Sampling and Analysis of Oxygen in the Void Volume of Ampuls and Vials

L. F. CULLEN and G. J. PAPARIELLO

Abstract □ A technique recognized as a considerable aid in stabilizing parenteral formulations sensitive to oxidative decomposition consists of replacing air in the container's headspace with an inert atmosphere. To monitor and investigate inert gas flushing techniques and to assist in evaluating antioxidant suitability, a procedure is described for the sampling and analysis of gaseous oxygen content in the void volume headspace of ampuls and vials. The procedure utilizes a paramagnetic oxygen analyzer in conjunction with a unique sampling cell and flow system which permit the collection of the headspace gas without atmospheric contamination. The sampling device and flow system were designed and evaluated to provide the necessary sensitivity, accuracy, and precision for the quantitative analysis of low air contaminant levels in the sample gas. With this method it is possible to analyze nine samples per hour with a relative standard deviation of 3.9% at a 19-μl. air level (*i.e.*, 5 mcg. of oxygen), which corresponds to approximately 2% of the theoretical gaseous void volume of a typical 1-ml. ampul product. Accuracy of the technique is demonstrated by experimentally confirming the relationship of headspace oxygen consumption to intact bisulfite and metabisulfite antioxidant content, following accelerated storage, in sealed ampuls.

Keyphrases □ Oxygen sampling, analysis—void volumes, ampuls, vials □ Sampling device, void volume oxygen—diagram □ Ampuls—oxygen determination □ Vials—oxygen determination

The adverse influence of atmospheric oxygen on both the physical and chemical stability of parenteral formulations and the numerous problems arising from the oxidative decomposition of drugs in the development of new products has been emphasized repeatedly in the past (1–6). The inclusion of a suitable antioxidant system to inhibit the oxidative process and the replacement of air in the headspace of a product's container with an inert atmosphere are generally recognized as considerable aids in stabilizing an injectable formulation. However, prior to the recent development of a method for measuring the oxygen level in the headspace of vial products (7), a survey of the literature failed to disclose any other analytical procedures specifically designed to monitor the efficiency of the inert gas flushing technique on the various parenteral containers. Thus, the pharmaceutical manufacturer has been in the dark with regard to the effectiveness of his oxygen exclusion process employed during the filling operation.

Clearly, a need existed for a sensitive, accurate, and rapid procedure for sampling and analysis of low air contamination levels in the void volume of inert gas flushed parenteral products, including ampuls. Such a technique could be employed to monitor and investigate the inert gas flushing processes and to assist in evaluating antioxidant suitability.

The accurate sampling of the void volume from a sealed, glass ampul within a controlled oxygen-free atmosphere and subsequent analysis, under similarly controlled conditions, for low oxygen levels is indeed difficult. Existing methods of oxygen analysis could not be readily modified to handle this problem. Several of the wide variety of methods which have been employed to determine gaseous oxygen vary from a highly sensitive luminescent-bacterial method (8), to colorimetric methods (9–12), microvolumetric (13, 14) and manometric (15) techniques, galvanic cell-type systems (16, 17), and coulometric procedures (18, 19). Many of these methods have the necessary accuracy and sensitivity at the oxygen level considered; however, they are either time-consuming and/or require meticulous care for satisfactory results. Furthermore, none of the procedures offered a convenient system for the sampling of the headspace gas within a sealed, glass ampul. Rapid and precise polarographic (7, 20) and gas chromatographic procedures (21, 22) which have found extensive use in oxygen analysis are also restricted by not affording systems readily adaptable to the collection and analysis of the headspace gas under anaerobic conditions.

Several commercial instruments are available which utilize the paramagnetic property of oxygen for its determination. This instrumentation is primarily employed for the continuous monitoring of the oxygen levels of expired air in respiratory physiology (23–25). The reported accuracy and sensitivity of the Beckman E2 Oxygen Analyzer (26), which has available a small volume internal analysis system equipped to measure nonflowing samples and an adaptable sample inlet port, suggested a promising approach. A consideration of the capabilities and limitations of this instrument led to the design of a unique sampling cell and flow system, of definite and critical specifications, which were evaluated to provide an accurate and rapid sampling technique for the analysis of the headspace gas of ampul products. The design of the sampling device permitted an extension of the procedure to the analysis of low air contamination levels in the void volume of inert gas-flushed injectable vial products. With this method it is possible to analyze nine ampuls and/or vials per hour.

