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A New Reagent System for Photometric Analysis of Selenium in Complex Materials

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A new reagent system consisting of 4-nitrophenyl hydrazine and 8-quinolinol is described for the photometric determination of selenium. 4-Nitrophenyl hydrazine is oxidised with selenious acid in 6 *M* hydrochloric acid to 4-nitrophenyl-diazonium chloride which is then coupled with 8-quinolinol to form an 'azoxine' dye. The dye gives intense purple colour in alkaline medium with an absorption maxima at 550 nm. The colour system obeys Beer's law in the range of 7-45 μg Se/25 ml. The molar absorptivity and Sandell's sensitivity are 3.2×10^4 l. mole^{-1} cm^{-1} and 0.0025 μg/cm^2 respectively. The optimum reaction conditions and other analytical parameters are evaluated. The effect of various ions is discussed. The method is applied for detection and determination of selenium in complex materials such as cabbage leaf and cigarette paper.

SELENIUM and its compounds are of wide occurrence and known for their toxicity[1]. Selenium may be inhaled as fumes, dust or absorbed through skin and gastrointestinal tract. When taken into body, it is rapidly absorbed in blood and gets accumulated in lower kidney and muscle tissue. Several reports list selenium as a carcinogen[2,3]. Intoxication due to increased dietary intake of selenium has been reported to cause bad teeth, jaundice, chlosema, vertigo, chronic gastrointestinal disorder, dermititis, hair loss, fatigue, etc[4]. Intake of selenium bearing plants causes chronic selenosis. It has been found that cruciferous plants accumulate much more selenium than other vegetables[5]. Selenium is also reported to be present in cigarette paper and tobacco[6-8]. These materials are largely consumed. Hence, sensitive methods are required for analysis of selenium in these materials.

Several analytical methods have been proposed for determination of selenium and they have been nicely reviewed[9-11]. Most popular spectrophotometric methods for determination of selenium are based on preparing sol of selenium and subsequent measurement of absorbance of the colloidal solution. Different reducing agents and stabilising agents have been proposed[12]. The methods are less sensitive and less specific. Other methods are based on the formation of complexes with *o*-diamines which form coloured or fluorescent paizselenol with selenious acid[13,14]. These methods lack sensitivity and selectivity. Some other methods make use of oxidising property of selenious acid[15]. The present investigation is based on oxidation of 4-nitrophenyl hydrazine to 4-nitrophenyl diazonium chloride with selenious acid in strong hydrochloric acid which is then coupled with 8-quinolinol in alkaline medium. The violet coloured dye is measured photometrically at 550 nm. The optimum reaction conditions and other analytical parameters are evaluated. The method has been successfully used for the analysis of selenium in cigarette paper and cabbage leaves.

Experimental

Apparatus and reagents : All spectrophotometric measurements were done at ECIL spectrophotometer model GS-865 and Carl Zeiss Spekol using 1 cm matched silica cells. An ECIL *p*H meter model 821 was used for *p*H measurements. A thermostated water bath was used for maintaining constant temperature.

The stock solution of selenium (1 mg/ml) was prepared by dissolving 0.22 g of sodium selenite in 100 ml distilled water. Working standards were prepared by appropriate dilution. 0.02% solution of 4-nitrophenyl hydrazine (4-NPH) in water and 0.2% solution of 8-quinolinol in 90% ethanol were used.

Procedure : An aliquot of sample solution (no more than 4 ml) containing 7-45 μg of selenium wa transferred to a test tube. To it was added 1 ml o 4-NPH and the acidity was maintained around 6*M* The test tube was kept at 60 ± 2° for 15 min in th thermostated water bath and the contents wer transferred to a 50 ml beaker. To it was addec 1 ml of the 8-quinolinol solution, 1 ml of 10% solution of disodium EDTA and 1 ml of 10% solution of sodium potassium tartrate and mixe well. The mixed solution was made alkaline b adding 5*M* sodium hydroxide solution drop by drop cooling it simultaneously to 25° to prevent th decomposition of the dye by the heat produce during neutralisation. The violet dye was transferre into a 25 ml volumetric flask and diluted to the mar with distilled water. The absorbance of this solu

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tion was measured at 550 nm and the amount of selenium was calculated from a calibration curve.

Preparation of sample solution : Into a 100 ml Kjeldahl flask 1 g of dried crushed cabbage leaf/ cigarette paper from 5 cigarettes was taken and to it was added 15 ml concentrated nitric acid. The solution was heated till reduced to 2-3 ml. This procedure was repeated for 3-4 times to ensure complete decomposition. Then 5 ml of conc. hydrochloric acid was added and heated till 2-3 ml was left. This procedure was also repeated 2-3 times to remove nitrous fumes. The digest was filtered, if necessary, and the volume was made upto 10 ml with distilled water. Selenium content was determined by the recommended procedure. Precaution must be taken to see that mixture does not dry up completely during digestion, otherwise there will be loss of selenium through volatilisation of oxides of selenium[16]. It was found that samples of cabbage had no selenium, therefore known amount of selenium was added to the sample before digestion. Selenium content was then determined by the recommended procedure. As concentrated nitric acid and hydrochloric acid was found to give nearly precise results, under the condition employed, the use of perchloric acid[17,18] was avoided for the reasons of safety. However, when perchloric acid was used, the results obtained were high, since oxidants interfere with this method.

