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DETERMINATION OF SELENIUM IN BIOLOGICAL MATERIALS WITH ASCORBIC ACID

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Selenium reacts with ascorbic acid to give a light orange colour having maximum absorbance at 375 nm. The colour reaction has 1 μg/ml as the visual limit of identification and provides the basis for a new spectrophotometric method for the microdetermination of selenium from pure solution and biological materials which are of vital importance. The quantitative assessment of the tolerable amounts of different ions which do not interfere with determination has been reported.

INTRODUCTION

It is well known fact that sulphur in cystine and methionine in many plants can be very easily replaced by selenium. Areas having high concentration of selenium in soil may be dangerous for human as well as animals due to selenium poisoning.

The determination of selenium in biological materials is consequently of great importance. Apart from toxicological aspect it has been found that selenium is also an essential trace element [1] in a number of animals. Disorders associated with vitamin E and white muscle disease in sheep can be prevented by administering selenium in trace amounts [2].

It is very important to devise some sensitive method for the determination of selenium in biological materials. Phenylene-diamine hydrochloride [3] has been used for the microdetermination of selenium in technical sulphuric acid. The procedure requires at least 3 hr. to perform and during the procedure there are many stringent conditions to be maintained, thus making the method almost inapplicable.

Thioglycolic acid has also been used for the determination of selenium [4] but there are many metal ions which interfere in the procedure. The absorbence of the ethyl acetate phase was measured at 260 nm and in UV the measurements are not very reliable.

Selenium was determined by heat treatment from concentrated sulphuric acid [5]. The method is not sensitive and has many interferences, thus making it inapplicable to biological materials.

Potassium permanganate and condensed phosphoric acid also give green colour with selenium but interferences in this method have not been checked. The procedure was only applied to pure solutions and it was not tested on biological materials which are of vital importance [6].

In the present investigation ascorbic acid has been applied for the microdetermination of selenium in pure and biological materials. It has been found that ascorbic acid produces a light orange colour with selenium. The oxidising potential of selenium is fairly low and not many metal ions and anions interfere in the procedure.

The method is convenient, rapid, precise, and accurate.

EXPERIMENTAL

Reagents. The selenium solution was prepared by dissolving 100 mg of pure selenium dioxide in 250 ml distilled water, standardized and diluted to a solution containing selenium 10 μg/ml.

The ascorbic acid solution was prepared by dissolving 250 mg of pure ascorbic acid in a little distilled water to which were added a few crystals of sodium meta-bisulphite and the resulting solution was made upto 250 ml in a volumetric flask.

Gelatin solution (3% in water) was prepared by dissolving gelatin in hot water and decolourising it with charcoal. Sulphuric acid and all other reagents used were of analytical grade.

Procedure: An accurately measured volume (0.7 ml) of the test solution was taken in a test tube. 2 ml ascorbic acid solution was added and the solution was allowed to stand for 2 min. Then 0.5 ml of gelatin solution was added and the total volume was made upto 5 ml with distilled water. The solution was thoroughly shaken and the resulting light orange colour was measured at 375 nm employing an Sp-600 spectrophotometer and 1 cm cell, using 0.5 ml of gelatin solution and 4.5 ml of distilled water as blank. The experiment was repeated with different volumes of selenium dioxide solution and a calibration curve was prepared

(Fig. 1). The colour reaction obeys Beer's law in the range of 5 μg to 100 μg.

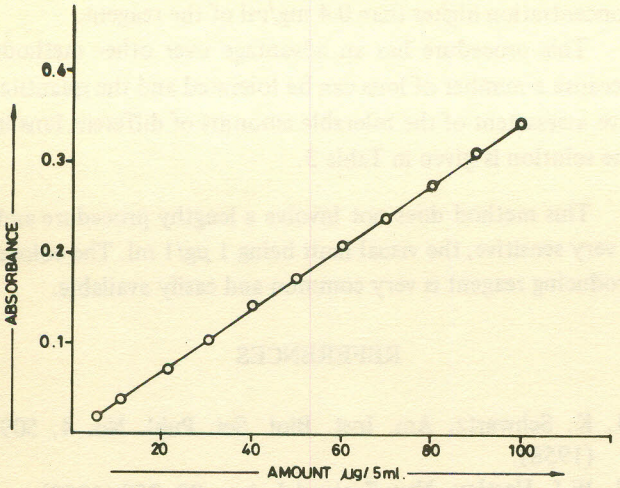

Fig. 1. Calibration curve

An accurately weighed amount of grass or leave of alfalfa or beaf kidney was taken in a round-bottomed flask and to it 25 mg of selenium dioxide was added along with 2 ml of pure concentrated sulphuric acid and 3 ml of pure hydrogen peroxide. The flask was fitted with a water condenser and heated. Digestion was carried out till the solution became colourless. The total volume was made up to 250 ml with distilled water and the amount of selenium was determined in the same way as for the pure selenium dioxide solution. This procedure was repeated for the other biological materials.

