

AD No. 23242

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A METHOD FOR THE DETERMINATION OF SMALL QUANTITIES
OF CARBON MONOXIDE IN AIR*

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*These studies were aided by a contract between the Office of
Naval Research, Department of the Navy, and the Trudeau-Saranac
Institute, NR 112-214.

There is a need for a precise method for determining low concentrations of CO in small gas samples¹. Methods²⁻⁶ which are accurate to within about 5% of the amount of CO present are either time-consuming or require that the gas sample volume be large (100-5000 ml.). These disadvantages were overcome to some extent by Polis et al⁷ using the CO-palladium-molybdate reaction in an acetone solution, but the accuracy of this method in our hands was less than that required for our physiologic studies. Starting with the procedure of Polis, we have developed a method for the determination of 0.002 to 0.01 ml. of CO in samples less than 50 ml. in volume with a mean error of 2%. The use of the dilution technique to be described will permit the determination of CO in the concentration range of 0.004% to 0.25%.

PRINCIPLE OF THE METHOD

A measured volume of the gas sample is introduced into a partially evacuated reaction tube containing a measured volume of palladium chloride-phosphomolybdic acid--methyl-ethyl ketone reagent. Molybdenum blue is formed. The optical density of the reacted liquid is proportional to the number of moles of CO in the tube and is determined with a photoelectric colorimeter.

REAGENTS AND EQUIPMENT:

Palladium chloride solution: One gm. of $PdCl_2 \cdot 2H_2O$ (Fisher Scientific Company) is dissolved in water containing 7 ml. of concentrated HCl and diluted to 500 ml.

Phosphomolybdic Acid solution: Twenty-five gm. of $20\text{MoO}_3 \cdot 2\text{H}_3\text{PO}_4 \cdot 48\text{H}_2\text{O}$ are dissolved and diluted with water to 500 ml. Phosphomolybdic acid as currently supplied by the Fisher Scientific Company, New York is satisfactory.

Five normal H_2SO_4 .

Solution A: Equal volumes of the above three reagents are mixed and stored in a stock bottle. The reagent is similar to that of Polis et al⁷ except for its higher concentration of H_2SO_4 .

Solution B: Three volumes of methyl-ethyl ketone (Eastman Kodak) are added to one volume of 95% ethyl alcohol.

CO reagent: One liter of Solution B is added to 250 ml. of Solution A and stored in a glass-stoppered (clear glass) bottle at room temperature in daylight for at least two weeks before use. During this period, the color of the mixture changes from yellow to dark blue-green and finally to greenish yellow, at which time its color usually becomes stable. When this point is reached, the properties of the reagent remain unchanged for several months. If the mixture becomes dark, direct sunlight or ultraviolet irradiation will lighten it without interfering with its ability to react with CO.

Reaction tubes: These have a capacity of approximately 100 ml. and are 25 mm. in diameter. At one or both ends, carefully ground stopcocks of 1 mm. bore are fused on so that the narrowed portions

of the tubes are short, wide and tapered to avoid the trapping of liquids near the cocks. At least one cock should be three-way. (See C in Fig. 1.) Rubber-free paraffin or silicone stopcock greases have been found satisfactory.

Gasometer: Two mercury-containing 50 ml. burettes, one with appropriate connections for delivering measured gas volumes into the reaction tubes. (Fig. 1.)

Water bath and revolving tube holder: The temperature of the bath is set at $37.2 \pm 0.2^\circ\text{C}$. The reaction tubes are clipped onto a revolving tube holder which rotates them about their long axis at 30 r.p.m.

Optical equipment: Matched unstoppered glass absorption cells with a 20 mm. light path have been found satisfactory. A photoelectric colorimeter (Lumetron, Model 402E) is used with a glass filter having a transmission band of 640 to 680 millimicrons.

PROCEDURE

Preparation of reaction tubes:

Five ml. of the CO reagent are delivered into each of a series of reaction tubes from a syringe fitted with polyethylene capillary tubing which is threaded through the bore of the reaction tube stopcock. The tubes are then evacuated to a pressure of 220-230 mm. Hg in order to allow the introduction of up to 50 ml. of gas sample and the subsequent heating to 37.2°C , without the development of a pressure greater than atmospheric within the tubes.

