Table I.—Comparative Results of Thyroid Assays

Material	Per Cent Iodine			
	U. S. P. XI Method		Micro-Method	
Thyroid Substance	0.2037		0.2090	
		0.2040 Av.		0.2024 Av.
	0.2043		0.1993	
C. T. Thyroid 1/2 gr. plain	0.0367		0.0366	
		0.0365 Av.		0.0365 Av.
	0.0362		0.0364	
C. T. Thyroid 1 gr. S. C. Brown	0.0782		0.0785	
		0.0786 Av.		0.0786 Av.
	0.0789		0.0786	
C. T. Thyroid 1 gr. Granulation	0.0738		0.0739	
		0.0738 Av.		0.0752 Av.
	0.0738		0.0766	
C. T. Thyroid 2 grs. Granulation	0.0831		0.0836	
		0.0834 Av.		0.0837 Av.
	0.0836		0.0838	

Since this method has proved itself to be comparatively simple and to take but a fraction of the time spent on other assays, its accuracy remains as the only point to be confirmed. The Research Surgery Department of Ohio State University found the alkaline ash method to yield consistently high results. Tippett's (2) finding that this was due to a personal equation and could be eliminated by equalizing the titration time of the blank with that of the assay proper made nearly perfect iodine recoveries possible. The authors, to prevent any personal favoring of results, enlisted the aid of their laboratory co-worker, Mr. Roger F. Maize, in checking their work. The results of three operators shown in the following table are in remarkably close accord. Although usually a matter of personal preference, the authors suggest that one-hundredth-normal sodium thiosulfate solution be prepared by careful dilution of a tenth-normal solution that has been accurately standardized against pure copper (3).

The single disadvantage of the method lies in the fact that it requires an all-Pyrex glass apparatus of the type shown. This should be easily made by any experienced glass worker or can be ordered from the Leonard Glass Works, 1432 Minnesota Avenue, Columbus, Ohio.

CONCLUSIONS

1. In skilled hands, the U. S. P. XI assay method for Thyroid is accurate, but has the disadvantage of being time-consuming because of the time required to titrate the blank.

2. The method described is fully as accurate and in addition is rapid and easily run.

3. The authors recommend that a study of the method be made with the view of its adoption as a standard Pharmacopœial procedure.

Credit is due Mr. H. W. Jones, Scientific Director of The Columbus Pharmacal Company, for his invaluable assistance in many phases of this investigation.

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(2)   Ind. Eng. Chem., Anal. Ed., 10 (1938), 612.

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An Interferometer Method for the Assay of Nitrous Oxide

By Frederick K. Bell and John C. Krantz, Jr.*

For a number of years, the gas interferometer has been used successfully for the analysis of carbon dioxide in air with an accuracy of 0.01 per cent. The fundamental factor which determines the feasibility of the method is the ratio of the indices of refraction of the gases involved and in this case the refractivity[1] of air is 2917×10^{-7} and that of carbon dioxide is 4498×10^{-7}. The value of this ratio also determines the degree of sensitivity that can be expected.

In a survey of the field of possible methods for the assay of nitrous oxide it occurred to the authors that the interferometer method should offer considerable promise. The

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[1] It will be recalled that the refractivity, R, is equal to the index of refraction, n, minus unity, therefore, $R = n - 1$.

purity rubrics of the United States Pharma-copœia for this gas eliminate appreciable amounts of all foreign substances with the exception of the gases of the atmosphere and of these only oxygen and especially nitrogen are of importance.

The refractivity of nitrous oxide is 5160×10^{-7}, that of air is 2917×10^{-7} and that of nitrogen is 2972×10^{-7}. It is thus seen that the conditions for the interferometric analysis of nitrous oxide are even more favorable than for the air-carbon dioxide system. Furthermore, whether the impurity is air or nitrogen or a mixture of these gases, there will be no significant effect, for pharma-copœial purposes, on the accuracy of the analysis.

These predictions have been verified by actual analyses which involved the examina-tion of a large number of analyzed nitrous oxide samples containing from 0.0 to 10 per cent nitrogen and also a number of com-mercial products. All of these samples have been previously analyzed by one or more of the following methods: (1) water solubility, (2) explosion and (3) condensation. Our results indicate that the interferometric method is accurate to within 0.2 per cent and therefore compares favorably with other methods of assay that have been sug-gested.

