Indirect micellar spectrophotometric determination of lead in various soybean based products using green chemistry

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ABSTRACT

The study was conducted for the purpose of quantifying lead in various samples of soy-products by micellar spectrophotometry using green chemistry approach. The ligand \( N_2P_4HB \) was synthesized for the purpose with the help of weak acid and is found to be soluble in alcohol, acetone and in chlorinated hydrocarbons such as chloroform. It dissolves in alkaline aqueous media but is practically insoluble in water at pH less than 6. In the presence of surfactants its solubility has been found to be increased significantly. On addition of solution of ligand in presence of surfactants to metal ion solution, colored complex were formed. The local nonpolar microenvironment of the micelles incorporates the non-polar colored complex. Secondly a marked increase in absorption intensity has also been observed. This phenomenon of micellar solubilization with increasing absorption intensity has been used to develop a novel, green, simple, inexpensive and sensitive spectrophotometric method for determination of heavy metal ion in various soy products.

Key words: Heavy metals, lead, Micellar spectrophotometric, soymilk and soy-paneer.

INTRODUCTION

A variety of acceptable foods can be developed from soybean to fit in the Indian dietary pattern. Coagulation of soymilk yields a white soft gelatinous mass. The product has bland taste and unique body and texture resembling paneer obtained from milk in appearance and physico-chemical characteristics. Thus it can serve as a substitute for milk paneer and can also be cheaper source of quality proteins (Ojha et al., 2014).

According to extensive research, heavy metals like lead can enter in our food cycle through soil or during processing operation and have the most damaging effects on human health. It can enter the human body maximum through uptake of food (65%) followed by air (15%). Processed foods such as RTE /RTS soy-products, meat, grains, seafood, soft drinks and wine may contain significant amounts of lead. Cigarette smoke also contains small amount of lead. As per Chemical Agent Briefing Sheet, 2006 - lead can enter (drinking) water through corrosion of pipes or contamination of the soil and further to the product during processing. This is more likely to happen when the water is slightly acidic.

Lead can enter a foetus through the placenta of the mother. Because of this it can cause serious damage to the nervous system and the brains of unborn children. In the human body, lead inhibits porphobilinogen synthase and ferrochelatase, preventing both porphobilinogen formation and the incorporation of iron into protoporphyrin IX, the final step in heme synthesis (Cohen et al., 1981).

Literature reveal a number of methods (Sunandamma et al., 2011, Magda, 2006, Rajesh and Manikandan, 2007, Prasad and Sharma, 2012) to determine lead spectrophotometrically. In the present investigation the author proposed a novel green, simple, inexpensive and selective methods for determination of heavy metals like lead in soy products – soymilk and soy-paneer using micellar spectrophotometry.

MATERIALS AND METHODS

To develop a standard formulation and process, the study involved several phases, one of them is the synthesis of ligand, preparation of stock solutions and reagents and the other is preparation of soymilk and soy-paneer samples. The manufactured soy-products were analysed for presence of heavy metals.

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Food grade soybean variety MAUS-47 supplied from Marathwada Agricultural University (MAU), Parbhani, was used in the study. All beans were stored in a cool dry storage room until used for preparation of soymilk and further soy-paneer. Coagulant (Calcium Chloride) food grade quality was procured from Scientific Trading, Mehsana. The non ionic surfactant Triton X-100 (Polyoxyethylenetetraoctylphenylether) with a molecular weight of 624 (product of CDH) was used as such. EC Double Beam UV-VIS Spectrophotometer, with quartz cell of 10 mm light path was used for Electronic spectral measurement at GCRC (Green Chemistry Research Center) Bikaner (Raj.). The Elico model pH meter was used to adjust the pH values of various solutions.

**Preparation of soymilk:** Soymilk was prepared using the method described by Kulkarni et al. (1985) with slight modification to produce smooth textured soy-paneer. Approximately 200 gm soybean split was prepared from cleaned beans by soaking in water for half an hour and then dried in sunlight for 2 days. The soybean split was prepared by breaking in stone miller. This soybean split was then cleaned. The purpose of soybean split making was to dehull the beans.

The dehulled soybean split were soaked in water at room temperature for a period of 16 hours and ground with water at a bean: water ratio 1:9. The resultant slurry was strained through 3 layered muslin cloth to remove soy residue (okara), to obtain soy milk of 11°Brix. The brix of soy milk was measured using hand refractometer. Two market samples of UHT packed soymilk samples were also procured for simultaneous study.

