STUDIES IN ORGANO-SELENIUM COMPOUNDS

PART I. DETERMINATION OF Se

BY D. DINGWALL AND W. D. WILLIAMS

From the School of Pharmacy, Royal College of Science and Technology, Glasgow

Received August 31, 1960

Selenium in some organo-selenium compounds has been determined colorimetrically by conversion to selenious acid and hence to a selenium sol stabilised with chlorpromazine hydrochloride. Non-ionic, cationic and anionic stabilisers have been compared with chlorpromazine hydrochloride but only cetomacrogol appeared to be as efficient.

WITH the development of studies in organo-selenium compounds in this School a simple method for the determination of selenium became essential. The available methods are generally based upon oxidation of selenium to selenious acid which can be determined by volumetric (Yoshimura, 1957; Bradt and Lyons, 1926), gravimetric (Banks and Hamilton, 1939; Drew and Porter, 1929), or colorimetric procedures. The most sensitive of the colorimetric methods appears to be that described by Hoste and Gillis (1955) involving the formation of a coloured piazselenol with diaminobenzidine. Relatively less sensitive reactions are those using such reducing agents as sulphurous acid, hydrazine and stannous chloride (De Meio, 1948) and ascorbic acid (Yoshimura, 1957), and one using codeine sulphate (Gortner and Lewis, 1939). Selenious acid in the presence of reducing agents yields golden brown sols which rapidly deposit red selenium, and for quantitative work a stabiliser must be present. Many substances have been used for this purpose, among them acacia (Robinson, Dudley, Williams and Byers, 1934), gelatin (Yoshimura, 1957), glycerol (Dolique, Giroux and Pérahia, 1946), mucilage from *Plantago psyllium* seeds (Gutbier, Huber and Eckert, 1923), saponin (Gutbier and Rhein, 1923) and starch (van der Meulen, 1934) in colorimetric and volumetric procedures.

A chance observation led to the discovery that chlorpromazine hydrochloride, and similar phenothiazine derivatives, stabilised selenium sols to give a stable colour suitable for spectrophotometric measurement at 420 mμ. The use of a pure chemical entity as a reagent offered a material advantage over complex colloidal material in the preparation of standard solutions and as glycerol proved unsatisfactory, chlorpromazine hydrochloride was used in preference to such substances as starch or gelatin. When it became evident that a complex of selenium and chlorpromazine was not involved (cf. palladium chloride and chlorpromazine, as described by Ryan, 1959), the efficiency of the reagent was compared with that of other substances.

EXPERIMENTAL

Reagents. Sulphuric acid. Nitric acid. Hydrochloric acid. Ascorbic acid solution, 1 per cent w/v. Chlorpromazine hydrochloride solution, 2 per cent w/v. Sodium hydroxide solution, 20 per cent w/v.

Standard selenium dioxide. Selenium dioxide (reagent grade, 50 g.) was treated with nitric acid (25 ml.) in an evaporating dish and heated in a heating mantle until the removal of nitric acid was complete. The surface-crust of colourless needle crystals (24·5 g.) was easily separated

TABLE I

PERCENTAGE OF SeO_2 WHEN DETERMINED BY VARIOUS CHEMICAL METHODS

Material	Method			
	Potassium permanganate (volumetric)*	Iodine/thiosulphate (volumetric)** (Method B)	Gravimetric (procedure A)†	Gravimetric (hydrazine)††
Original	99·6 99·6	94·6 94·4	93·0 93·1 96·3	91·7
Standard	99·6 99·6	99·7 99·7	99·6 100·0	99·2 99·3

* Vogel (1951a). ** Vogel (1951b). † Vogel (1951c). †† Gutbier, Metzner and Lohmann (1904).

from an amorphous grey residue by means of a spatula, stored in a desiccator and used for the preparation of standard selenious acid solutions. Analyses of original and standard selenium dioxide are recorded in Table I.