It is the purpose of this communication to describe a simple method for the calibra-tion of the interferometer and to detail a procedure for the assay.

EXPERIMENTAL

The interferometer used was a standard portable Zeiss instrument. The optical system of this com-pact instrument is so arranged that the optical path traverses the gas chamber twice so that the effective length of the gas cell is twice the actual length. Our first experiments were made using a 50-cm. cell (effective length 100 cm.) which represents the opti-mum sensitivity for the instrument. It was soon found that in order to take full advantage of this opti-mum sensitivity considerable refinement and careful regulation of the procedure were required. However, we were able to determine the minimum cell length which could be expected to give the desired sensitiv-ity and a 10-cm. cell (effective length 20 cm.) was selected. The instrument for this size of cell is con-siderably more compact and less expensive and re-presents a definite advantage in the ease and ra-pidity of the procedure.

For the calibration of the interferometer, we have adopted the method of Edwards.[2] A con-venient and simple arrangement is shown schemati-cally in Fig. 1. One arm of the glass manometer, M, having a length of not less than 50 cm. is open to the atmosphere. The other arm is connected through a glass T-tube to the inlet of cell F of the gas chamber of the interferometer. The other arm of the T-tube is connected to another T-tube one arm of which leads to the inlet of cell E of the gas cham-ber. U-tubes containing calcium chloride and soda lime, respectively, are connected to this T-tube as shown. The outlet tubes of both cells of the gas chamber are joined by a Y-tube which leads to a calcium chloride tube. A glass stop-cock is placed at A and B, C and D are screw clamps.
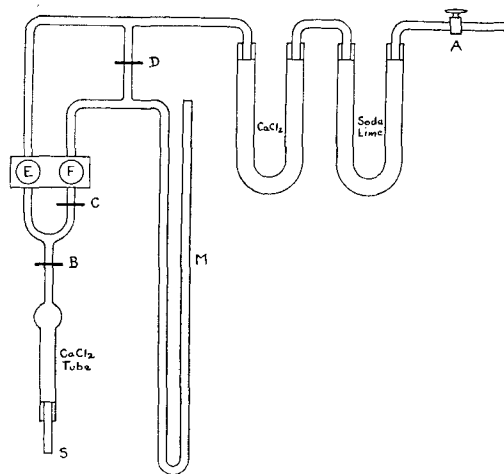

Fig. 1—Apparatus for calibration of interferom-eter.

The manometer M is equipped with a millimeter scale and is filled with any convenient liquid having a low vapor pressure and a known density of ap-proximately unity.

With stop-cock A and screw clamps B, C and D open, suction is applied at S. A moderate flow of dry, CO_2-free air is drawn through the system for thirty minutes. Clamp B is then closed and stop-cock A is opened.

At this point the zero reading of the interfer-ometer is determined. Several separate readings should be made and these should check within one division of the drum. The zero reading will be found to lie within a few divisions (plus or minus) of the actual zero of the drum. All interferometer readings are made to the nearest drum division.

To proceed with the calibration, stop-cock A is closed and clamp B is opened and gentle suction is carefully applied at S until the manometer registers approximately 50 cm. pressure difference. Clamps B, C and D are closed and stop-cock A is opened.

[2] Edwards, J. D., J. A. C. S., 39, 2382 (1917) and Bureau of Standards, Technologic Paper No. 131 (1919).

The manometer pressure is read to the nearest millimeter and the interferometer reading is made immediately thereafter.   By carefully regulating clamp *D* a new pressure is established in cell *F*.   This new pressure is read on the manometer and the interferometer reading is taken at once.   Proceeding in this manner at pressure intervals of from 35 to 40 mm. a series of drum readings of the interferometer and corresponding pressure readings is obtained.   The drum readings are corrected for the zero readings and the pressure readings are converted into millimeters of mercury.   The room temperature is recorded.

The above data are applied to the equation:

$$\Delta R = \frac{273 \times 0.0002917}{760 \times T} (P_1 - P_2) \qquad (1)$$

where *T* = the room temperature (absolute); 0.0002917 is the refractivity of dry, CO_2-free air; $P_1 - P_2$ represents the difference in pressure between the two cells of the interferometer and ΔR is the difference in refractivity between the gases in the two cells of the interferometer.