**Preparation of Soy-paneer:** A 500gm portion of soymilk was heated to a temperature of 80°C under stirring. The time period of heating was kept constant for every soy-paneer preparations. Soy-paneer was prepared by coagulating the soymilk using coagulant calcium chloride @ 0.5% (Murdia, et al., 2010).

Coagulant dissolved in 20 ml of cold water and was used immediately. The hot soymilk and coagulant solution was poured simultaneously into a glass container ensuring good mixing. The coagulant soymilk suspension was allowed to stand undisturbed for a period of 20 minutes to ensure coagulation. The curd formed was broken thoroughly and pressed in muslin cloth. The whey was drained off for 10 minutes and then pressed uniformly (Gangopadhyay, et al., 1992) The Soy-paneer in muslin cloth was transferred to plastic bag and stored in refrigeration. Simultaneously two more ready to use market samples of tofu were procured from Ahmedabad market. The products obtained were evaluated for their heavy metal contents.

**Reagents:** In the Green synthesis of N-(2'-Pyridyl)-4-hydroxybenzamide (N2P4HB) 4-hydroxybenzoic acid (1 mmol) and 2-aminopyridine (1 mmol) mixed in minimum amount of ethanol and then irradiated under microwave (600 W) for 20 min, the mixture was taken out filtered and filtrate was concentrated and amide was precipitated by the addition of water and crystallized from ethanol (Garg, et al., 1999). It is a weak acid and is found to be soluble in alcohol, acetone and in chlorinated hydrocarbons such as chloroform.

Stock solution of lead (II) was prepared by dissolving appropriate amount of analytic grade lead nitrate in double distilled water and stored in a coloured bottle. The solution was standardized by titrating against standard sodium chloride solution using K2CrO7 as indicator.

**Experimental:** A series of solutions, containing 2.5 ml of 1.85X10⁻³M lead(II), 2.5 ml buffer solution of different pH, 2.5 ml 1.8X10⁻³M Triton X-100 and 0.5 ml of 9X10⁻¹⁰M N2P4HB were prepared and their pH values were adjusted at different levels in a total volume of 25 ml. The absorption spectra of these solutions were recorded against reagent blank. A plot of pH versus absorbance at λmax 485 nm showed that the absorbance increased from pH 5.0 to 7.0 slowly, then increased rapidly up-to pH 7.5 after that it remained constant upto pH 8.5 and finally decrease. In further studies a pH 8.0 was selected.

Absorbance of solutions containing varying amounts of the N2P4HB, fixed amount of lead(II) 2.5 ml of 1.85X10⁻³M and triton X-100, 2.5 ml of 1.8X10⁻³M were recorded at pH 8.0. It is revealed that at least 4 times molar excess of N2P4HB is required to get maximum and constant absorbance.

Further effect of concentration of Triton X-100 was studied by preparing various solutions having increasing concentration of Triton X-100 at a fixed concentration of lead(II) 2.5 ml of 1.85X10⁻³M and maintaining the pH at 8.0 in 25 ml with double distilled water and absorbance was measured at lmax 445 nm. A plot of observation revealed that the absorbance remains constant from 1.8 X 10⁻³ M, and in further study its concentration is fixed at 1.8 X 10⁻³ M.

**RESULTS AND DISCUSSION**

Absorbance of the solutions containing increasing amounts of lead(II), fixed N2P4HB concentration 0.5ml of 9X10⁻¹⁰M, Triton X-100 concentration 2.5ml of 1.8X10⁻³M and at pH 8.0 were prepared in 25 ml with double distilled
water and absorbance measured at \(\lambda_{\text{max}}\) 485 nm. A plot of Pb(II) concentration in ppm versus absorption intensity at \(\lambda_{\text{max}}\) 485 nm has been represented in Fig. 1. A linear curve was obtained in the range 0.2 – 2.4 ppm.

In order to investigate the stability of the complex, absorbances were measured at different time intervals at \(\lambda_{\text{max}}\) 485 nm. A plot of time in minutes versus absorbance in Fig 2, showed that the complex was stable up to 60 minutes in ordinary conditions.