Introduction of standard and unknown gas samples:

Into two reaction tubes accurately measured volumes of between 4-50 ml. of a known CO (0.02-0.1%) in air mixture* (saturated with water vapor) are introduced via the gasometer. (See Fig. 1.) A measured volume of each unknown (saturated with water vapor) is similarly transferred to each of the remaining reaction tubes. The following procedure is used: connections C, D, and E are filled with mercury from chamber B and from burette K, and part of the sample in A is transferred to K. Connection L is rinsed with 3-4 ml. of the sample via cocks H and M and the sidearm N. A reading is made of the mercury level in K with the sample at atmospheric pressure and cock M closed. Any desired volume up to 50 ml. is then introduced into the previously evacuated tube O. The mercury levels are in the position indicated in Fig. 1 during admission of the sample. With cock M closed, a second reading of the mercury level in K is made with the remaining sample in K under atmospheric pressure. The barometric pressure and room temperature are recorded and all gas volumes are reduced to volumes at 0°C. and 760 mm. Hg.

Equilibration:

The tubes containing the samples and reagent are clipped onto a tube holder in a water bath at 37.2°C. and rotated at 30 r.p.m. for exactly 30 minutes. After removal from the bath,

*Obtained from the Mine Safety Appliances Company, Pittsburgh, Pa.

the tubes are allowed to cool with the cocks still closed and the external arms are wiped free of any water.

Optical measurements:

The colored solutions from the tubes are transferred to matched cuvettes and the optical density of each solution is determined against water as a blank. The densities of the two solutions from the tubes containing known amounts of CO are plotted against the number of ml. of CO introduced and a straight line is drawn connecting these two points. From this line, the number of ml. of CO in the unknown tubes is determined graphically. The percent of CO in the sample can then be calculated.

The absorption spectrum of the reagent after one-half hour of rotation with CO at 37.2°C. was determined with a Beckman spectrophotometer. (Fig. 2.) The reagent has light transmission characteristics similar to that prepared by Polis et al⁷.

RESULTS

For a given lot of reagent, the optical density of the reacted solution was found to be proportional to the number of ml. of CO in gas samples when from 0.002 to 0.01 ml. of CO were introduced into the reaction tubes.

The results of 39 consecutive analyses of 4 to 25 ml. samples of a known CO-in-air mixture are shown in Table I. The mean error was 2% of the amount present with a standard deviation of 1.5%. The maximum error was 6%.

DISCUSSION

The nature of the physical and chemical processes by which molybdenum blue is formed by CO in the presence of palladium is not definitely understood^{8, 9}. It is thought that Pd⁺⁺ is first reduced by CO to form Pd⁰. Additional CO is then adsorbed by the metallic palladium, thereby becoming activated. Phosphomolybdic acid is then reduced by the adsorbed CO to produce molybdenum blue, rapidly at first, and then at a reduced rate for a period of several hours thereafter⁷. Ketones accelerate this process in an unknown fashion.

In the method of Polis et al⁷, an acetone reagent was equilibrated with CO in glass-stoppered tubes at 60°C. for one hour. Using this method, we were unable to avoid leaks of both gas and reagent from the tubes, since neither glass stoppers nor stopcocks were found to be competent to contain gas at the high pressure (300-400 mm. Hg above atmospheric) developed when the acetone reagent was brought to 60°C. in a closed vessel. In investigating several ketones with lower vapor pressures than acetone, we have found that methyl-ethyl ketone* accelerates the CO-palladium-molybdate reaction and allows it to proceed at temperatures less than 60°C. and hence at considerably lower reacting tube pressures. Advantage has been taken of these characteristics in the present method.

*Methyl-n-propyl and n-butyl-ethyl ketone reduce phosphomolybdic acid at room temperature in the absence of CO.

The procedure used by Polis et al⁷ for introducing measured volumes of the gas sample was unreliable in our hands and did not permit the sample volume to be conveniently varied. We therefore used a vacuum technique by which the sample volume could be varied from 5 to 50 ml. and measured with an error of less than one percent. This technique also makes it possible to have a sub-atmospheric pressure in the ketone-containing reaction tubes at the beginning of and during the reaction period so that conventional stopcocks can be used with a greatly lessened chance for leaks.

When methyl-ethyl ketone is added to the aqueous palladium phosphomolybdate mixture, a two-phase system results, and the color formed in the presence of CO is concentrated in the ketone phase. Despite the advantage of increased sensitivity in the color reaction under these circumstances, we have found that the two phases are difficult to separate for colorimetric determination, and have therefore added ethyl alcohol to the reagent to form a single phase.

The CO reagent is sensitive to contact with reducing agents and also tends to turn blue on standing in the dark. Either event renders it too dark for use. Ultraviolet light reverses the alteration that occurs when the reagent stands in the dark without destroying its capacity to react with CO in the range desired. Each lot of mixed reagent can be made suitable for use by exposure to ultraviolet light or sunlight until the blue color has disappeared after which the reagent undergoes no further changes for several months.

