SPECTROPHOTOMETRIC DETERMINATION OF ANTIMONY AND BISMUTH IN THEIR MIXTURE AFTER SEPARATION BY SOLVENT EXTRACTION AND PAPER CHROMATOGRAPHY

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INTRODUCTION

DETERMINATION of antimony and bismuth which usually accompany each other, mostly in biological materials, is of particular importance. McChesney[1] described a method for the determination of both in biological materials, using varying concentrations of the iodide ion. Bismuth, in presence of 200 p.p.m. or below of antimony, was determined by complexing antimony with fluoride or tartaric acid[2]. Besides, several other methods[3-6] are known for the determination of bismuth in samples containing antimony and other metal ions where bismuth was separated first by solvent extraction. West and Hamilton[7], on the other hand, described a method for the separation of antimony from many elements by solvent extraction. Antimony in aqueous solution containing potassium iodide and sulphuric acid was extracted with benzene which was then identified with rhodamine B.

Based on this and also from the fact that antimony and bismuth can be determined separately by developing their colour with potassium iodide in sulphuric acid, an extraction spectrophotometric determination of both was tried.

Large number of investigators[8-11] have effected separation of many metal ions by paper chromatographic method and in many cases determined their R_f values. As an alternative to the extraction photometric method, chromatographic separation followed by spectrophotometric determination as their iodo complex was also studied.

EXPERIMENTAL

Reagent.—An 11·2% solution of potassium iodide containing 2 gm per 100 ml ascorbic acid was used as chromogenic reagent.

Standard Solutions.—For the extraction method of separation, antimony and bismuth sulphate solutions were prepared in a mixture of dil. sulphuric and nitric acids. The resulting solutions was diluted to contain 100 ppm in each.

For separation by chromatographic method, a synthetic mixture of both in HCl containing 1000 ppm of each was prepared.

A. *Extraction spectrophotometric method.*—Synthetic mixtures of chloride free bismuth and antimony were prepared by taking aliquots containing 30, 25, 20, 15, 10 and 5 μg of bismuth in 50 ml separating funnels and adding to these 5, 10, 15, 20, 25 and 30 μg of antimony respectively. Each of these solutions were diluted to 5 ml by adding 0·75 ml of 27 N sulphuric acid (solutions should be 4 N with respect to sulphuric acid), 2·5 ml of potassium iodide reagent and distilled water. Solutions were allowed to stand for few minutes and then shaken with 5 ml portion of benzene for 5 minutes. Aqueous layers were transferred to another set of funnels and extractions, in this manner, were repeated 3–4 times. Combined benzene extracts, in each, was then evaporated on a water bath and the residue was treated with sulphuric acid and potassium iodide as above making the final volume 5 ml. The resulting solutions which gave clear spectrum of iodoantimonite ion (Fig. 1) were read at 425 nm.

Aqueous layers were slightly warmed on water bath to remove any dissolved benzene and finally volumes were made up in 5 ml standard flasks. Characteristic spectrum of the iodobismuthite ion (Fig. 1) was obtained in the aqueous layer. Extinctions were measured at 460 nm. The results, as calculated from the individual calibration curve, are shown in Table I.

TABLE I

Extraction spectrophotometric determination of bismuth and antimony

Bismuth μg		% Error	Antimony μg		% Error
Present	Found		Present	Found	
30·0	29·9	0·4	5·0	4·3	14·0
25·0	24·9	0·4	10·0	9·1	9·0
20·0	20·0	0·0	15·0	14·0	6·6
15·0	14·8	1·3	20·0	19·2	4·0
10·0	9·8	2·0	25·0	23·8	4·8
5·0	5·0	0·0	30·0	28·6	4·6

B. *Spectrophotometric determination after separation by paper chromatography.*—Whatman No. 1 chromatographic paper was cut into strips 30 × 2·5 cm. Equal volumes of mixture (containing 1000 ppm bismuth and antimony) were then applied (keeping the spot area to a minimum) to two chromastrips with the help of a micro pipette at a distance of about 5–6 cm from one end of the strip.

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Paper strips, after drying the spot with a hair drier, were kept for an hour or two in air before subjecting to chromatographic development.

