

Thioacetamide I

Substitute for Hydrogen Sulfide in Medicinal Chemicals for Identity and Purity

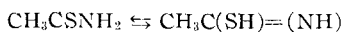
By JACK JUE and C. LEE HUYCK†

Identification tests and tests for heavy metals in inorganic chemicals using thioacetamide solution and hydrogen sulfide solution gave identical results. Difficulty was encountered only when solutions of organic drugs in volatile flammable solvents interfered with the hydrolysis of thioacetamide. In this case, the length of time for the completion of the test was increased in order to insure complete hydrolysis of the thioacetamide. In testing acetylsalicylic acid, methenamine, and salicylic acid contaminated with the minimum amount of solution of lead salt sensitive to hydrogen sulfide test solution, the color of the precipitate was brown instead of black.

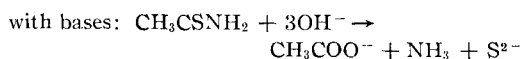
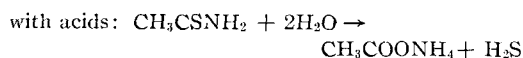
THE PURPOSE of this study is to review the previous work done on the use of thioacetamide test solution as a substitute for hydrogen sulfide test solution in identifying salts of heavy metals, to detect heavy metal impurities in chemicals, and to investigate the possibility of using thioacetamide test solution as a substitute for hydrogen sulfide test solution.

Hydrogen sulfide from generators or compressed hydrogen sulfide is troublesome compared with aqueous solutions of a solid compound such as thioacetamide as a source of the gas. An added disadvantage of hydrogen sulfide is unpleasant odor, with both systemic and local toxicity.

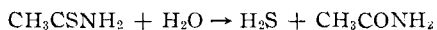
Thioacetamide (1) is available in the form of white crystals. It is soluble in water, alcohol, benzene, and sparingly soluble in ether. Its aqueous solution is neutral and stable at room temperature. A slight sediment may develop upon standing, but the liquid still has precipitating power. In solution, thioacetamide is a mixture of the amide and its enol form in equilibrium



Hydrolysis occurs in both alkaline and acid solutions. Both acids and bases catalyze the hydrolysis. The equations for the reactions are as follows



The hydrolysis reaction may be expressed as follows



Thioacetamide hydrolyzes slowly in the cold but more rapidly at elevated temperature; therefore, the rate of hydrolysis can be controlled by regulating the temperature. The thioacetamide is introduced into the solution to be analyzed and is heated to approximately 80° on a water bath to cause hydrolysis. Hydrogen sulfide is liberated, causing metal sulfides to be precipitated. The precipitates formed with thioacetamide are coarser and more easily separated by centrifuging or filtering than those formed with hydrogen sulfide. The cations are precipitated in nearly stoichiometric amounts and very little of the hydrogen sulfide ever escapes into the air. There is no need for delivery tubes, safety traps, or hoods. The thioacetamide is usually used in the form of a 10% aqueous solution, the most common being 1M or 7.5% w/v concentration.

Vavilov (2) showed that bismuth can be detected in the presence of metals of group IV with thioacetamide. When a drop of bismuth solution is treated with an alcoholic solution of thioacetamide, a yellow coloration is produced. It is probable that $(\text{BiO})_2\text{SCNHCH}_3$ is formed as a result of the reaction between the univalent BiO and the thioacetamide. Cations of group III and copper show evidence of behaving similarly.

Barber and Grzeshowiak (3) used thioacetamide in place of gaseous hydrogen sulfide for precipitation of insoluble sulfides. Thioacetamide was successfully used in place of gaseous hydrogen sulfide in the development of new methods of qualitative analysis of group II and group III. A water solution of thioacetamide was added directly to the solution of cations. No great ex-

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cess of thioacetamide was required. Nearly equivalent amounts of the organic sulfide and the cation brought about complete precipitation of the insoluble sulfide. The hydrolysis of thioacetamide gave a relatively low concentration of the sulfide ions favoring the rapid coagulation and filtration of the insoluble sulfides. The time required for complete precipitation of the insoluble sulfides by thioacetamide was less than the overall time required when gaseous hydrogen sulfide was used. The advantages of thioacetamide solution are tolerable odor, good stability, low cost, and a high degree of solubility in water.