The order of mixing of various solutions had no significant effect except in few cases where precipitates appeared though these were dissolved or settled down immediately by the addition of surfactant solutions. The general order followed was buffer solution, metal solution, surfactant solution and reagent solution.

**Recommended procedure:** To a suitable aliquot of solution containing 0.2-2.4 mg/ml of lead(II) add 2.5 ml borate buffer of pH 8.0, 2.5 ml of \(1.8 \times 10^{-3}\) M Triton X-100 and 0.5 ml \(9 \times 10^{-3}\) M N2P4HB solution. Dilute the solution to 25 ml with double distilled water and measure the absorbance at 445 nm against the reagent blank.

**Effect of diverse ions:** The selectivity of the method was investigated for the determination of 2 ppm of lead in the presence of other ions. An ion was considered to be interfering, if the absorbance obtained differed more than \(\pm 5\%\) from that of lead(II) alone. Most of the cations and several anions did not interfere. Only copper, zinc and cadmium ions interfered seriously.

**Analytical application:** The optimum analytical conditions and characteristics for the determination of lead with N2P4HB in triton X-100 micellar medium are mentioned in Table 1. The proposed method was applied for the determination of lead in soy-products. After pretreatment 25 gm of soymilk and soy-paneer samples were taken into a slowly heated silica crucible when all the moisture was removed the temperature was raised to approximately 450-500°C avoiding loss of sample by foaming and swelling. At this temperature ignition was continued until grey ash was obtained. The crucible was allowed to cool. The ash was dissolved in minimum amount of concentrated nitric acid and evaporated to dryness and ignited again at 450-500°C for 1h. Dissolved the resulting white ash in minimum amount of dilute nitric acid and the amount of lead was determined by following the recommended procedure. The results for the estimations are presented in Table 2.

**CONCLUSION**

<table>
<thead>
<tr>
<th>(\lambda_{\text{max}})</th>
<th>485 nm</th>
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<tbody>
<tr>
<td>pH</td>
<td>7.5 to 8.5</td>
</tr>
<tr>
<td>[N2P4HB]</td>
<td>&gt; 8 \times 10^{-4} M</td>
</tr>
<tr>
<td>[Triton X-100]</td>
<td>&gt; 1.8 \times 10^{-3} M</td>
</tr>
<tr>
<td>Beer’s law (in ppm) optimum concentration</td>
<td>0.2 to 2.</td>
</tr>
<tr>
<td>Molar extinction coefficient ((\varepsilon)) 1 mol(^{-1}) cm(^{-1})</td>
<td>5.1 \times 10^4 M</td>
</tr>
<tr>
<td>Sandell’s sensitivity ((\mu g\text{ Pb(II)/cm}^2))</td>
<td>0.0012</td>
</tr>
</tbody>
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<p>| TABLE 2: Lead content in different samples of soymilk |</p>
<table>
<thead>
<tr>
<th>Sample</th>
<th>Amount of Lead present (mg/g)</th>
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<tbody>
<tr>
<td>Soya milk (Market sample – 1)</td>
<td>0.23 ± 0.005</td>
</tr>
<tr>
<td>Soya milk (Market sample – 2)</td>
<td>0.24 ± 0.008</td>
</tr>
<tr>
<td>Soya milk prepared at lab</td>
<td>0.20 ± 0.007</td>
</tr>
<tr>
<td>Tofu (Market sample – 1)</td>
<td>0.42 ± 0.02</td>
</tr>
<tr>
<td>Tofu (Market sample – 2)</td>
<td>0.44 ± 0.02</td>
</tr>
<tr>
<td>Soy-paneer prepared at lab</td>
<td>0.34 ± 0.01</td>
</tr>
</tbody>
</table>

± means standard error. Each value is mean of values in triplicate (n=3). Values with different superscript show significant difference (p<0.05)
The present investigations have revealed that N2P4HB is suitable for determination of lead, as spectrophotometric reagent. The method is quite sensitive, simple and rapid in Triton X-100 micellar medium. Although copper, zinc and cadmium ions interference makes it less selective but it ranks amongst the most sensitive reagents known so for the purpose. The developed methods have been applied successfully for the determination of these lead in soya milk and soy-paneer. It was also concluded that the market samples of both soya milk and tofu contains the lead within permissible limit. The product made in lab contains significantly lower amount of lead than that of market samples. This may be due to the lower exposure of product (made in lab) towards different unit operations in industry.

REFERENCES