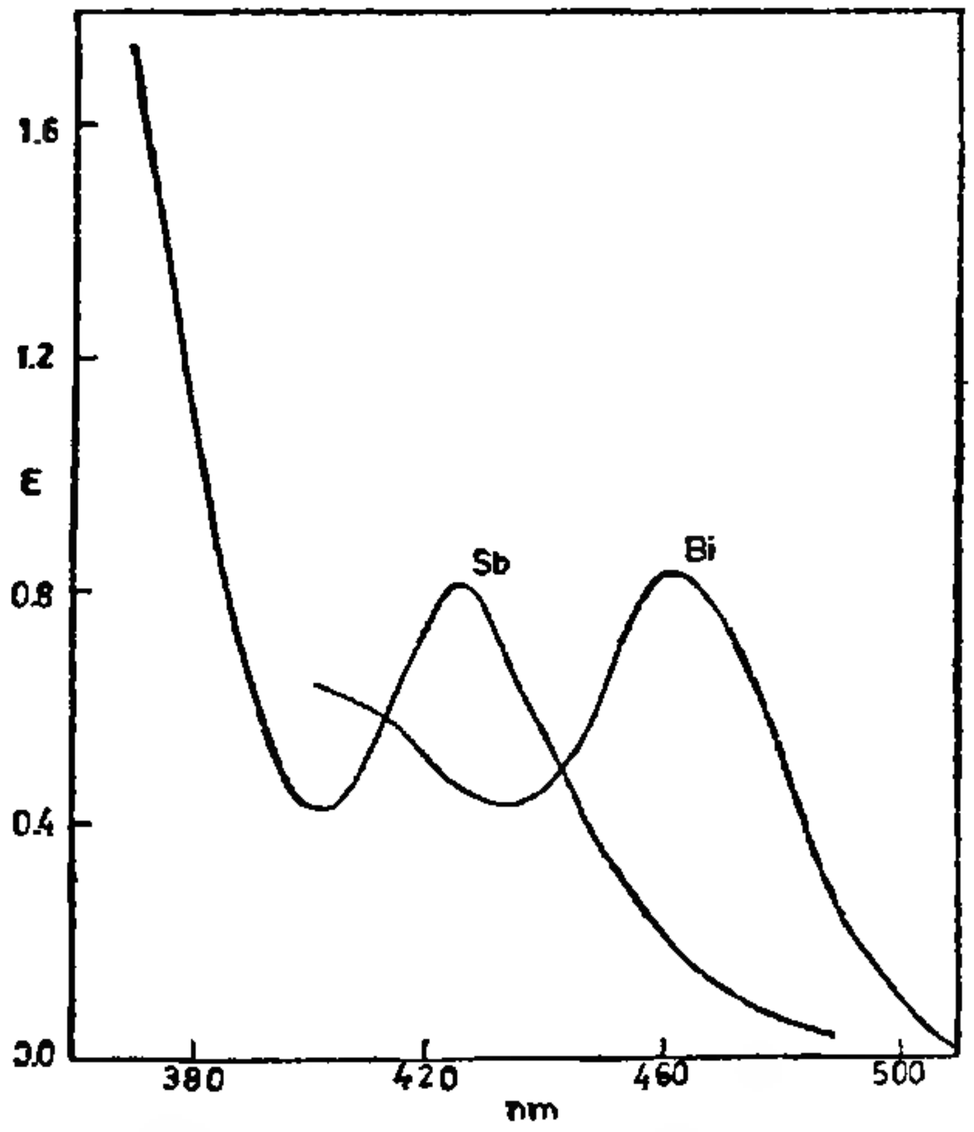

FIG. 1. Absorption spectra of Bi and Sb as their iodo complex after separation by solvent extraction. (Bi = 15 μg/ml ; Sb = 20 μg/ml).

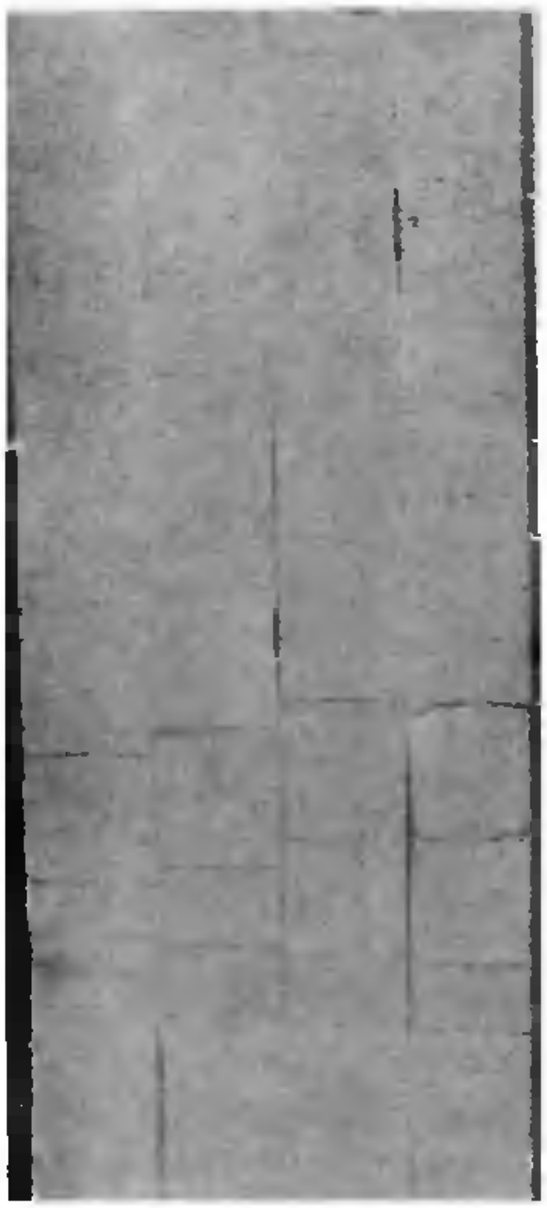

PLATE 1. Paper chromatographic separation of Bi and Sb. Upper spot—Sb ; Lower spot—iB.