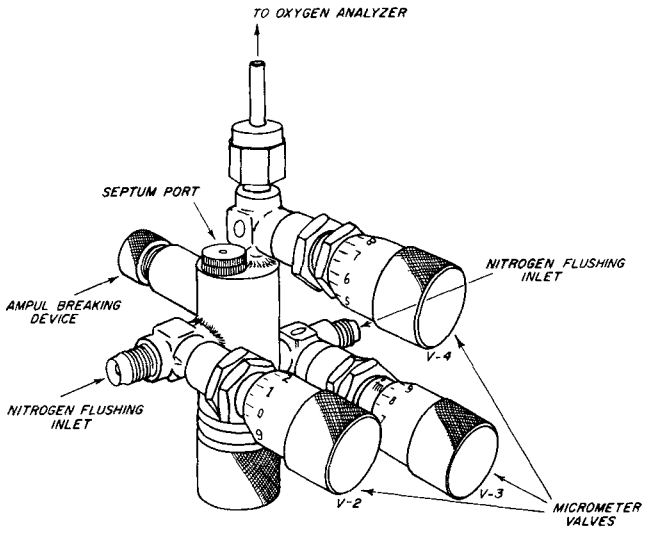

Figure 1—*Headspace gas sampling device.*

EXPERIMENTAL

Sampler Construction—The sampling device, designed to collect the headspace gas in an ampul without atmospheric contamination, is illustrated in Fig. 1. Dimensional details of this cell are given in Fig. 2. The O-ring-sealed components of the sampler, the sleeve adapter and chamber plug were machined from brass rods. For fine control of the flushing gas during deaeration of the sampling cell and to prevent damage to the detector of the analyzer from pressure surges, minimum void volume metering valves[1] were fitted to the sample chamber. Following the removal of a port connector on each of the metering valves, the valves were soldered directly onto openings in the cell, as shown in Fig. 1. This reduced the increase in total void space of the sampler introduced by the micrometer valves and eliminated the need for additional fittings. The septum inlet assembly is used for calibration purposes in the ampul procedure and as the injection port for a gas-tight syringe containing headspace gas sampled from vial products.

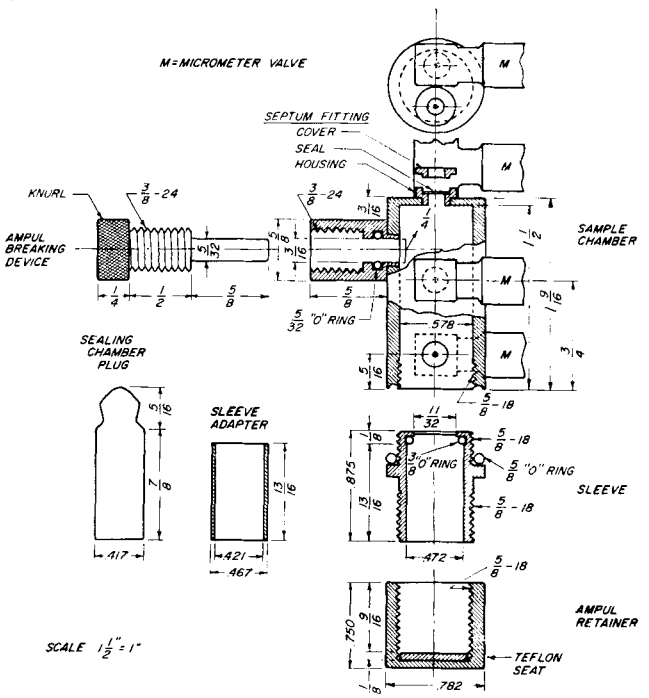

Figure 2—*Dimensional details of sampling device.*

[1] Nupro model B-2S, Nupro Co., Cleveland, Ohio.