Reaction time. The extinction of the sols obtained when selenious acid was treated with ascorbic acid solution (1 ml.) reached a maximum within

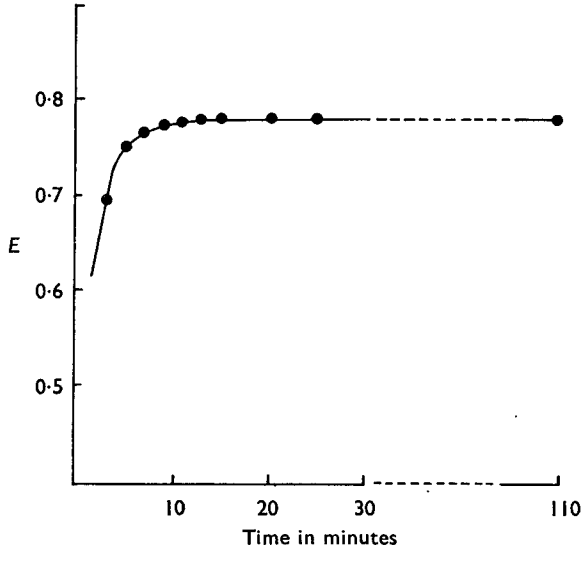

FIG. 1. Development and stability of the extinction of a selenium sol.

20 min., indicating the reaction was complete. A standard reaction time of 30 min. was therefore adopted. Fig. 1 illustrates the results obtained in the presence of chlorpromazine hydrochloride solution (1 ml.) as stabiliser.

Effect of Acidity on the Stability and Extinction of Se Sols

Nitric acid (traces) present. (*a*) Weighed quantities of selenium dioxide were examined as described in the Method for the Determination of Selenium (below) omitting the neutralisation with sodium hydroxide and addition of hydrochloric acid. The development of the colour when chlorpromazine hydrochloride and cetomacrogol were used as stabilisers was slow and the results were variable.

(*b*) Aliquot portions of the selenious acid solutions obtained in (*a*) above were carefully neutralised and treated as described in the Method for the Determination of Selenium (p. 15) from the words "add hydrochloric acid (1 drop) . . .". No abnormality was observed in the development

TABLE II

EFFECT OF A MODERATE EXCESS OF ACID ON EXTINCTIONS

Excess of hydrochloric acid ..	As in method	1 drop	2 drops
Extinction	0·883	0·878	0·886

TABLE III

EFFECT OF SODIUM SULPHATE ON EXTINCTIONS

Extinction (Na_2SO_4 absent)	0·344	0·528	0·684	0·844
Extinction (Na_2SO_4 present)	0·356	0·538	0·714	0·895

TABLE IV

EFFECT OF TEMPERATURE ON EXTINCTIONS

Temperature (° C.) ..	13	22	25
Extinction	0·292	0·289	0·284

of the colour or the constancy of the readings. A similar result was obtained when cetomacrogol solution (1 per cent, 1 ml.) was used in place of chlorpromazine hydrochloride solution.

(*c*) A solution of selenious acid obtained during one of the assays was examined in the presence of one and two drops of hydrochloric acid in excess of that stated in the Method for the Determination of Selenium. The results are recorded in Table II.

Nitric acid absent. Aliquot portions of a selenious acid solution (0·208 mg./ml.) were treated with sulphuric acid (0·2 ml.), cetomacrogol (0·01 per cent, 1 ml.), ascorbic acid solution (1 ml.) and made up to 10 ml. with water. The extinctions reached a maximum within 30 min. and remained stable.

Effect of Sodium Sulphate on the Extinction of Se Sols

Colours were developed from selenious acid solutions in the presence of sodium sulphate (anhydrous, 0·5 g.) and in its absence. The results are recorded in Table III.

Effect of Temperature on the Extinction of Se Sols

Colours were developed from a selenious acid solution at 13°, 22° and 25°. The results are recorded in Table IV.

Comparison of Stabilisers

Varying quantities of stabilisers were added to slightly acid solutions of selenious acid and a sol developed by the addition of ascorbic acid solution (1 ml.). The sols were adjusted to 10 ml. with water and the extinctions were measured after 30 min. at 420 mμ in 1 cm. cells. The results are recorded in Table V.

TABLE V

EXTINCTIONS OF SELENIUM SOLS IN THE PRESENCE OF STABILISERS

Stabiliser	SeO_2 (mg.)	ml. of 1 per cent solution of stabiliser						
		0·005	0·01	0·2	0·5	0·6	1·0	2·0
Chlorpromazine hydrochloride	1·0	—	—	Turbid	Unstable	0·860	0·832	0·820
Cetomacrogol 1000 B.P.C.	0·604	0·490	0·490	0·480	0·476	—	0·468	0·463
Cetrimide	0·604	—	0·479	—	—	—	0·474	—
Gelatin	0·604	—	—	0·530	0·520	—	0·504	—
Sodium dodecylsulphate	0·604	—	Turbid	Turbid	—	—	0·468	—
Starch	0·604	—	—	0·695	0·620	—	0·567	—
Glycerol	0·5	—	Turbid at all concentrations even up to 1 ml. in 10 ml. of final mixture.					

