

# THE BEHAVIOUR OF SOME THIOSEMICARBAZONES TOWARDS SILVER NITRATE AND A GRAVIMETRIC ESTIMATION OF 4-ACETAMIDOBENZALDEHYDE THIOSEMICARBAZONE

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FOLLOWING reports by Domagk<sup>1,2,3</sup> on the tuberculostatic activity of 4-acetamidobenzaldehyde thiosemicarbazone,  $\text{CH}_3\text{CONH} \text{---} \text{C}_6\text{H}_4 \text{---} \text{CH}=\text{NNHCSNH}_2$ , which have since led to clinical trials in this country, the analytical behaviour of several aromatic thiosemicarbazones has been investigated in order to establish a satisfactory method of assay. A review of the literature shows that little work has been carried out on the analysis of this type of compound, although several methods are described for the estimation of semicarbazones. These methods<sup>4,5,6</sup> based on the quantitative estimation of the ammonia formed during hydrolysis of the compound have given very unsatisfactory results when applied to the analysis of 4-acetamidobenzaldehyde thiosemicarbazone. The variations appear to be due to the partial hydrolysis of the acetamido group to give free ammonia under the conditions necessary for the complete hydrolysis of the thioamide group. Using the Kjeldahl method of determining nitrogen, the ammonia liberated corresponded to only 2 of the 4 nitrogen atoms. Furthermore, in the presence of sugar or starch the method gave abnormally high values for the nitrogen content, probably as a result of some reduction of the hydrazine formed in the reaction<sup>5</sup>. Thus this method could not be applied to the estimation of the compound in the form of tablets containing carbohydrates, and alternative methods of assay were therefore investigated.

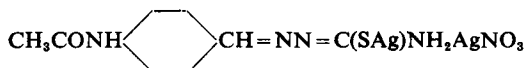
In an attempt to find an improved procedure for the isolation of aldehydes and ketones, Neuberg and Neimann<sup>7</sup> examined the metal derivatives of several thiosemicarbazones. Of these, the silver derivatives appeared to be the most useful and the authors found that the complexes obtained corresponded to the general formula  $\text{RR}'\text{C}=\text{NN}=\text{C}(\text{SAg})\text{NH}_2$ . In all cases, the Volhard estimation of silver showed that one silver atom combined with one "thiosemicarbazide residue." Later, Harlay<sup>8,9</sup> re-examined these complexes and found that, in addition to the Neuberg form, other combinations of thiosemicarbazones and silver nitrate could be obtained in which more than one thiosemicarbazone group was associated with each silver atom. For example, by using  $\frac{2}{3}$  of the theoretical volume of 0.1N silver nitrate solution, acetone thiosemicarbazone yielded a crystalline precipitate consisting of a combination of 3 molecules of the thiosemicarbazone and 2 molecules of silver nitrate. In the present investigation we have precipitated the silver derivative from a solution of 4-acetamidobenzaldehyde thiosemicarbazone in methyl alcohol

using excess of a saturated methyl alcoholic solution of silver nitrate. Using aqueous silver nitrate the precipitate tended to be gelatinous but in methyl alcohol and particularly in the presence of a few drops of nitric acid the precipitate was denser, more crystalline in appearance and easily filtered. The silver derivatives of several other aromatic thiosemicarbazones have been prepared and the silver content determined by Volhard's method. The stability of the silver derivatives varied considerably and many were not sufficiently stable towards light and heat to permit quantitative estimation of the silver. Those complexes which were examined are given in Table I and show that in each case more than

TABLE I  
THE SILVER DERIVATIVES OF SOME THIOSEMICARBAZONES

Thiosemicarbazone	Mol. Wt.	M. Pt.	Characteristics of silver derivatives		
			Silver content per cent.	Thiosemicarbazone per cent.	No. of silver atoms/mol. of thiosemicarbazone
Benzaldehyde ... ..	179·2	159 to 160°C.	46·34 46·50	39·60 39·66	1·94 1·90
4-Nitrobenzaldehyde ... ..	208·2	250°C.(d)	38·98 38·91	52·39 50·84	1·44 1·48
Salicylaldehyde ... ..	195·2	220°C.	40·48 40·44	49·23 49·09	1·49 1·49
4-Ethylsulphonyl-benzaldehyde...	269·3	235°C.(d)	35·61 35·12	55·89 56·59	1·59 1·55
4-Acetamidosalicylaldehyde ...	251·3	252°C.(d)	34·65 34·32	54·94 55·55	1·47 1·44