For the term $P_1 - P_2$ we may substitute the manometer pressure *Pm* (in millimeters of mercury) and then, for a definite temperature, we may introduce a constant *C* and the equation becomes

$$\Delta R = C\, Pm \qquad (2)$$

Using this relation, a value of ΔR is calculated for each manometer pressure of the calibration series to which there is also a corresponding drum reading. The values of ΔR are then plotted in rectangular coördinates against the corresponding drum read-
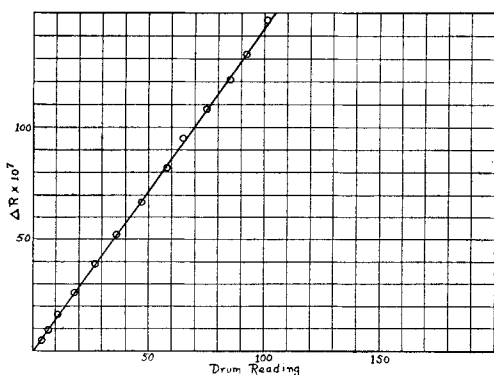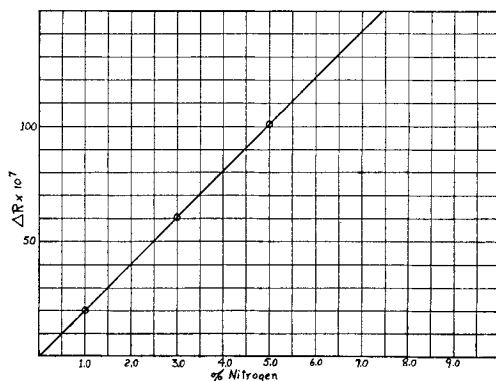

Curve I—Drum readings and refractivity.

ings corrected for the zero reading (Curve I). The points thus obtained should be very nearly on a straight line which passes through the origin and the most probable straight line is drawn through them and the origin.

A second curve is plotted from values obtained from the following equation:

$$\Delta R = \frac{273}{T} \cdot \frac{P}{760} \cdot \frac{a}{100} (R_1 - R_2) \qquad (3)$$

where *T*   =   temperature (absolute)
   P   =   barometric pressure
   R_1 =   refractivity of nitrous oxide
   R_2 =   refractivity of nitrogen
   a   =   per cent of nitrogen
   ΔR =   difference in refractivity between nitrous oxide and nitrous oxide containing a per cent of nitrogen.

The value of $R_1 = 0.0005160$ and $R_2 = 0.0002972$. Since this equation is also that of a straight line through the origin, a few points will definitely locate its position.   Therefore the value of ΔR is calculated for three values of *a*, *e. g.*, 1, 3 and 5, and these values of ΔR are plotted on rectangular coördinates against the corresponding values of *a* and the straight line is drawn through the points (Curves II and III).

Curve II—Per cent nitrogen and refractivity.

Curve I and Curve II are now combined so as to yield a third curve in which the drum reading of the interferometer is plotted against the percentage of nitrogen (Curve III) and it is this curve which is to be used in this assay.

The assay is carried out in the following manner. A commercial cylinder of the gas which passes the Tests of Purity of the Pharmacopœia is inverted and, with the valve open wide, liquid nitrous oxide is collected in a suitable container.   After approximately one-half of the liquid nitrous oxide has boiled away, the container *C* is connected to the interferometer system as is shown in Fig. 2.   A vigorous flow of nitrous oxide is allowed to pass through the interferometer for several minutes and then an insulating jacket is brought up around the container. Under these conditions there should be a small but steady flow of nitrous oxide through cell *E* of the interferometer.   The exit tube, consisting of 5-mm. glass tubing, proceeds vertically from the container *C* for approximately 60 cm. and thence horizontally for 20 cm. and finally downward for approximately 90 cm. ending in a mercury seal, *S*, having a mercury head of 2–3 mm.   Approximately 20 cm. above this point a side arm leads to a calcium chloride drying tube and thence to cell *E* of the interferometer. The exit tube of this cell is connected directly to an anti-diffusion and overflow device as shown.   A vertical length of 2.5-mm. glass tubing approximately 70 cm. long is spliced to a short length of 5-mm.
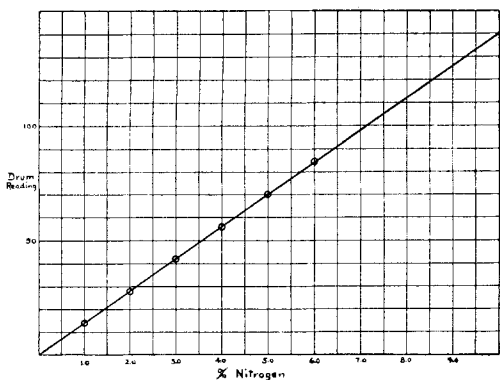
Curve III—Per cent nitrogen and drum reading.

tubing into which is sealed a side arm carrying a mercury seal, the mercury head being 2–3 mm.