Apparatus—A Beckman E2 Oxygen Analyzer[2] equipped with 0–1 and 0–10% oxygen percentage ranges was employed in all experiments. The sensitive detection system of the analyzer utilizes the magnetic susceptibility of oxygen to provide the basis for a quantitative evaluation of the oxygen content in the sample gas. Detailed discussions of this characteristically strong property of oxygen and how it is applied to the paramagnetic oxygen analyzers were described by Catton (27) and Johnson (28).

Accurate flow control of nitrogen during deaeration of the sampling device was provided by a flowmeter unit.[3]

A vacuum pump[4] in conjunction with a fine metering valve[5] permitted a complete and graduated evacuation of the flow system and analysis cell of the oxygen analyzer. A McLeod-type vacuum gauge[6] was inserted into the manifold to monitor the efficiency of the evacuation process.

Rubber vacuum tubing (0.32 cm. [0.125 in.] i.d. × 0.32 cm. [0.125 in.] wall thickness)[7] was connected to each of the threaded fittings of the metering valves and sample ports of the oxygen analyzer with 0.95 cm. (0.375 in.) o.d. tubing clamps. To minimize the total void volume of the actual analysis system, only 3.81-cm. (1.5-in.) lengths of this vacuum tubing were employed.

Pressure-Lok gas-tight syringes of 0.5-ml. and 1.0-ml.[8] capacity were used to calibrate the system in the analysis of both ampul and vial products and to sample the headspace gas in the analysis of vial products.

Analytical Procedures—*Ampul Products*—A diagram of the flow system indicating the equipment arrangement for the analytical method is shown in Fig. 3. The collection of an uncontaminated sample of headspace gas followed by conveyance of the gaseous sample from the sampling device to the sensitive detector of the oxygen analyzer consists of the following steps: (*a*) deaeration, with nitrogen,[9] of the sampling device containing the sealed ampul; (*b*) complete evacuation of the analysis cell to purge it of air; and (*c*) the introduction of the sample or calibration gas into the cell. Exact flow rate conditions are described to avoid sudden pressure changes which can damage the magnetic unit.

In operation, the sealed ampul is placed into the sleeve component of the sampler and pressed into firm contact with the 0.95 cm. (0.375 in.) O-ring seal with the ampul retainer. This assembly is inserted into the sample chamber and tightened. In preparation for deaeration of the sampling device and flow system, as illustrated in Fig. 3, the flowmeter needle valve (V-1) is closed, and the second stage of a two-stage regulator on the nitrogen cylinder is set to deliver 10 psig Needle valves V-2 and V-3 are opened to provide a nitrogen gas flow rate of approximately 125 ml./min. Valves V-4 and V-5 are set to permit a flow rate of 250 ml./min., with the T-bore stopcock (T-6) vented to the atmosphere. To nitrogen-flush the sampling device, slowly open flowmeter needle valve (V-1) until a flow rate of 220 ml./min. is established as indicated by a 105 mm. metering-tube reading with the Pyrex float. After a 2-min. flush close valve V-1, followed by valves V-2, V-3, and V-4 in successive order. Break ampul, then evacuate flow system and detector by closing valve V-5 to produce a flow rate of 70 ml./min., setting stopcock (T-6) for system evacuation, and drawing a vacuum for 1.5 min. To ensure a relatively complete deaeration process, a vacuum reading after approximately 1.25 min. of this evacuation period should indicate a system pressure of less than 0.4 mm. Hg. To convey sample gas mixture to the evacuated detector of the analyzer for compositional analysis, close valve V-5 and slowly open valve V-4 to a flow rate of 100 ml./min. Following sample readout, the magnetic unit is gradually adjusted to atmospheric pressure by venting stopcock (T-6) to the atmosphere and slowly opening valve V-5 to deliver a flow rate of 70 ml./min. The sampling cell pressure