The slope and position of the calibration line varies slightly from day to day and from one lot of CO reagent to another. Satisfactory results can be obtained by running two standards with each series of unknowns. It is best to have the standards fall closely on either side of the unknown. Hence, the standard volumes are chosen with respect to the anticipated concentrations of the unknown. This procedure was found to be preferable to using a blank since less than 0.002 ml. of CO produced color out of proportion to the CO introduced and since the blank tube tended to be relatively high, having density readings of .15 to .25 when determined against water.

The present method has proved sensitive and reliable for frequent determinations of CO in small gas samples, but it has the following disadvantages. 1) A "curing" period of two weeks is required in preparing the CO reagent. 2) The CO reagent does not give reproducible results if used more than 2-3 months after it is prepared. 3) The requirement that two standard gas samples be used for calibration with each series of unknowns is time-consuming. 4) As in other analytical methods involving molybdenum blue, even minor variations in the conditions under which reduction occurs may alter the results⁹. 5) Reagents made with different lots of phosphomolybdic acid vary considerably in their reaction with CO. The following lots of phosphomolybdic acid have been found satisfactory: Lot #473976 (Fisher Scientific Company), #52282 (Merck and Company) and #ZJZ-1 (Mallinckrodt Chemical Works). The CO reagent made from these materials gives

reproducible color reactions with CO when H_2SO_4 is present but no color is formed when HCl is substituted in equal concentration. On the other hand, the reagent made from Lot #463985 (Fisher Scientific Company) is easily reduced by CO in the presence of HCl while the use of H_2SO_4 results in a high blank and instability of color. 6) The method is not specific for CO since other gases, such as H_2S and acetylene, form molybdenum blue on contact with the reagent. The expired gas of human beings, whether smokers or non-smokers, either contains no such gases or they are present in such small amounts that they are not detectable by the present method. Use may therefore be made of the method for studies of CO uptake in men inspiring the gas in low concentrations.

SUMMARY

A colorimetric technique for the determination of low concentrations of CO is described. Measured volumes of CO containing standard and unknown gas samples are reacted with a palladium chloride phosphomolybdic acid methyl-ethyl ketone reagent and the optical densities of the reaction products are determined. With this procedure, 0.002 to 0.01 ml. of CO can be determined with a mean error of 2% of the amount present in gas samples of 4 to 50 ml. in volume.

The authors are grateful for the advice and suggestions given by Dr. Friedrich Klemperer and Dr. Lester Scheel.

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TABLE 1

Analyses of 4 to 25 ml. Samples of a Known CO-in-Air Mixture

ml. CO Present	ml. CO Found	ml. CO Present	ml. CO Found
0.0019	0.0018	0.0063	0.0062
.0020	.0020	.0063	.0064
.0027	.0028	.0065	.0064
.0030	.0030	.0065	.0066
.0032	.0032	.0066	.0068
.0035	.0035	.0066	.0064
.0035	.0033	.0069	.0068
.0037	.0036	.0076	.0076
.0039	.0038	.0079	.0079
.0039	.0037	.0083	.0082
.0039	.0039	.0084	.0084
.0043	.0044	.0088	.0088
.0046	.0046	.0091	.0090
.0047	.0047	.0093	.0093
.0051	.0053	.0093	.0094
.0054	.0055	.0101	.0100
.0054	.0053	.0101	.0100
.0055	.0056		
.0055	.0054	Mean Percent Error	2%
.0056	.0055	S. D. of Mean Error	±1.5%
.0060	.0060	Maximum Error	6%
.0060	.0060		

