh waters are generally 3 μg/L (Forstner and Wittmann, 1979). An increased prevalence of short term memory loss was seen in smelter workers exposed to lead (Valciukas *et al*., 1980). Soil with a high organic content is the important sink of lead in polluted areas (Kabata and Pendias, 1984). Centre for disease control concluded that blood lead levels in excess are associated with increased risk of neurotoxicity (CDC, 1991) Fine particles of lead are either transported or deposited on land and vegetation causing significant contamination (Senapati and Mishra, 1996).

Lead is readily transferred across the placenta and the concentration of lead in the blood of new born children is similar to the of their mothers (Abadin *et al*., 1997). Water lead concentration in some fresh water areas were below the water Quality standard for water lead (Nakagawa *et al*., 1998). 56% of the surface soil of the waste water irrigated agricultural soil showed pollution indices of lead and other heavy metals (Wang, 1998). Heavy metal primarily Pb, uptake by waste water sludges was also reported (Urasa and Macha, 1999). Accumulation of lead and other heave metals in vegetables was observed (Fytianos *et al*., 2001).

Lead was determined by Anodic stripping voltammetry with a detection limit of 8×10^{-12} M (Omanovic *et al*., 1994), by flame atomic absorption spectrometry with a detection limit of 10 μg/l (Ma and Adams, 1996), by graphite furnace atomic absorption spectrometry with a detection limit of 0.07 μg/l (Manzoori and Saleemi, 1996), by electro thermal atomic absorption spectrometry with a detection limit of 0.07 ng (Narukawa *et al*., 1997), by cyclic and stripping voltametry with a detection limit of 8×10^{-7} M (Osipova et al., 1997), by electro thermal atomic absorption spectrometry with a detection limit of 1.2 μg/l (Acar *et al*., 2000).

These above non-enzymatic or chemical methods are not affordable in villages or primary health centres. These equipments are available only in Central Government Laboratories or Universities only. Hence a low cost and reasonably sensitive method using enzyme inhibition technique is reported.

MATERIALS AND METHODS

The healthy female chick head was procured from slaughter house immediately after decapitation and brought to laboratory in a ice jacketed flask. The brain was isolated from head and was kept in a deep freezer. A 5% (w/v) chick brain homogenate was prepared in cold distilled water using an electrical homogenizer with teflon pestle.

The homogenate was filtered through a four layered cheese cloth. The filtrate was stored in a refrigerator and was used immediately as succinate dehydrogenase enzyme source.

All chemicals used were of analytical reagent grade and were provided by Loba Chemie, Bombay, India. Sodium succinate substrate (2.5%), 2- (4- Iodophenyl) –3-(4-nitrophenyl)-5 phenyl tetrazolium chloride (INT) (0.4%) and N-methyl phenazonium metho sulphate (PMS) (0.1%) were prepared in distilled water and mixed in 10:10:2: ratio respectively as chromogenic reagent.

Sodium chloride (0.5%), ethylene diamine tetraacetic acid (EDTA) (5%), acetone were prepared in distilled water in 9:0.5:0.5 ratio respectively as solvent system.

Lead nitrate, lead acetate, sodium carbonate and glacial acetic acid were prepared in appropriate concentrations.

Whatman No.3 filter paper strips for paper chromatography were used (Prameela Devi and Nanda Kumar, 1981).

Fortified Water sample and clean-up procedure

20 mg/l (20 ppm) of lead nitrate or lead acetate was dissolved in potable water (river water); then, 100 ml of this water sample was employed in the present investigation for clean-up. This sample was subjected to concentration by precipitation with sodium carbonate (Svehla, 1987). Equal volume of 0.5 M sodium carbonate solution was added to the above samples and were kept at room temperature for 10 minutes. These samples were transferred into centrifuge tubes and centrifuged at 2500 rpm for 15 minutes. The precipitate formed was redissolved by adding 0.5 ml of 0.5 N glacial acetic acid. After the dissolution of precipitate, the solution is diluted to 7.5 ml by adding distilled water to achieve finally 0.01 N of glacial acetic acid in the final solutions. This normality of glacial acetic acid has no effect on succinate dehydrogenase (SDH) enzyme. The final solution was used for a paper chromatographic enzymatic method of detection (Fig.1).

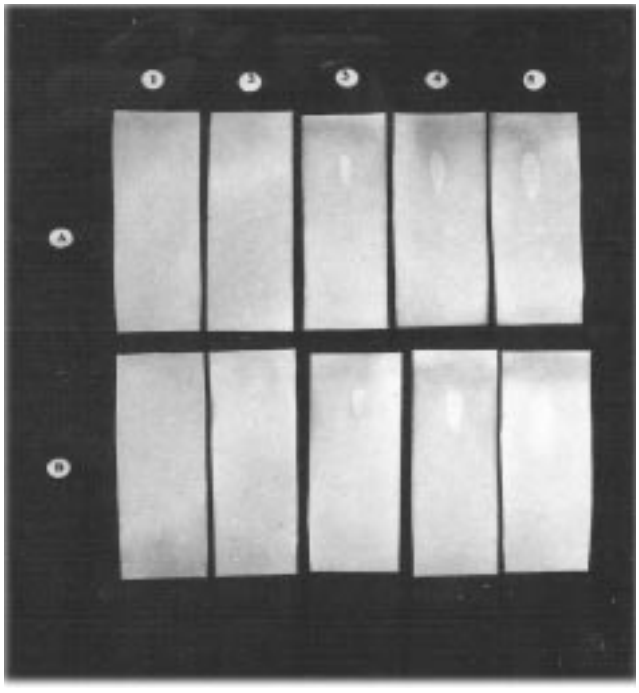

Figure 1: Detection of lead compound from fortified water sample by paper chromatographic- chick brain succinate dehydrogenase inhibition method. (A) lead nitrate fortified water sample; (B) lead acetate fortified water sample; (1-5) different concentrations of lead compounds from 1 to 15 μg.