[2] Beckman catalog No. 118521, Beckman Instruments, Inc., Fullerton, Calif.
[3] Matheson model 620BBV with No. 602 metering tube, Matheson Co., Inc., East Rutherford, N. J.
[4] Welch model 1400B, W. M. Welch Scientific Co., Skokie, Ill.
[5] Nupro model B-2S.
[6] Nester/Faust model 68, Nester/Faust Manufacturing Corp., Newark, Del.
[7] Thomas catalog No. 8844, Arthur H. Thomas Co., Philadelphia, Pa.
[8] Models 306000-A and 306001-A, Precision Sampling Corp., Baton Rouge, La.
[9] Prepurified grade, Matheson Co., Inc.

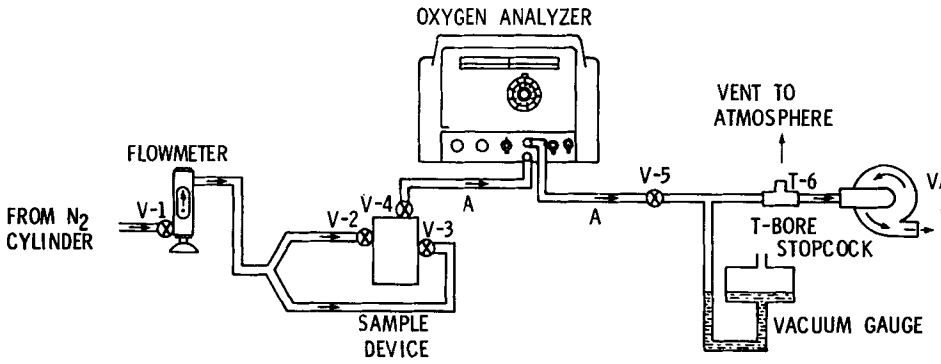

Figure 3—*Flow system for oxygen analysis.*

is brought to atmospheric by opening valve V-4 to provide a flow rate of 250 ml./min.

The exact flow system conditions described above for the analysis of the headspace gas are used in calibration of the system. Calibration is effected by injecting, in duplicate, known volumes of air at the appropriate level into the nitrogen-purged sampling device, which contains an ampul. Standardization is repeated at the end of a series of 20 samples in order to minimize the effects of instrumental variations. Calculations are made using corresponding instrument responses of standards and headspace gas of ampul products.

Vial Products—The flow system, as illustrated in Fig. 3, and exact analytical conditions described under *Ampul Products* for conveyance of the sample gas from the sampling device to the detector were employed in the analysis of vial products. The chamber plug, dimensionally detailed in Fig. 2, is inserted to seal the sampling chamber. In sampling, an accurately measured portion of the headspace gas is drawn into a gas-tight syringe and locked in the syringe prior to withdrawal from the vial. This is followed by injection into the nitrogen-flushed sampling cell after penetration of the septum and opening the actuation valve of the syringe. The volume of the headspace gas mixture sampled should, preferably, contain the equivalent of 0.02–2.5 ml. of air. It should be noted that during the vial sampling process a partial vacuum is created. Consequently, a correction must be made for this sampling effect in order to determine the actual sample volume.

The system is calibrated by injecting appropriate levels of air, sampled directly from the atmosphere, into the nitrogen-purged sampling device. Using this standardization technique and the Eq. 1 which corrects for the previously described sampling effect, the percent air in the sample can be calculated.

$$\% \text{ air (v/v)} = \frac{(R_V)(A_S)(V_T)100}{(R_S)(V_V)} \qquad \text{(Eq. 1)}$$

where R_V = response of sample; R_S = response of standard; A_S = volume of air injected for calibration; V_V = measured void volume of vial; V_T = measured void volume of vial (V_V) plus the indicated sample volume of the gas-tight syringe.