The cylinder of gas to be tested, placed in a vertical position and allowed to come to room temperature, is connected to the calcium chloride tube which leads to the inlet tube of cell F of the interferometer. The outlet of this cell is connected to an anti-diffusion and overflow device similar to the one just described.

The valve of the cylinder is opened gradually until an over flow is observed at the mercury trap and the gas is allowed to flow at this rate for 15 seconds, after which time the cylinder valve is turned off and clamp D is closed. After an interval of 15 seconds the interferometer reading is made and then clamp D is opened again. This same procedure is repeated for subsequent determinations either on the same gas sample or on a different sample. The actual time required to completely flush out cell F
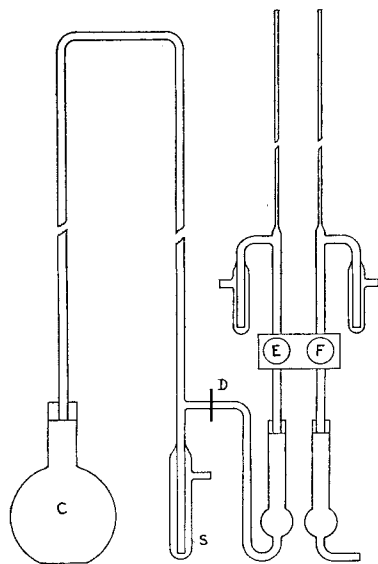

Fig. 2—Schematic interferometer system.

will, of course, vary with the characteristics of the individual set-up and can be readily determined by varying the time until consecutive readings can be checked within 2 drum divisions.

The barometer pressure and the room temperature are recorded. From the drum reading, corrected for the zero reading, of the interferometer, the percentage impurity, as nitrogen, is read off directly to the nearest tenth of a per cent from Curve III.

The results of a series of analyses are shown in Table I. Four determinations were made on each

Table I.—Percentage Impurity as Nitrogen

$t = 22°$; barometer $= 764$. Zero reading $= +3$

Sample	Drum Read-ing	Drum Read-ing Cor-rected	Im-purity, %	Purity, %
No. 52, Commercial	19	16	1.1	98.9
	20	17	1.2	98.8
	19	16	1.1	98.9
	19	16	1.1	98.9
No. 50, 9.2 per cent N₂	135	132	9.4	90.6
	135	132	9.4	90.6
	134	131	9.3	90.7
	135	132	9.4	90.6
No. 61, 100 per cent N₂O	5	2	0.2	99.8
	4	1	0.1	99.9
	5	2	0.2	99.8
	4	1	0.1	99.9
No. 95, 8.5 per cent N₂	120	117	8.3	91.7
	119	116	8.3	91.7
	120	117	8.3	91.7
	119	116	8.3	91.7
No. 70, Commercial	17	14	1.0	99.0
	17	14	1.0	99.0
	17	14	1.0	99.0
	17	14	1.0	99.0
No. 27, 4.9 per cent N₂	74	71	5.1	94.9
	74	71	5.1	94.9
	73	70	5.0	95.0
	74	71	5.1	94.9
No. 23, 0.2 per cent N₂	4	1	0.1	99.9
	4	1	0.1	99.9
	5	2	0.2	99.8
	4	1	0.1	99.9
No. 50, 9.2 per cent N₂	134	131	9.3	90.7
	135	132	9.4	90.6
	134	131	9.3	90.7
	135	132	9.4	90.6
No. 61, 100 per cent N₂O	4	1	0.1	99.9
	5	2	0.2	99.8
	4	1	0.1	99.9
	4	1	0.1	99.9
No. 27, 4.9 per cent N₂	73	70	5.0	95.0
	73	70	5.0	95.0
	73	70	5.0	95.0
	74	71	5.1	94.9

sample before proceeding to the next sample and the determinations are tabulated in the order in which they are made. This series is typical except for the fact that the samples were deliberately selected in a sequence that would show alternately high and low degree of purity. The results indicate that the 15-second period of flushing out the interferometer is adequate and therefore an actual analysis can be performed in approximately one minute.

