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Titration of Small Amounts of Mustard and Other Gases with Bromine and Methyl Red.*

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The quantitative determination of mustard gas and other war gases is difficult since very minute quantities must be analyzed. The method used should also be simple and rapid since it is often necessary to analyze a large number of samples. Mustard gas and the arsenic gases are known to react rapidly with bromine, a reagent which allows extremely accurate titration in very dilute solutions. The usual starch-iodide indicator is not sufficiently accurate for titration with 10^{-4} molar bromine and no satisfactory indicator has been reported.

A number of different oxidizing agents and different indicators have been tried but bromine and methyl red are the most satisfactory in 10^{-4} M solution. Hypochlorite solution may be used in place of bromine. It is much more stable, but reacts with many compounds with which bromine does not.

The method finally developed determines mustard gas, Lewisite, and ethyl or phenyl dichlorarsine within an error of $\pm$ 0.02 ml 10^{-4} molar bromine per ml final volume of the titrated solution. It should be useful for the titration of any compounds which react rapidly with bromine in acid solution.

1. *Description of Samples of Gases Tested.*

Mustard (I)—1 technical, prepared by Levinstein method (Sartori, M., The war gases, New York, D. Van Nostrand Co., Inc., 1940, 223) and redistilled; one C.P. No difference noted.

Ethyldichlorarsine (II)—one C.P. and one technical. Technical titrates slightly lower.

Lewisite (III)—2 technical samples.

Phenyldichlorarsine (IV) — one technical sample.

2. *Preparation of Solutions.* Weighed or measured amounts of I, III, and IV were dissolved in alcohol to give a 10^{-2} M solution. From these, 10^{-4} M solutions were prepared by dilution with water. Solutions of III and I were also prepared in water alone in the same way as those prepared from 10^{-2} M in alcohol.

A 10^{-4} M solution of II was prepared by addition of the gas to water, since II is partially oxidized in alcohol.

These aqueous solutions are referred to as "solutions of the gases," although actually they are solutions of the hydrolysis products of the gases.

3. *Bromine Solution.* Saturated stock solution: 50 ml one M hydrochloric acid is shaken with 10 ml liquid bromine and the solution is kept in a dark glass bottle in the presence of excess bromine. This solution is 0.5 M. The concentration remains constant.

Standardization of bromine solution for titration: When the saturated solution is diluted to 10^{-4} M there is usually a loss of 10-30% in strength due, presumably, to traces of reducing substances in the acid or water used. It is necessary, therefore, to standardize the dilute bromine. This may be done by titration against a solution of 10^{-4} M mustard gas or thiodiglycol in water, or with potassium iodide and 10^{-3} M thiosulfate in the usual way. Ten ml dilute bromine in one N sulfuric acid is added to one ml 10^{-2} M potassium iodide and the liberated iodine titrated with 10^{-3} M thiosulfate, with starch as indicator. One ml 10^{-3} M thiosulfate is equivalent to 0.5 ml 10^{-3} M bromine.

An approximately 10^{-4} M bromine solution was obtained by diluting the saturated bromine solution 1/4,000 with the 0.5 M sulfuric acid used in this work. This dilute bromine solution remains constant within 10% for 24

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TABLE I.
Titration of the Various Gases in 0.1 M Sulfuric Acid with Methyl Red and 10^{-4} M Bromine. Initial volume 1 ml.

Gas	Quantity, ml 10^{-4} M	Ml 10^{-4} M bromine Corrected for blank	
—	0	0.05 (blank)	
I	0.1	0.11	
	0.3	0.30	
	1.0	0.95	
	3.0	3.1	
III	0.1	0.11	
	0.3	0.31	
	1.0	1.00	
	3.0	2.95	
		Aqueous sol.—II	10^{-4} M aqueous sol.prepared from 10^{-2} M ETOH sol.
II	0.1	0.09	0.035
	0.3	0.33	0.10
	1.0	0.96	0.40
	3.0	3.10	1.10
IV	0.1	0.09	
	0.3	0.31	
	1.0	1.05	
	3.0	2.95	

hours if kept in dark glass-stoppered bottles. It loses strength rapidly in strong light or if left open. For this reason the burette should be refilled shortly before titrating.