As an alternate technique in standardization of the system, known volumes of either air or air-inert gas calibration mixtures are sampled from vial packages filled with water to yield a headspace volume equivalent to the vial products. Employing the gas-tight syringe to collect, store, and transport the headspace gas as described previously, the withdrawal of identical volumes of sample and reference gas from equal voids eliminates a consideration of the dilution factor introduced in the sampling process, as required in Eq. 1. Calculations are made using the corresponding instrument responses of standards and samples of the headspace gas of vial products.

RESULTS AND DISCUSSION

Design of Sampling Device—The major consideration in the development of the sampling system was to design a sampling cell of a sufficiently low void volume to provide the necessary sensitivity for accurately monitoring inert gas flushing efficiency at levels greater than 98%, *i.e.*, less than 2% air contamination. Two percent air contamination corresponded to a 20-μl. air level (*i.e.*, 5 mcg. of oxygen) in the typical 1-ml. ampul product investigated. At such levels of oxygen, the analyzer has a reported accuracy of ±1.7 μl. air (±0.4 μl. oxygen) and a limit of detection of 0.2 μl. air in the sample gas with its 3.5-ml. internal analysis system at 760 mm. pressure (28). However, with a gaseous void volume of approximately 1 ml. for most ampul products, insufficient sample gas is available to satisfy the detector at atmospheric pressure. Thus, it was realized that a special flow system, as described under *Analytical Procedure*, would be required which results in the analysis of the sample gas at a reduced pressure. Under these operating conditions, the accuracy and sensitivity characteristics of the detector are reduced, necessitating an evaluation of pressure effects in establishing the optimum sample chamber volume for the sampling device.

In designing the cell for optimum accuracy and sensitivity conditions, a balance had to be considered between: (*a*) satisfying the pressure requirements of the detector by increasing the void space of the sample chamber in order to approach atmospheric conditions in the analysis cell; (*b*) reducing the volume of the sample chamber to prevent a loss in sensitivity from an unnecessary high dilution with the inert gas. Employing the ideal gas laws to calculate the effect of sample concentration and pressure on instrument readout (28), the dimensional parameters of the sampling system were derived. A graphic representation of the effect of sample cell volume on the obtainable theoretical accuracy is shown in Fig. 4.

It was calculated that a sample chamber and associated metering valves with a 5.2-ml. void volume provided the optimum analysis conditions. A sampling device of these dimensions has an ultimate, theoretical accuracy of ±7 μl. air and a limit of detection of 0.7 μl. air. The sampling device and flow system have been fabricated to these specifications (see Figs. 2 and 3). Thus, the indicated accuracy and sensitivity to monitor inert gas flushing efficiency at levels greater than 98% has been built into the system.

Linearity and Sensitivity—A typical calibration curve obtained by injecting known volumes of air into the sampling device in the

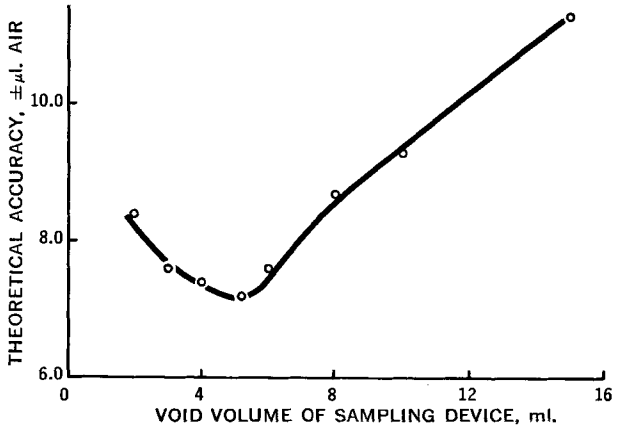

Figure 4—*Effect of void volume of sample device on the theoretical accuracy of system.*