Fales and Kenny (4) gave alternative thioacetamide precipitation procedures which have been used for the last 3 years in our classes.

Bloemendal and Veerkamp (5) found that thioacetamide can fully replace hydrogen sulfide and ammonium sulfide in the qualitative test for heavy metals. A simple analysis procedure was given. They also found that other sulfide compounds like thioformamide are less stable, but usable. Thiourea, thioglycollate, thioacetate, thiosalicylate, and thiophosphate, however, are useless as a replacement for hydrogen sulfide.

Flaschka (6) and Flaschka and Abdine (7) made a study of the use of thioacetamide. Qualitative procedures using thioacetamide were fundamentally identical with those using hydrogen sulfide and differ only in manipulative details. Some precipitation and separation procedures were outlined.

Flaschka and Abdine (7) were among the first to study thioacetamide in quantitative analysis. They found that since thioacetamide formed coarser precipitates, the precipitates are less contaminated by foreign ions, easier to filter, and therefore more suitable for gravimetric analysis than those formed by hydrogen sulfide.

Flaschka and Abdine (7, 8) studied some thioacetamide separations in the presence of ethylenediaminetetraacetic acid (EDTA). The EDTA prevented the precipitation of some metals as their sulfides through chelation. Equilibrium of the metal-EDTA-thioacetamide system resulted by the addition of the calcium ion. Metals were separated as sulfides by precipitating some with thioacetamide in EDTA solution and separating the remainder by the addition of a large amount of calcium ions.

Amin (9) used thioacetamide with EDTA titrations in complexometric analysis of different ores and alloys. Also thioacetamide was used with ion-exchange resins in the analysis of various ores.

Swift and Butler (10) do not agree that thio-

acetamide solutions can be substituted for hydrogen sulfide without modification of procedure. Rate of reaction measurements have shown that the hydrolysis of thioacetamide to hydrogen sulfide and acetamide is a first-order reaction with respect to both thioacetamide and hydrogen ion concentration, and only a small fraction of the acetamide formed is hydrolyzed to acetic acid and ammonia. In solutions having pH values of less than 3, the precipitation of lead sulfide by thioacetamide is preceded by hydrolysis and the rate of precipitation equaled the rate of hydrolysis. In solutions having pH values from 3.5 to 5.1, the precipitation is first order with respect to both thioacetamide and lead ion concentration. At pH 5, the rate of precipitation of lead sulfide with hydrogen sulfide was found to be 1000 times greater than the formation of lead sulfide by hydrolysis of thioacetamide.

Monte Bovi (11) who first suggested the use of thioacetamide in the testing of pharmaceuticals found it to be a satisfactory replacement for hydrogen sulfide in certain tests used to establish the identity of mercury, bismuth, zinc, and manganese salts, and to detect the presence of such impurities as mercury (II) in merbromin and nitromersol, mercuric iodide in yellow mercurous iodide, copper or zinc in methylene blue, iron in zinc oxide, and heavy metals in sodium chloride and potassium nitrate. Monte Bovi concluded "there is need for more fundamental studies, especially on a quantitative basis, to establish the sensitivity of thioacetamide and the mechanism of reaction, before it can be adopted as a direct replacement for hydrogen sulfide in all pharmaceutical testing. Until then, the indiscriminate use of thioacetamide as a replacement for hydrogen sulfide is not justified."

EXPERIMENTAL

Every identification test for heavy metal salts and every test for heavy metal impurities in chemicals of the U.S.P. (12) was performed with both hydrogen sulfide test solution and thioacetamide aqueous solution. The hydrogen sulfide test solution was made by passing an excess of hydrogen sulfide into cold distilled water. The solution was stored in a small, dark amber-colored bottle filled nearly to the top and kept in a cool dark place. It was not used unless it possessed a strong odor of hydrogen sulfide and unless it produced a copious precipitate with an equal volume of ferric chloride test solution.

The thioacetamide was 1 *M* or 7.5% w/v. The commercial reagent grade thioacetamide was used. The solution was stored in a dark amber-colored bottle similar to the bottle used to store the hydrogen sulfide test solution. An equal volume of this solution, previously heated on a water bath to approximately 80° for a minimum of 5 minutes (in order to hasten the hydrolysis of the thioacetamide) was

used along with the hydrogen sulfide solution in all the tests for comparative purposes.