one silver atom is associated with each thiosemicarbazone molecule. By far the most stable complex was that obtained from 4-acetamidobenzaldehyde thiosemicarbazone, and a complete examination of the derivative was possible. Elemental analysis indicated that it had the formula  $C_{10}H_{11}O_4N_3SAG_2$  while determination of the nitrate group with nitron suggested a possible structure:—



The derivative, which is stable at 100° to 105°C., remains unchanged after boiling with water, but treatment with boiling N nitric acid, results in the elimination of one silver atom. The compound can also be estimated in the form of tablets by extracting with methyl alcohol, filtering off the excipient (e.g. starch) and precipitating the silver derivative quantitatively from the filtrate. These results together with the assay figures for several samples of 4-acetamidobenzaldehyde thiosemicarbazone are summarised in Table II.

During the course of our investigation it was found necessary to obtain

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a simple and rapid method of assaying thiosemicarbazide. Again a review of the literature has shown that few details are given on the estimation of this compound<sup>8,11</sup> but semicarbazide has received more

**TABLE II**  
THE GRAVIMETRIC ESTIMATION OF 4-ACETAMIDOBENZALDEHYDE THIOSEMICARBAZONE

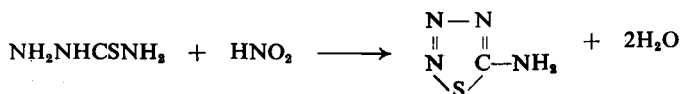
Sample	Melting Point*	Assay per cent.
1	226 to 227°C. (d)	99·63
2	228 to 229°C. (d)	100·0
3	227 to 228°C. (d)	99·82 99·86
4	226 to 227°C. (d)	99·51 99·79

THE ASSAY OF TABLETS (NOMINAL ACTIVE CONTENT 50 MG.)

Sample	Assay—per cent.	Wt. of drug/tablet
1	33·45	49·2 mg.
2	33·49	49·8 mg.
3	32·04 32·09	49·9 mg.

\*M.pt. 230°C. (d) Behnische<sup>10</sup>.

thorough investigation<sup>12,13,14</sup>. We have evolved a novel method for the estimation of thiosemicarbazide using the known reaction with nitrous acid to form 5-amino-1,2,3,4-thiotriazole<sup>15</sup>



The reaction is quantitative and proceeds rapidly at room temperature to give a sharp end-point with starch-iodide indicator. We have compared this method with that referred to by Harlay<sup>8</sup> in which the silver derivative was precipitated from acid solution. The results, which are given in Table III, show that the volumetric assay gives more consistent

**TABLE III**  
THE ESTIMATION OF THIOSEMICARBAZIDE

Sample	Melting point*	Assay as silver complex per cent.	Sodium nitrate assay per cent.
1	176 to 178°C.	98·86 99·82	98·88 98·83
2	182 to 183°C.	99·74 99·42	100·1 99·93
3	182·5 to 183°C.	99·97 99·86	100·0 99·93

\* M.pt. 181° to 183°C. Freund and Imgart<sup>14</sup>.

figures and is unaffected by the presence of hydrochloride in the thiosemicarbazide.

#### EXPERIMENTAL

*Silver derivative of 4-Acetamidobenzaldehyde thiosemicarbazone.* 0.2 g. of 4-acetamidobenzaldehyde thiosemicarbazone, accurately weighed, was dissolved in 60 ml. of aldehyde-free methyl alcohol, warmed on a steam bath to about 60°C. and treated with excess of a hot saturated methyl alcoholic solution of silver nitrate. The white colloidal precipitate which formed, rapidly became crystalline and separated readily. After cooling, the precipitate was collected on a No. 3 sintered glass filter, washed with methyl alcohol until free from silver and dried to constant weight at 100° to 105°C. Found: C, 23.5; H, 2.15; N, 13.6; S, 6.18; Ag, 42.1; NO<sub>3</sub>, 11.3. C<sub>10</sub>H<sub>11</sub>ON<sub>4</sub>SAg·AgNO<sub>3</sub> requires C, 23.4; H, 2.16; N, 13.65; S, 6.25; Ag, 42.1, NO<sub>3</sub>, 12.1 per cent.