4. *Methyl Red Solution.* Five mg per liter in water.

5. *Concentration Range and Accuracy.* All of the determinations have been carried out with samples of 5 ml or less of a 10^{-4} M solution of the gas titrated with 10^{-4} M bromine, delivered from a 5 ml burette graduated to 0.02 ml. Under these conditions the titrations are accurate to about $\pm$ 0.02 ml of 10^{-4} M bromine per ml final volume of solution, i.e., if the volume at the end of titration is 2 ml the end point is sharp to $\pm$ 0.04 ml. If the final volume is 10 ml the end point is sharp to $\pm$ 0.20 ml. If larger quantities of gas are to be titrated stronger bromine solutions should be used.

Technique of the Determination. A sample of one to 5 ml of the solution is put into a 1.5 x 15 cm test tube and adjusted to approximately 0.1 M sulfuric acid by addition of a few drops of concentrated sulfuric acid. One drop of methyl red is added for each ml of solution. Dilute (10^{-4} M) bromine solution is run in from the burette until the solution becomes nearly colorless. Another drop of methyl red is introduced and bromine added again until the solution is colorless. The end point can be judged best by holding a tube of water alongside the tube of solution which is being titrated. Both tubes should be allowed to rest vertically on white paper and observed from an angle of about 45° to the vertical. The end point is not reversible so care must be taken not to overstep. It is preferable to add the methyl red in two steps owing to partial irreversible oxidation of the indicator by local excess bromine during the titration. Determination of the end point is greatly facilitated by the use of a Fisher fluorescent titration lamp (Fisher Scientific Co., Pittsburgh, Pa.).

The concentration of sulfuric acid or methyl red may vary $\pm$ 50% without affecting the results.

Results. Examples of titrations carried out as described are shown in Table I.

Sources of Error. Any substance which reacts with bromine will evidently interfere with the titration. Hydrogen sulfide and sulfides in general are the most common such substances.

Since rubber tubing and stoppers contain large amounts of sulfides the solutions must not come in contact with them.

Hydrogen Sulfide in Air. Hydrogen sulfide is not absorbed in one M sulfuric acid so that in analyzing samples of air, hydrogen sulfide is not titrated. It may be removed from acid solution by boiling for several minutes. Old sulfide solutions, however, cannot be freed of reducing substances in this way.

Summary. Mustard (I), ethyldichlorarsine (II), Lewisite (III), and phenyldichlorarsine (IV) may be titrated with 10^{-4} M bromine using methyl red as indicator. The method is accurate to about $\pm$ 0.02 ml 10^{-4} M bromine per ml of solution titrated. The reaction of these compounds is that of one mole of bromine per mole of gas.

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Carbonic Anhydrase in the Pallium of Primates Compared with that of Lower Mammals.*†

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Carbonic anhydrase reversibly catalyzes the change of carbon dioxide and water to carbonic acid. It occurs in the central nervous system in amounts ranging from a doubtful positive in the cord of the hog[1] to a content in either the cerebellum or cerebrum approximately equal to one-tenth of that found in the blood.[2] None has been found in peripheral nerve of man and dog (unpublished). The content in the spinal cord of mature animals in which it occurs in any considerable amount is well below that of the medulla, pons and higher centers.[3]

Previously reported data indicated a well defined difference in the pattern of the quantitative distribution of carbonic anhydrase in the pallium of the lower animals studied, the dog, the hog and the cat, from that found in man. In the lower animals the cortex held the greater content. In man the pattern was reversed; the white matter immediately below the cortex had a markedly greater enzyme content than the cortex. Exception was found in the motor cortex where more definite approximation to the animal pattern appeared in the leg area.[4]

The present study is an extension of work previously reported. The brains of 4 Rhesus monkeys were examined to see whether the pattern of man is found also in these primates; and additional data on other of the lower mammals, the horse, the steer, the sheep and the rabbit are given. Data on the guinea pig are not included as the subcortical white matter of the brain was too small in amount to be separated successfully from the cortex.

Technique. The technique employed for the determination of carbonic anhydrase was essentially that of Philpot and Philpot[5] as modified by Keilen and Mann.[6] Specific procedures employed[4] and the method used to determine the enzyme activity attributable to the blood in the tissue[7] are given elsewhere.

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