In the tests for identification, the procedure of the U.S.P. was followed with both solutions. In testing for heavy metals, contamination was accomplished by addition of 0.001 mg. lead in the form of a water-soluble salt dissolved in 1 ml. distilled water. This amount of lead is the minimum amount that gives a color with hydrogen sulfide test solution and was the standard of comparison for the thioacetamide solution.

Where applicable, official tests for identification were conducted as directed in the U.S.P. with the two solutions, and no difference in color or appearance could be detected. See Table I.

TABLE I.—CHEMICALS WHICH YIELDED EQUALLY POSITIVE IDENTIFICATION TESTS WITH HYDROGEN SULFIDE AND THIOACETAMIDE TEST SOLUTIONS

Antimony potassium tartrate	Mercuric chloride
Antimony sodium thioglycolate	Yellow mercuric oxide
Bismuth potassium tartrate	Mersalyl
Bismuth subcarbonate	Zinc peroxide
Bismuth subsalicylate	Zinc stearate
Carbarsonc	Zinc sulfate

Tests for heavy metals impurities were conducted as directed with the two solutions, after contaminating them with lead as directed above. Differences were noted with acetylsalicylic acid, methenamine, and salicylic acid. Solutions of these chemicals required a slight amount of heat in addition to the heat supplied by the reagent. After warming slightly on a water bath, instead of a black precipitate, a brown precipitate was observed. No differences in color or appearance could be detected when the two solutions were used in testing the chemicals contaminated with heavy metals. See Table II.

TABLE II.—CHEMICALS CONTAMINATED WITH HEAVY METALS WHICH YIELDED EQUALLY POSITIVE TESTS WITH HYDROGEN SULFIDE AND THIOACETAMIDE TEST SOLUTIONS

Aluminum sulfate	Magnesium oxide, heavy
Ammonium carbonate	Magnesium sulfate
Ammonium chloride	Magnesium trisilicate
Barium sulfate	Potassium bicarbonate
Boric acid	Potassium bromide
Calcium carbonate	Potassium chloride
Calcium chloride	Potassium hydroxide
Calcium gluconate	Potassium iodide
Calcium hydroxide	Potassium nitrate
Calcium lactate	Sodium bicarbonate
Calcium mandelate	Sodium biphosphate
Dibasic calcium phosphate	Sodium bisulfate
Ferrous sulfate	Sodium borate
Diluted hydriodic acid	Sodium bromide
Hydrochloric acid	Sodium chloride
Diluted hydrochloric acid	Sodium citrate
Hydrogen peroxide solution	Sodium iodide
Magnesium carbonate	Sodium phosphate
Magnesium oxide	Sodium salicylate
	Sodium sulfate

Likewise, U.S.P. organic chemicals examined for heavy metals were contaminated with a lead solution as previously described and tested with the two solutions as directed. No difference in color or appearance could be detected. See Table III.

TABLE III.—ORGANIC CHEMICALS CONTAMINATED WITH HEAVY METALS WHICH YIELDED EQUALLY POSITIVE TESTS WITH HYDROGEN SULFIDE AND THIOACETAMIDE TEST SOLUTIONS

Amylene hydrate	<i>p</i> -Aminobenzoic acid
Barbital sodium	Pentobarbital sodium
Carbachol	Phenobarbital sodium
Citric acid	Phenolphthalein
Dextrose	Propylene glycol
Ethyl aminobenzoate	Saccharin
Gelatin	Sodium saccharin
Glucose	Sucrose
Glycerin	Tartaric acid
Iodoalphonic acid	Theobromine calcium salicylate
Lactic acid	Thiophental sodium
Lactose	Urea
Methyl salicylate	Urethane
Nicotinamide	
Nicotinic acid	

In addition, the official pharmaceuticals and their preparations were contaminated with lead solution and tested for heavy metals by the two solutions as previously described. No difference in color or appearance could be detected. Pharmaceuticals and preparations tested are given in Table IV.