The effect of variations in room temperature and barometric pressure on the accuracy of the method

can be readily determined from the equations given above. In Equation 1, it is seen that the barometric pressure does not appear, but a change in 3° in temperature causes a variation of approximately 1 per cent of the value of ΔR. This effect is vanishingly small in the case of purer samples of nitrous oxide and in the case of a 5 per cent nitrogen impurity where the drum reading for the Zeiss instruments is approximately 70, the variation is considerably less than the limit of reproducibility of the drum reading. Two calibrations carried out at different selected temperatures should therefore cover the range of normal seasonal temperature variations.

Equation 3 can be solved for any desired temperature and pressure. The variations in ΔR with temperature are the same as in the case of Equation 1. A variation of 8 mm. in the barometric pressure causes a variation of approximately 1 per cent of the value of ΔR.

The total volume of the interferometer system should be restricted as far as practicable in favor of the efficiency and rapidity with which it can be swept out. The inner walls of all rubber tubing connections should be freed of dust and the exits of the calcium chloride tubes should be well plugged with cotton to filter out all calcium chloride dust.

The actual reading of the interferometer is doubtless a matter of individual variation. We have found, however, that in making readings, eyestrain is greatly reduced if the reading is made as accurately as possible within ten or fifteen seconds. After the eye is rested a few seconds, the final reading is made. Prolonged staring into the instrument is to be avoided. In making several determinations on the same gas sample it is desirable to rotate the drum 10 or 20 divisions in either direction from the previous reading before making a subsequent reading.

The zero reading of the interferometer should be checked from time to time especially when any unusual temperature changes have occurred. Any gas or gas mixture can be used for this purpose, the requirements being that both cells of the interferometer contain the same gas at the same temperature and pressure.

SUMMARY

1. A method for the assay of nitrous oxide based on the use of the gas interferometer has been described.

2. A simple method for the calibration of the interferometer has also been indicated.

3. The interferometric method of assay yields results accurate to within 0.2 per cent and therefore compares favorably with other proposed methods in this respect. The method is unusually rapid.
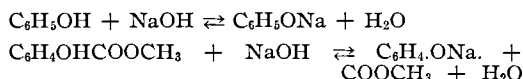
Determination of Free Phenols in Methyl Salicylate

By R. W. Towne, R. M. Hitchens and M. S. McCauley*

INTRODUCTION

On numerous occasions during the last several years need has arisen for an accurate, sensitive method for determining traces of phenolic impurities in methyl salicylate. The Dodge (1) method, used extensively, is limited in value since it is sensitive to only 0.02 per cent phenol and since it does not preclude errors caused by volatilization of some salicylic acid along with the phenols. Because of these faults this method is unsatisfactory in cases where it is desired to know the exact phenol content.

EXPERIMENTAL

Principle of Proposed Method.—Since methyl salicylate is itself a phenol, the determination of traces of phenol in this product resolves itself into the separation of two phenols. Fortunately, methyl salicylate is a much weaker acid than phenol itself, although both are extremely weak acids. It should be possible to effect a concentration of the phenol present by extracting the ester with a dilute solution of sodium hydroxide, thus removing all of the phenol together with a little of the methyl salicylate. The reactions:

$$C_6H_5OH + NaOH \rightleftarrows C_6H_5ONa + H_2O$$
$$C_6H_4OHCOOCH_3 + NaOH \rightleftarrows C_6H_4.ONa. + COOCH_3 + H_2O$$

The small amount of methyl salicylate extracted may be converted into sodium salicylate and methanol by saponification:

$$C_6H_4.ONa.COOCH_3 + H_2O \rightarrow C_6H_4.OH.COONa + CH_3OH$$
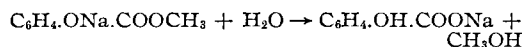
The excess alkali may be removed by acidification to a p_H value of 9, at which point the phenol will be present largely as such with a small amount of the sodium salt, the salicylate as sodium salicylate and the methanol as such. If the solution is buffered at this p_H value the phenol may be distilled quantitatively and thus separated completely from the salicylate. The phenol in the distillate may be determined by the customary volumetric conversion to tribromphenol.

Details of Proposed Method.—Solutions and Reagents:

(1) Sodium hydroxide solution, 1 Gm. per 100 cc. water.

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