Results and Discussion

Spectral characteristics : Absorption spectra of reagent and azoxine dye are shown in Fig. 1. The dye shows λ_{max} at 550 nm. The reagent has negligible absorption at this wavelength. However, a reagent blank was used to compensate for any change in the colour of the reagent on storage.

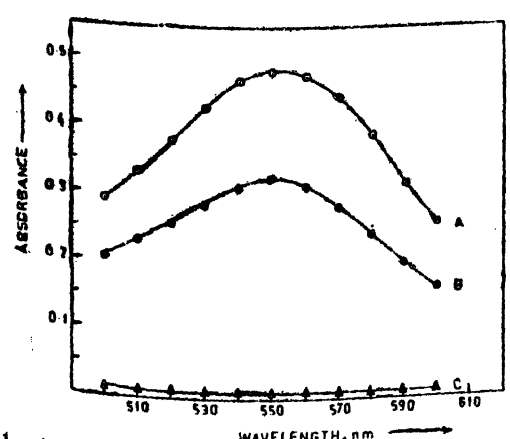
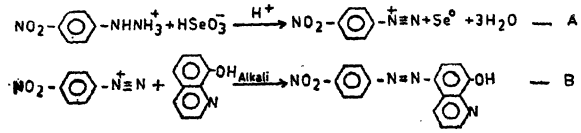


Fig. 1. Absorption spectra of azoxine dye and reagent blank in alkaline medium against water.
A. Se = 30 μg/25 ml.
B. Se = 20 μg/25 ml.
C. Reagent black.

Reaction : The reaction is given below. The reaction B is identical with the reaction proposed by Nair and Gupta[19].



Effect of variables :

Acidity : The effect of acidity on oxidation of 4-NPH was studied by maintaining different molarities of hydrochloric acid ranging from 1 to 7 *M* and developing the colour by the recommended procedure. This revealed that atleast 5 *M* hydrochloric acid was required for complete oxidation. Constant absorbance values were obtained when the acidity was above this. It was also found that wave length of maximum absorption remains constant in the range of acidity.

Effect of time, temperature and reagent concentration : The oxidation reaction is dependent on temperature. To determine the effect of temperature and time, the reaction was carried out at temperatures varying from 25 to 60°. The maximum time required for complete oxidation and stability of the diazonium chloride at respective temperature were evaluated by carrying out the oxidation reaction for different time and developing the colour. It was found that higher temperature accelerates the reaction, as the same intensity of colour is reached in 10 min at 60°, but in 45 min at 25°. However, the stability of diazonium chloride decreases at higher temperature. In this investigation the reaction was carried out at 60±1° for 15 min.

The amount of reagent is not critical. For 7-45 μg of selenium per 25 ml, 1 ml of 4-NPH and 1 ml of 8-quinolinol gave reproducible results. At higher concentration 4-NPH itself gave colour with sodium hydroxide and it should be avoided.

Effect of pH and stability of dye : It was found that for coupling reaction *p*H 11 is required to attain maximum absorbance. This *p*H was attained with 5 *M* sodium hydroxide.

The dye was stable for 3-4 hr. Some turbidity was observed after this period.

Beer's law, molar absorptivity and Sandell's sensitivity : Beer's law was obeyed in the range of 7 to 45 μg/25 ml. The molar absorptivity and Sandell's sensitivity were 3.2×10^4 1. mole^{-1} cm^{-1} and 0.0025 μg/cm^2 respectively. The detection limit was 0.1 μg/ml. The standard deviation and relative standard deviation were ±0.0026 and ±0.814%, respectively.

Validity of the method : To check the validity of the method, 20 μg of selenium was determined in presence of known amounts of interferants. 100 fold excess of Br^-, CN^-, CNS^-, CO_3^{2-}, PO_4^{3-}, SO_4^{2-}, citrate and tartrate, 50 fold excess of Be^{2+}, Ba^{2+}(a), Co^{2+}(a), Ca^{2+}(a), Ni^{2+}(a), Mg^{2+}(a), Zn^{2+}(a), Bi^{3+}(b), Li^+, K^+, Pb^{2+}(a), Al^{3+}(a), 20 fold excess of Te^{4+}, Hg^{2+}(a) and 5 fold excess of Fe^{2+}, Cr^{3+} do not interfere. Many ions (marked by a and b) interfere by precipitation as hydroxide. These are masked with 1 ml of 10% disodium salt of EDTA (a) and

1 ml of 10% sodium potassium tartrate (b), respectively. Cu^{2+}, oxidants such as potassium permanganate, potassium dichromate, Fe^{3+} and reductants such as sulfide, thiosulphate, Sn^{2+} interfere. The interference of Cu^{2+} and Fe^{3+} can be eliminated by separating them as their cupferronates[20].

The method was then successfully applied for the analysis of selenium in cabbage leaves (Table 1) and cigarette paper.

TABLE 1—DETERMINATION OF SELENIUM IN CABBAGE LEAVES

Selenium added μg	Selenium found* μg	Recovery %
40	39.00	97.50
20	19.50	97.50
10	9.70	97.00

*Average of three determinations.

Cigarette paper of different brands were found to contain different amounts of selenium. It was also found that the selenium content varied with different lot of the same brand. It was found that even one cigarette paper contained sufficient selenium to give positive test for selenium by this method.

The method is simple, sensitive, free from rigorous control of *p*H and can be compared favourably with other methods[12-15]. The coupling reagent 8-quinolinol used in proposed method is non-toxic as compared to α-napthylamine used in Kirkbright's method[15]. The method can be applied for the analysis of selenium in complex materials with reproducible results.

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