Solvent systems for lead compounds (lead nitrate and lead acetate)

For chromatographic movement of soluble lead compounds on whatman No.3 filter paper, a number of solvent systems in different combinations were tried. 15 different solvent systems for both lead nitrate and lead acetate in different combinations and ratio's were examined (Table 2). The recommended solvent systems was 0.5% Nacl – 5% EDTA – acetone (9: 0.5:0.5,v/v) for both lead nitrate and lead acetate. It allows appropriate solubility, movement of compounds on chromatographic paper and compactness of spots. The R_f value was 0.78 $\pm$ 0.06 for both lead nitrate and lead acetate (Fig. 1). The detection limit was 5 μg.

Chromatographic – enzymatic detection

The solutions containing 1-15 μg amounts of lead were spotted through a capillary and were dried either by blowing air or by using a hot air blower (hair drier) to minimise the widening of the spot. The whatman No.3 paper strips were placed in a glass jar (12 cm x 4 cm) containing 0.5% Nacl – 5% EDTA–acetone (9 :0.5:0.5, v/v) solvent system and the chromatographing was done as per the standard method (Prameela Devi and Nanda Kumar, 1981). Pink formazan colour represents enzyme activity and the white spots represents the lead compounds (Fig.1).

RESULT AND DISCUSSION

Chick brain succinate dehydrogenase showed 0.4 μg minimum detectable limit and is sensitive than other sources (Table 1). Hence chick brain as enzyme source is recommended for the detection of lead compounds. A procedure was also developed to extract lead from fortified water sample. Specific enzyme compatible solvent system was also developed for detection of lead compounds. This solvent system not only useful for the detection and separation of lead compounds as distinct and compact chromatogram but also useful for detection and separation of interfering from other heavy metal compounds. The

minimum detection limit for both lead nitrate and lead acetate by a solvent run on whatman No.3 paper is 5 μg with R_f value of 0.78 $\pm$ 0.06 (Fig.1). The detection of lead nitrate and lead acetate in fortified water samples were confirmed by running a standard technical grade lead compound which also showed 0.78 $\pm$ 0.06 R_f value.

Table 1: Minimum detectable amounts of lead compounds by succinate dehydrogenace inhibition method from various animal tissues on whatman No.3 filter paper without solvent run (spot test).

S. No.	Animal source	Minimum detectable amount of lead nitrate or lead acetate (μg)	Remarks
1.	Sheep liver (fresh)	10	Less sensitive than chick brain
2.	Pig liver (liver acetone powder)	6	Less sensitive than chick brain
3.	Rat liver (fresh)	8	Less sensitive than chick brain
4.	Beef liver (liver acetone powder)	2	Less sensitive than chick brain
5.	Horse liver (liver acetone powder)	6	Less sensitive than chick brain
6.	Calotes liver (fresh)	5	Less sensitive than chick brain
7.	Frog liver (fresh)	4	Less sensitive than chick brain
8.	Pila globosa hepato pancreas (fresh)	7	Less sensitive than chick brain
9.	Chick brain (fresh)	0.4	More sensitive (recommended)

The principle of detection of lead by enzyme inhibition on paper chromatography is as follows. The succinate dehydrogenase is a kreb's cycle enzyme converts sodium succinate substrate into a metabolite fumarate in a dehydrogenation reaction (Mayes, 2000). During this chemical reaction elections are liberated and are accepted by tetrazolium salt (Selighman et al., 1956) namely INT, reducing it into a pink coloured formazan. Pink colour formazan indicates the enzymatic reaction. (Prameela Devi and Nanda Kumar, 1981; Rajendra Babu and Nanda Kumar, 1982). White inhibition spots indicates the inhibition of succinate dehydrogenase activity. Specific R_f value confirms the presence of lead compound. The enzymatic method is simple, sensitive, less cumbersome and might find application in the qualitative detection of trace amounts of lead. This method is an alternative method to the chemical methods for qualitative detection.

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Table 2: Lead compound (Lead nitrate or Lead acetate) chromatogram appearance and movement in different solvent systems detected by chick brain succinate dehydrogenise inhibition method on whatman No.3 filter paper.

S. No.	Solvent system	Ratio	Movement of chromatogram On Whatman No.3 filter paper	Recommendations
1	Distilled water	-	No proper movement of chromatogram	x
2	Acetone	-	No movement of chromatogram	x
3	0.1% NaCl	-	Chromatogram showed trailing	x
4	0.5% NaCl	-	Chromatogram showed trailing	x
5	1% NaCl	-	Chromatogram showed trailing	x
6	Chloroform	-	No movement of chromatogram	x
7	N-hexane	-	No movement of chromatogram	x
8	5% EDTA : acetone	0.5:4.5	No movement of chromatogram	x
9	5% EDTA : acetone	1:4	No movement of chromatogram	x
10	0.5% NaCl : acetone	9:1	Chromatogram showed trailing	x
11	1%NaCl: acetone	9:1	Chromatogram showed trailing	x
12	0.5%NaCl : 5% EDTA : acetone	9:0.5:0.5	Compact chromatogram	□
13	1%NaCl : 5% EDTA : acetone	9:0.5:0.5	Chromatogram moved upto solvent front	x
14	0.5%NaCl : 5% EDTA : acetone	0.5:0.5:9	No movement of chromatogram	x
15	1%NaCl : 5% EDTA : acetone	0.5:0.5:9	No movement of chromatogram	x

Note : □ : Recommended solvent system
x : Not recommended solvent system

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