TABLE VI

EFFECT OF ORGANIC MATTER ON THE RECOVERY OF SELENIUM

Organic compound	SeO_2 weighed (mg.)	SeO_2 found (mg.)	Recovery (per cent)
Glucose	3·260	3·21	98·5
Glucose	2·350	2·35	100·0
Methylene blue	3·360	3·32	98·8
Phenothiazine	3·348	3·28	98·0
Phenothiazine	3·310	3·32	100·3
Phenobarbitone	3·040	3·06	100·7
Thiouracil	2·990	2·97	99·3
	1·038	1·04	100·2

Method for the Determination of Selenium

Dissolve an accurately weighed quantity of the organic compound, equivalent to about 2–3 mg. of Se, in sulphuric acid (1 ml.) and nitric acid (2 ml.) in a Kjeldahl flask of about 40 ml. capacity. Boil off the nitric acid carefully to leave a colourless residue (7–15 min.)* If the residue acquires a red or brown tint towards the end of the reaction add 1 drop of nitric acid and remove the excess by heating. Cool the residue, dilute with water (10 ml.) and neutralise with sodium hydroxide solution using 1 drop of phenolphthalein solution as indicator. Cool the mixture to 20°, transfer quantitatively to a 25 ml. graduated flask, make up to volume and mix well. Transfer 5 ml. of the solution to a 10 ml. graduated flask, add hydrochloric acid (1 drop) from a teat pipette to make slightly acid, chlorpromazine hydrochloride solution (1 ml.) water (2 ml. if necessary

* The digestion can also be carried out by electrical heating as used in the normal micro-Kjeldahl process but a period of 2 hours is necessary to remove the nitric acid.

to give a clear solution) and ascorbic acid solution (1 ml.). Make up to volume with water, mix well and measure the extinction of the sol at 420 mμ in a 1 cm. cell after 30 min. using a blank of reagents treated in the same way. Calculate the percentage of selenium in the compound by reference to a calibration curve prepared by using known amounts of selenium dioxide in the presence of sodium sulphate (anhydrous, 0·5 g.) and hydrochloric acid (1 drop) in a final volume of 10 ml.

Calibration Curve

The curve obtained showed no deviation from Beer's law up to the maximum amount of selenium dioxide used (1·0 mg.).

Effect of Organic Matter on the Recovery of Selenium

Accurately weighed quantities of selenium dioxide were treated as described in the Method for the Determination of Selenium in the presence of 7–10 mg. of organic compounds. The results are recorded in Table VI.

Discussion

Standard Selenium Dioxide

The required product for the formation of a colour is selenious acid, H_2SeO_3, which is formed when selenium dioxide dissolves in water. Sublimation of the dioxide always gave traces of red selenium in the product and proved unsatisfactory in preparing a standard. The method using nitric acid, however, gave colourless crystals and was similar to that of Lenher (1898) except that condensing funnels were omitted.

The standard gave satisfactory analyses when examined by four methods whereas the apparent content of SeO_2 in the original material depended upon the method used. The results are deemed to be of sufficient interest to be given in Table I.

Oxidation of Organic Matter

Oxidation of the organic compounds with a mixture of sulphuric and nitric acids proceeded smoothly and without the significant loss of selenium which is liable to occur when such an oxidation mixture is used (Fogg and Wilkinson, 1956; Gorsuch, 1959). This is no doubt explained by the very much smaller quantity of organic matter present (less than 10 mg.) as compared with 0·2–10 g. when traces of selenium are determined in vegetable material (Gorsuch, 1959; Williams and Lakin, 1935). Perchloric acid, which yields quantitative recovery of selenium (Fogg and Wilkinson, 1956), could not be used in this instance because selenious acid is oxidised further to selenic acid which is not reduced under the conditions of the assay. Control experiments with selenium dioxide in the presence of glucose and compounds which are difficult to oxidise gave reasonable recoveries of Se (Table V).

Reduction of Selenious Acid

Reduction of selenious acid to selenium is more conveniently carried out by ascorbic acid rather than by hydrazine, stannous chloride or sulphurous acid.

Although reduction can be carried out in strongly acid media (approximately N) the results of the experiments described under Effect of Acidity on the Stability and Extinction of Se Sols showed that the presence of appreciable traces of nitric acid in the final solution slowed the rate of colour formation and gave erratic results. Neutralisation of the oxidation mixture was therefore essential in order that some degree of control could be exercised over the final acidity. One and two drops of hydrochloric acid in excess of that used in the method caused no marked change in extinction and allowed a reasonable latitude in the size of drops.