The two strips (forming a pair) having equal amount of Bi^{3+} and Sb^{3+} were then fixed along a glass "T" tube, keeping the spots in the two strips in parallel position. These strips were then lowered inside a cylinder of 6 cm diameter and chromatography was performed in an ascending manner using ethyl methyl ketone + 10% concentrated hydrochloric acid as developer. Mouth of the cylinder was closed with a rubber stopper. Development was allowed to continue for 5–6 hours till the solvent front moved to a distance of 20–25 cm. Paper strips were then taken out and dried, and one of the two strips (reference strip) was then sprayed with 0·5% oxine reagent, dried, and finally exposed to ammonia. A dark spot near the solvent front and a yellow spot below this (shown in Plate 1) was observed in this reference strip. These spots appeared as dark and purple respectively under ultraviolet light.

Corresponding regions from the unstained papers were then cut and put inside 50 ml conical flasks. These were then eluted with a mixture of 0·75 ml of 27 N sulphuric acid, 2·5 ml of potassium iodide reagent and 1·75 ml of distilled water. Solutions were allowed to stand for 5 minutes. Eluates of the first and the second spot gave characteristic spectra of antimony and bismuth respectively similar to the ones shown in Fig. 1. Their extinctions were measured at 425 nm and 460 nm and the amount of antimony and bismuth were read from the calibration curve. Results are shown in Table II.

TABLE II

Determination of antimony and bismuth after paper chromatographic separation

Antimony μg		% Error	Bismuth μg		% Error
Present	Found		Present	Found	
5·0	4·6	8	5·0	4·6	8
6·0	5·7	5	6·0	5·4	10
7·0	6·5	7	7·0	6·9	2
8·0	7·3	9	8·0	7·9	2
9·0	8·6	5	9·0	8·9	2
10·0	9·8	2	10·0	9·6	4

Discussion.—Of all the methods, the iodide method is the simplest and yet give satisfactory result. However, in their simultaneous determination, a double reading was necessary, once at 460 nm and then at 425 nm for bismuth using 1·6% potassium iodide reagent and another at 425 nm for total amount of bismuth and antimony using 11·2% potassium iodide reagent. Antimony was determined by subtraction after necessary correction for bismuth. In the present method 'A' the same concentration of the reagent is used and the determination is

carried out after separation by solvent extraction. Good recovery was achieved as shown by the average of a number of determinations in Table I. Antimony, could not be determined directly in the benzene layer as the layer showed faint iodine colour even in the reducing atmosphere of ascorbic acid.

Number of developer liquids like water, ethanol + 10% 5 N HCl, butanol + 10 N HCl, pyridine water were tried for chromatographic separation of bismuth and antimony. With the first two developers, both the metals formed a mixed spot. With butanol + 10 N HCl, although the separation was achieved, the spots eluted with KI did not give their characteristic spectra. Antimony and bismuth could be separated with pyridine-water as developer when applied as their nitrates. But when applied as chlorides, a mixed spot was obtained. However, high acid concentration in ethyl methyl ketone containing 10% concentrated HCl reduced the possibility of oxysalt formation and thus resulted in clean separation. The ketone escaped on drying the paper and the spot eluted with KI gave characteristic spectra facilitating their spectrophotometric determination with an error less than 10% in the ppm range.

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NUTRITIONAL EVALUATION OF SOME INDIAN NONCULTIVATED WILD LEGUMINOUS SEED PROTEIN ISOLATES

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The protein isolates from some uncultivated wild leguminous seeds when supplemented with the limiting amino acids, methionine and tryptophan and fed to animals, no untoward symptoms were noticed and the diets proved capable of promoting growth and maintained positive nitrogen balance in them. Also they did not appear to induce any deleterious physiological after-effects on the animals as evinced by liver protein and several liver enzyme assays.

IN a previous communication[1] a number of wild leguminous seeds were analysed for their chemical composition and their essential amino acid content. Finding their protein and amino acid contents only slightly inferior to casein it was considered pertinent to supplement them with the inadequate amino acids and test their efficiency as growth promoters. However, as their unpalatability, bad odour and toxicity in some cases disallowed feeding of the entire seeds to experimental animals, their soluble proteins were extracted and tested with and without supplementation. The present communication describes the extraction, isolation and purification of proteins from these wild leguminous seeds and their evaluation by animal feeding experiments.

Incidentally, as liver tissue and liver enzymes are the most sensitive to respond to alterations in dietary protein, both qualitatively and quantitatively, liver protein depletion and repletion studies and assays of some important liver enzyme systems that respond significantly during altered conditions of protein feeding, were carried out.

Materials and Methods

Purified protein isolates were prepared as described earlier[2].

Evaluation of Proteins by Animal Experiments.—The biological values of the isolated proteins and their protein efficiency ratios were determined by animal experiments on 12 albino rats per group, 4–5 weeks old and weighing 40–48 g. Both the balance sheet method[3,4] and the rat growth method[5,6] were employed.

Experimental Diets.—A diet practically nitrogen-free but adequate in all other respects was prepared. It contained soluble starch (analytical reagent grade) 80 parts; sucrose, 10 parts; groundnut oil, 6 parts;