*Estimation of content of 4-Acetamidobenzaldehyde thiosemicarbazone in tablets.* 10 tablets were powdered as finely as possible and 0.3 g. of the powder (equivalent to 0.1 g. active material), accurately weighed, was extracted with 50 ml. of methyl alcohol by warming on the steam bath for 2 hours. The insoluble residue was filtered off, washed 5 to 6 times with small portions of methyl alcohol to give a total volume of 70 to 80 ml. An excess of methyl alcoholic silver nitrate was added to the hot filtrate and the precipitate which formed was filtered, washed free from silver with methyl alcohol and dried to constant weight at 100° to 105°C. 1 g. of residue = 0.4606 g. of C<sub>10</sub>H<sub>12</sub>ON<sub>4</sub>S.

*Estimation of Thiosemicarbazide.* (a) *Sodium nitrite titration.* A standard solution of thiosemicarbazide was prepared by dissolving 1 g. in 100 ml. of 0.25N hydrochloric acid. 25 ml. of this solution was titrated with 0.1N sodium nitrite solution at room temperature. The nitrite was added fairly rapidly with vigorous shaking until near the end point, then drop by drop until the solution gave an immediate reaction with starch-iodide paper. 1 ml. of 0.1N sodium nitrite = 0.009106 g. of CH<sub>5</sub>N<sub>3</sub>S.

(b) *Precipitation of silver complex.* 0.3 g. of thiosemicarbazide, accurately weighed, was dissolved in 40 ml. of 0.5N nitric acid. 12.5 ml. of 5 per cent. aqueous silver nitrate solution was added slowly in the cold to give an excess of about 10 per cent. The crystalline precipitate was allowed to settle, filtered through a No. 3 sintered glass crucible and washed free from silver with 0.5N nitric acid. The precipitate was dried to constant weight at 45°C for 18 hours. 1 g. of residue = 0.3489 g. of CH<sub>5</sub>N<sub>3</sub>S.

#### SUMMARY

(1) A gravimetric method for the estimation of 4-acetamidobenzaldehyde thiosemicarbazone as its silver derivative is described.

(2) The silver complexes of the thiosemicarbazones of benzaldehyde, 4-nitrobenzaldehyde, salicylaldehyde, 4-ethylsulphonylbenzaldehyde and 4-acetamidosalicylaldehyde have been examined. Estimation of their

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silver contents showed in each case that the ratio of silver to thiosemicarbazone was greater than 1.

(3) A rapid volumetric method of estimating thiosemicarbazide is described.

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### DISCUSSION

MR. B. W. MITCHELL read an abstract of the paper.

DR. E. F. HERSANT (Dagenham) said that the authors stated that in the sodium nitrite titration they titrated until the solution gave an immediate reaction with starch-iodide paper. In most cases one then obtained a false, early end-point. The B.P., in all the other similar titrations, advised that the titration be continued until the blue colour was given after allowing the solution to stand for one minute. He thought that this was the usual practice in all sodium nitrite titrations.

MR. G. F. HALL (Nottingham) agreed that the assay of thiacetazone was very troublesome from the nitrogen aspect, and said that since receiving the paper they had done only a few determinations by the silver precipitation method, but so far it looked very promising. They had used the sulphur determination, and had generally found it preferable to the nitrogen assay.

MR. B. W. MITCHELL in reply, said that it was correct to say that the end-point with starch-iodide paper was taken after 2 or 3 minutes. Actually, it was found, and was stated in the paper, that a very sharp end-point was obtained, i.e., the first indication of a blue colour was very sudden and seemed to be repeatable after 2 or 3 minutes. He did not know whether there was any reason for that, but comparing it with the titration of sulphonamide they had found it much easier. He did one or two sulphur determinations at the beginning, but did not get very good results.