RESULTS AND DISCUSSION

The colour developed in this reaction has maxima at 375 nm; hence all absorbence measurements were carried out at this wave length and the colour reaction obeys Beer's law.

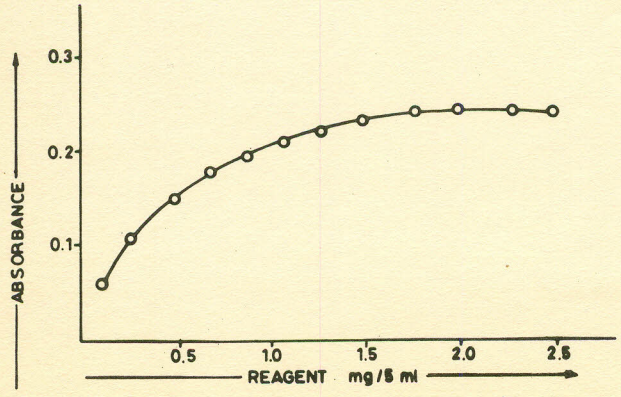

Fig. 2. Effect of reagent concentration.

Table 1. Determination of selenium in pure solution

Amount taken μg	Amount found μg	Standard deviation
2.0	2.0	0.1
5.0	5.0	0.0
10.0	10.0	0.1
20.0	20.0	0.1
30.0	29.6	0.5
40.0	40.2	0.5
50.0	49.7	0.4
60.0	59.8	0.3
70.0	70.0	0.6
80.0	79.0	0.7
90.0	89.5	0.8
100.0	100.6	0.7

Table 2. Recovery of added selenium from biological materials

	Amount added μg	Recovery μg	Standard deviation
Grass			
	5.0	4.7	0.7
	10.0	9.8	0.5
	20.0	19.6	0.5
	30.0	29.5	0.6
	40.0	39.3	0.4
	50.0	50.0	0.3
	60.0	60.5	0.3
Leaves	5.0	5.0	0.6
	10.0	9.5	0.6
	20.0	19.7	0.4
	30.0	29.9	0.5
	40.0	39.8	0.4
	50.0	49.6	0.2
	60.0	59.0	0.4
Kidney			
	5.0	4.6	0.8
	10.0	10.2	0.6
	20.0	19.6	0.7
	30.0	29.8	0.4
	40.0	39.5	0.3
	50.0	50.0	0.5
	60.0	60.5	0.5

Table 3. Quantitative assessment of tolerable amounts of different ions

Ions	Maximum amount of non-interfering ions %	Ions	Maximum amount of non-interfering ions %
Mg^{2+}	160	Ni^{2+}	200
Ca^{2+}	400	Co^{2+}	80
Th^{2+}	40	Sn^{4+}	200
Cu^{2+}	80	Zn^{2+}	400
Pb^{2+}	200	Sb^{5+}	20
Bi^{3+}	400	Ti^{4+}	200
Mn^{2+}	200	Cl^{-}	400
Tl^{3+}	80	Br^{-}	400
Hg^{2+}	80	NO_3^{-}	300
Sr^{2+}	100	SO_4^{2-}	300
Zr^{4+}	300	SO_3^{2-}	200
W^{6+}	80	SCN^{-}	200
As^{3+}	400	CH_3COO^{-}	200

The absorbtion measurements were carried out below 34° and low results were obtained at higher temperature. The colour was stable for 3 hr. after which turbidity appeared.

The effect of the reagent concentration is shown in Fig. 2 and the maximum colour intensity is obtained at concentration higher than 0.4 mg/ml of the reagent.

This procedure has an advantage over other methods because a number of ions can be tolerated and the quantitative assessment of the tolerable amounts of different ions in the solution is given in Table 3.

This method does not involve a lengthy procedure and is very sensitive, the visual limit being 1 μg/1 ml. The colour producing reagent is very common and easily available.

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