TABLE IV.—PHARMACEUTICALS CONTAMINATED WITH HEAVY METALS WHICH YIELDED EQUALLY POSITIVE TESTS WITH HYDROGEN SULFIDE AND THIOACETAMIDE TEST SOLUTIONS

Aluminum acetate solution	Ammoniated mercury ointment
Aluminum hydroxide gel	Orange flower water
Aluminum phosphate gel	Orange oil
Diluted ammonia solution	Ringer's solution
Caffeine and sodium benzoate	Lactated Ringer's solution
Cinnamon oil	Stronger rose water
Clove oil	Sassafras oil
Dextrose injection	Isotonic sodium chloride solution
Milk of magnesia	Distilled water
Zinc peroxide	

DISCUSSION OF RESULTS

Identification tests for heavy metals showed that thioacetamide test solution and hydrogen sulfide test solution, with few exceptions, gave identical results. Difficulty was encountered in testing water-insoluble organic salts for heavy metals because the volatile flammable solvents used had to be heated. In this case, the length of time of standing on a hot water bath could be increased in order to insure the complete hydrolysis of thioacetamide.

Plans are made to compare thioacetamide with hydrogen sulfide in the identification and detection of heavy metals in chemicals and pharmaceuticals

of the National Formulary. Comparative quantitative analyses with thioacetamide and hydrogen sulfide are also planned for chemicals of both the United States Pharmacopeia and the National Formulary where hydrogen sulfide is directed as the quantitative precipitating reagent.

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Kinetics of Reaction between Molecular Oxygen and 2,3-Dimercapto-1-propanol in Aqueous Solution

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Autoxidation of BAL is shown to take place in the absence of metallic catalysts. Although the iodometric sulphydryl concentration in this system was found to decrease linearly with time, the reaction was found to approach true zero order only at higher initial BAL concentration. The observed rate of oxidation was found to decrease with increased hydrogen ion concentration and to be of a mixed order with respect to oxygen tension.

BARRON, *et al.* (1), have suggested that the observed reaction in aqueous solution between 2,3-dimercapto-1-propanol, BAL, and molecular oxygen in absence of added catalyst could be entirely attributed to trace quantities of catalytic copper. In this communication, experimental evidence which supports the existence of the uncatalyzed reaction and kinetic dependencies of this oxidative reaction on pH, oxygen tension, and initial substrate concentration are presented.

EXPERIMENTAL

Reagents and apparatus employed in the work were the same as those reported in previous work on the copper catalyzed oxidation of BAL (2). The procedure used was also essentially the same except that potassium cyanide was added to all reaction solutions at a concentration of $1 \times 10^{-3}M$. While catalase is known to be inhibited by cyanide (3), sufficient catalase was found to remain to destroy satisfactorily any peroxide which may have been formed. The reacting systems were sufficiently stirred (2) so that the solutions were always kept in equilibrium with the gas phase.

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RESULTS AND DISCUSSION

Evidence for Uncatalyzed Reaction.—There appears to be ample evidence which points to reaction in the absence of copper between molecular oxygen and BAL in aqueous solution. As has already been shown (2), significant copper concentration, *ca.* $2 \times 10^{-6}M$, is apparently necessary to account for the observed rate. If such high concentrations of the metal were introduced by chance contamination, it would be expected that variation in sources of buffer salts, water, and substrate would produce significant changes in the observed rates of oxidation. This was not the case. Quite reproducible rates were observed for different lots of buffer salts, BAL, and water.

Further evidence supporting the existence of uncatalyzed reaction is presented in Fig. 1. These plots show the residual sulphydryl concentration of aqueous solutions of BAL as a function of time when exposed to oxygen at 1 atm. The uppermost curve shows the kinetic dependency for a system free of copper and cyanide as far as was possible experimentally. The second line shows essentially the same system but with $1 \times 10^{-3}M$ potassium cyanide added. It is evident that these two functions are quite similar, both qualitatively and quantitatively. The third and fourth curves show the effects of addition of copper, 2×10^{-6} and $5 \times 10^{-6}M$, respectively, on the initial system. The kinetic behavior of these last two runs is obviously quite different from those of the preceding two. Although the data are not shown, addition of $1 \times 10^{-3}M$ cyanide to the latter two systems yielded rate curves identical with the first two.