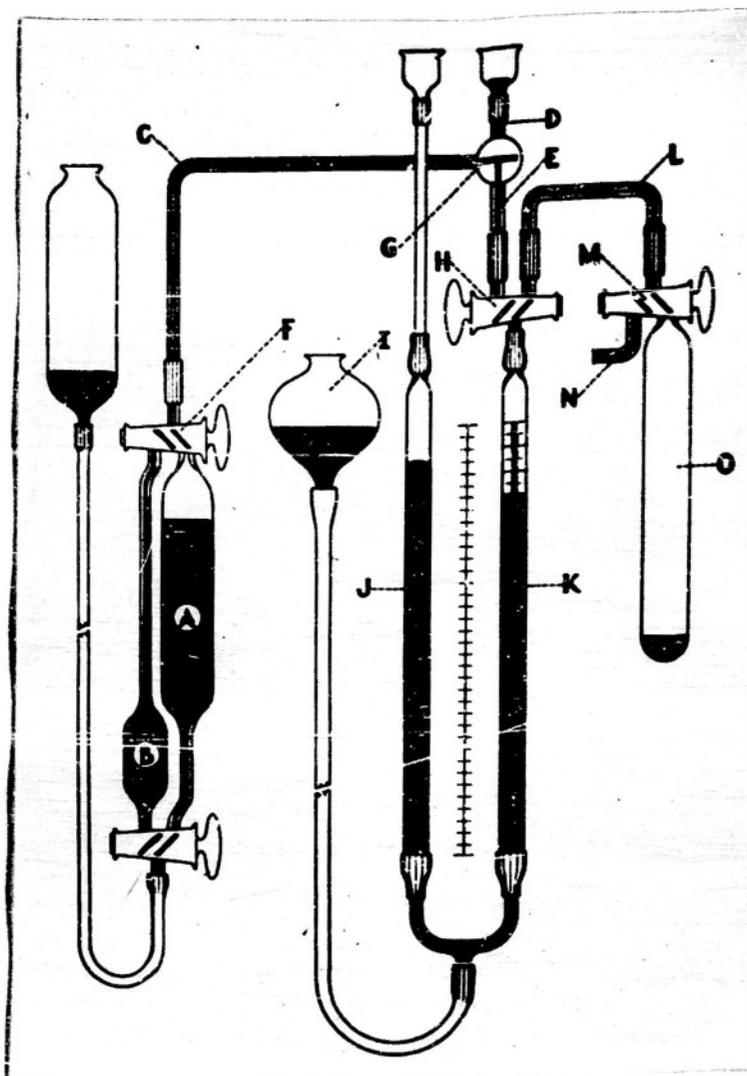


Fig. 1. Gasometer for the quantitative transfer of gas from sample tube A to the partially evacuated reaction tube O.

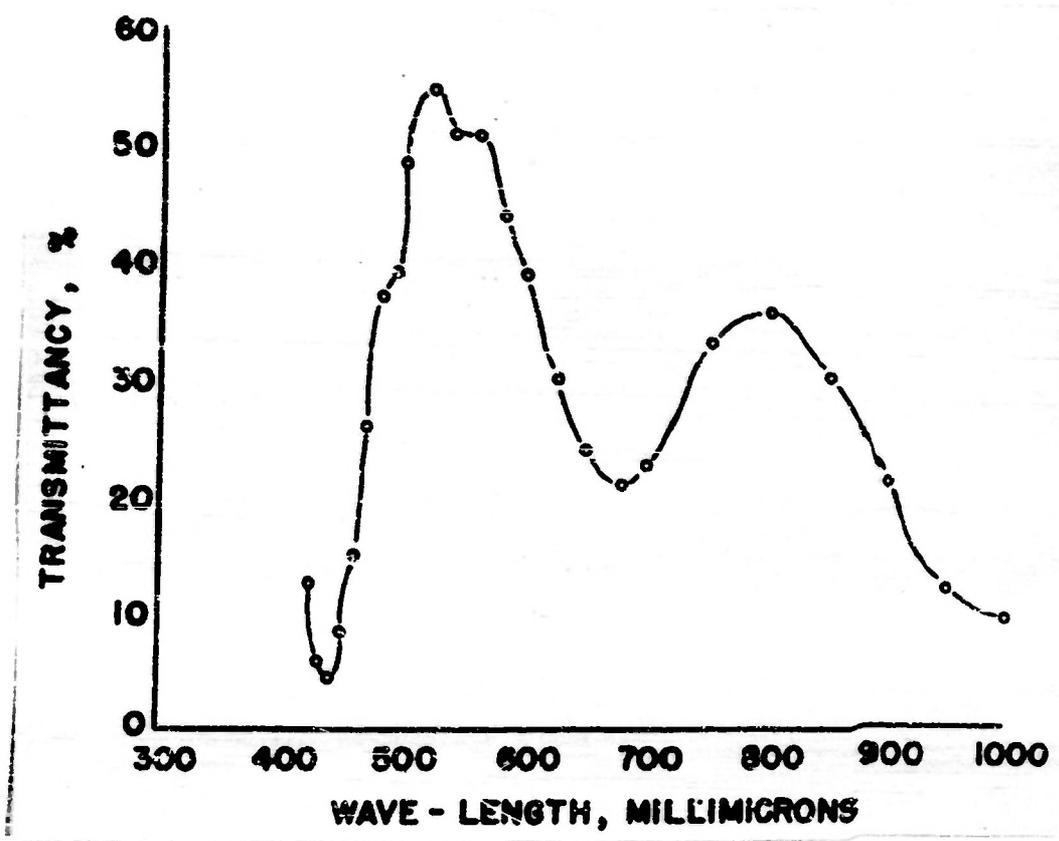


Fig. 2. Transmittancy curve of mixed reagent after reaction with CO. (Read against reagent blank)