These results quite definitely indicate that the U. S. P. XI method of assay determines only those alkaloids in fluidextract of ergot which are sparingly soluble in water.

A method for the determination of total alkaloids in fluidextract of ergot has been given in detail.

The author is indebted to C. E. Powell for the Broom and Clark and to C. C. Hargreaves for the Cock's Comb assay results appearing in this paper. He also wishes to express his appreciation to E. J. Hughes for his friendly criticism.

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A FURTHER STUDY OF THE ASSAY OF NITROGEN MONOXIDE.*[1]

BY FREDERICK K. BELL, C. JELLEFF CARR AND JOHN C. KRANTZ,[2] JR.

INTRODUCTION.

The tenth revision of the United States Pharmacopœia gave neither a purity rubric nor an assay for nitrogen monoxide. In a previous communication to this JOURNAL two of the authors with Reindollar (1) set forth a method of assay for nitrogen monoxide which depended upon the preferential solubility of the gas in water chilled to 0° C. Commercial samples were assayed and a standard sample of gas was assayed repeatedly. The probable error of a single determination of this series was 0.23 per cent. The method was made official in the eleventh revision of the Pharmacopœia and during the last two years has been subjected to careful scrutiny in the hands of many different analysts.

It is the purpose of this paper to present certain minor modifications of the method and further to compare it with the other procedures extant for assay of this gas.

EXPERIMENTAL.

Several samples of nitrogen monoxide of known N_2O content mixed with N_2 were prepared for us by Dr. Wardell of the Ohio Chemical Co. These gas mixtures were not compressed sufficiently so that a liquid and gaseous phase obtained in the cylinder as is present in the commercially

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compressed gas. These specimens were analyzed by the method now official in the Pharmacopœia with the following modifications. (1) The volume of gas employed was 50 cc. instead of 100 cc. This facilitates complete solution and thus diminishes the laboriousness of the determination and also the time required. These were criticisms which have been made against the method. (2) Into the upper end of the nitrometer was fused a 5-cc. pipette graduated in tenths of a cubic centimeter in order to permit a more accurate reading of the residual gas. (3) The balance bulb of the gas pipette was kept filled completely during the vigorous agitation.

Employing these modifications on gas mixtures of known N_2O content the following results were obtained.

(*a*)	N_2O content 99.8 per cent	11 determinations mean = 99.8	σ = 0.05 per cent
(*b*)	N_2O content 96.3 per cent	10 determinations mean = 96.8	σ = 0.30 per cent
(*c*)	N_2O content 94.6 per cent	10 determinations mean = 94.6	σ = 0.31 per cent
(*d*)	N_2O content 90.8 per cent	11 determinations mean = 90.9	σ = 0.34 per cent

DISCUSSION OF RESULTS.

Despite the laboriousness of the method the foregoing assays indicate that reasonably accurate and concordant results can be obtained by this method. The absolute accuracy of the method and the concordance of results are optimum when the purity of the gas is very high. In our previously reported experiments with a sample of gas assaying approximately 98 per cent σ = 0.34 per cent and in this series omitting the very concordant results obtained in the pure sample of gas the average of σ is 0.32 per cent.

The authors are fully cognizant of some of the shortcomings of this method. Our assays are run on samples where the impurity present is constantly nitrogen. Mixtures of other gases as impurities with varying degrees of solubility in water at 0° C. will affect the assay results. Besides, if concordant results are to be obtained the utmost meticulosity must be exercised in all of the details of the experiment, especially with reference to the absence of dissolved gases in the water used for the solution of the nitrogen monoxide.

Commercially in addition to the pharmacopœial method two other methods are in general use. The first which is official in the British Pharmacopœia of 1932 depends upon the freezing of the gas by means of liquid air and measuring the volume of the impurities which remain in the gaseous state. We have not yet had experience with this method. The second method depends upon exploding a measured volume of nitrogen monoxide after admixture with a definite volume of hydrogen and measuring the residual nitrogen. The Ohio Chemical Co. sent the authors complete equipment of this method and in this laboratory it has been given careful study. Very concordant results were obtained on standard mixtures of N_2O and N_2. However, it was observed that reversing the order of introducing the two gases into the explosion chamber influenced the result. Differences as high as 0.3 per cent were obtained by reversing the order of mixing the two gases. For pharmacopœial purposes the chief objection that the authors see to the method as it is now employed is the high degree of purity required for the hydrogen and the fact that its absolute percentage strength must be ascertained before using it in the assay, especially since the principal impurity in commercial hydrogen is oxygen. It is readily seen that any oxygen present will combine with its equivalent of hydrogen under the experimental conditions prevailing in the explosion method. Thus, the presence of 0.1 per cent oxygen in the hydrogen employed will cause an error in the final assay

of the nitrogen monoxide of 0.3 per cent. Therefore, if this assay were made official, hydrogen would have to be recognized as a reagent and a method of determining its purity within 0.1 per cent would be necessary.

SUMMARY.

1. The water solubility method of assay of nitrogen monoxide has been further studied and with certain minor modifications it was found again to yield reasonably accurate and concordant results on standard mixtures of N_2O and N_2.

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DRUG EXTRACTION. XV. A STUDY OF FRACTIONAL PERCOLATION.*[1]

BY WILLIAM J. HUSA[2] AND C. L. HUYCK.

In previous experiments by the present authors it was found that fractional percolation was successful for fluidextract of belladonna root and fairly successful for fluidextract of nux vomica (1).

Further research has been carried out to determine the efficiency of U. S. P. XI fractional percolation and N. F. II fractional percolation as compared with ordinary percolation. The relative efficiency of the different processes was determined by comparing the reserve percolates and finished fluidextracts as to content of alkaloids and extractive matter and by noting the time required in each case. In some instances analyses were also made of various fractions of weak percolate in order to throw further light on the progress of the extraction at various stages of fractional percolation.

HISTORICAL REVIEW.

In 1833, M. Boullay (2) of France suggested an extraction process for difficultly extractable substances, called "continuous displacement," in which the drug was divided in several containers and the liquid allowed to pass successively from one to another. E. R. Squibb (3) modified this process in 1866 by collecting a reserve percolate and several fractions of weak percolate from each portion of drug. At first Squibb called this method "divided percolation," but in 1867 he introduced the term "repercolation" (4). The name "fractional percolation" was applied by Diehl (5) to a repercolation process in which no weak percolate was left when the fluidextract was completed.

The greatest disadvantage of Squibb's repercolation process was that when a fluidextract was prepared some weak percolate remained which had to be kept in storage until the next time the fluidextract was made. After further experiments, notably by Squibb (6), Diehl (5), Lloyd (7) and Hallberg (8), a fractional percola-

* Scientific Section, A. PH. A., New York meeting, 1937.

[1] This paper is based on part of a dissertation presented to the Graduate Council of the University of Florida by C. L. Huyck, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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