Colour

The colour is attributed to colloidal selenium rather than to a complex of selenium and chlorpromazine because it is similar to that obtained when other reagents are used. This is confirmed by the precipitation of selenium on addition of ethanol to the reaction mixture.

The sols showed no absorption maximum and the nominal wavelength of 420 mμ was selected because this region is conveniently obtained on a filter absorptiometer if a spectrophotometer is not available. Further, the extinction of the blank in chlorpromazine hydrochloride is small in this region.

Stabilisers

Three of the stabilisers were quickly eliminated from consideration by the appearance of the final sols. Starch and gelatin gave slightly opalescent sols which showed a marked fall in extinction when the concentration of colloid was increased. These disadvantages could well give rise to difficulties when different batches of stabiliser are used. Cetrimide was more satisfactory but the Tyndall effect was still evident under the assay conditions. The remarkably small effect of increasing cetrimide concentration is of interest (Table IV). Although highly efficient in slightly acid, salt-free solutions it could not be used without preliminary neutralisation of the oxidation mixture.

Cetomacrogol 1000 B.P.C. was very efficient (Table V) and a determined effort was made to confirm it as the stabiliser of choice. Encouraging results were obtained in the presence of sulphuric acid, but, as shown in the experimental section, neutralisation of the oxidation mixture could not be avoided. As a consequence, the selenium sols showed an obvious Tyndall effect at low concentrations of cetomacrogol and an increased concentration was necessary to give the degree of clarity obtained when chlorpromazine hydrochloride was used. Thus cetomacrogol under the assay conditions lost two possible advantages, viz., elimination of one step in the assay and a low concentration of reagent. In addition, the composition of cetomacrogol may vary slightly from batch to batch so that a check on the effect of using different batches of material would be necessary before suggesting its use in the colorimetric assay. Consequently there appeared to be no advantage in reverting to cetomacrogol as stabiliser although it was realised that other analysts, bearing in mind such factors as cost and nature of materials, might well prefer it.

Chlorpromazine hydrochloride was therefore retained as stabiliser and no difference in results was noted when using different batches of reagent. A concentration effect was observed as with other stabilisers but it was not regarded so seriously since standard solutions can be reproduced more easily than with colloids.

Sodium dodecylsulphate was not examined in detail but was included to show the effect of an anionic surface-active agent.

Salt and Temperature Effects

As might be expected, the presence of sodium sulphate affected the extinction of the sols (Table II). It must therefore be included when

TABLE VII

SELENIUM IN ORGANIC COMPOUNDS

Indent. No.	Type of compound	Se (per cent) Found	Theory
1	Amino-selenazolone, hydrochloride	39·4	39·64
2	Amino-selenazolone, picrate	20·2	20·13
3	Amino-selenazolone, hydrobromide	30·8	30·66
4	Amino-selenazolone, picrate	19·2	19·45
5	Amino-selenazolone, hydrobromide	29·0	29·09
6	Amino-selenazolone, picrate	18·6	18·78
7	Se-Benzyl iso-selenourea hydrochloride	31·4	31·70
8	Selenothiazine	31·6	32·0
9	Selenothiazine	27·7	28·15
10	Selenothiazine	24·5	24·1
11	Benzyldiselenide	45·9	46·19
12	Selenazolidine	41·6	41·11
13	Selenazolidine	43·9	44·34
14	Selenazolidine	37·8	38·30
15	Selenazolidine	40·9	41·11

preparing the calibration curve. The effect of temperature (Table III) indicated that a wide range for development of colour should be avoided and solutions were generally used at about 20°.

RESULTS

The results obtained on synthetic compounds are given in Table VII. With the exception of compounds 7 and 11 (Table VII) only the class of compound is given but satisfactory elemental analyses were obtained for all.

In view of the results in Table VI those in Table VII would appear to be reliable figures for the content of Se in the organic compounds.

The method has the advantage over the volumetric method of Gould (1951) in that it is applicable to compounds which contain iodine. Any iodate produced in the oxidation step is reduced by the chlorpromazine.

Acknowledgements. We wish to thank Dr. J. B. Stenlake for helpful discussion, Mrs. D. Harvey and Miss M. Buchanan for technical assistance and Smith, Kline and French Ltd., for the supply of compounds 8, 9 and 10 and the award of a maintenance grant to one of us (D.D.).

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