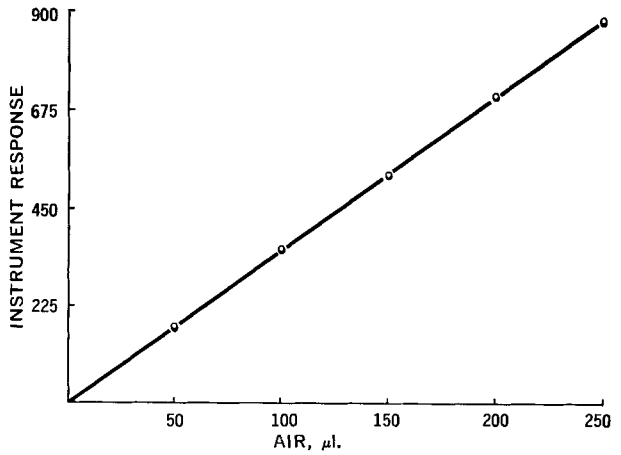

Figure 5—*Relationship of instrument response to air level.*

50–250 μl. range, equivalent to 14–68 mcg. of oxygen, demonstrates the linearity of the instrument response-air level relationship (Fig. 5). These data had been collected with the oxygen analyzer set at its most sensitive operating range, *i.e.*, the 0–1% oxygen percentage range. The 0–10% range on the instrument extends the applicability of the sampling and flow system (Fig. 3) to include the analysis of most ampul and vial parenteral products at any level of inert gas flush efficiency by permitting an evaluation of headspace gas which contains 0.25 to 2.5 ml. of air. A linear relationship exists between instrument response and air level at these higher concentrations.

The actual measured lower limit of sensitivity of both the ampul and vial procedures is 2 μl. of air in the sample gas mixture. This air level represents 0.2% of the total void volume of the typical 1-ml. and 2-ml. ampul products. The determined sensitivity of 2 μl. of air approaches the theoretical sensitivity of 0.7 μl. of air.

Ampul Procedure—*Precision*—Repeatability of the procedure was determined by assaying the headspace gas of 20 individual ampuls flushed with a 0.4% (v/v) oxygen-nitrogen calibration gas mixture.[10] Experimentally, 1.0-ml. aliquots of water were introduced into 1-ml. ampuls under an atmosphere of the oxygen-nitrogen gas mixture within an inflatable polyethylene glove chamber. Using the gas mixture the ampuls were flushed for exactly 20 min., then immediately sealed under the oxygen-nitrogen atmosphere with approximately 1 ml. of headspace above the water. Following the analysis of the headspace gas, the void volumes of the individual ampuls were measured and calculated into the results. This eliminated any differences in the void volumes of the ampuls produced by the sealing process. Reported in terms of air at 25° and 760 mm. Hg, a relative standard deviation of 3.9% was determined at the 19-μl. air level. The 19-μl. air level, which corresponds to 98.1% nitrogen flushing efficiency, demonstrates the precision of the procedure at a high level of inert gas layering.

Accuracy—Accuracy of the ampul procedure was established by experimentally determining the relationship of oxygen consumption to intact bisulfite and metabisulfite content after interaction in sealed ampuls. In this study, 1.0 ml. of an 0.1% solution of the antioxidant (sodium bisulfite or sodium metabisulfite) was introduced into 1-ml. ampuls and flushed with air-nitrogen gas mixtures in order to obtain either 60 or 500 μl. air per 1 ml. of ampul void volume. A gas proportioning unit[11] provided accurate metering of air and nitrogen to the desired ratio during this flushing process. The ampuls were sealed to contain a void volume of about 1 ml. under the air-nitrogen atmosphere, then immediately assayed for the initial antioxidant content (29) and headspace air. The ampuls were stored at temperatures from 35 to 60° to accelerate oxygen consumption and assayed for antioxidant and headspace air content at periodic time intervals. Typical data collected in this study are summarized in Table I.

The theoretical consumptions of sodium bisulfite and sodium metabisulfite by the oxygen content of 1.00 ml. of air at 25° and 760 mm. are 1.77 and 1.62 mg., respectively (6). The results indicate the experimental values are in excellent agreement with theory. Data obtained on ampuls containing less than 10 μl. of air in the headspace gas, *i.e.*, less than 1% air in the typical 1-ml. and 2-ml. ampul products, demonstrate that the technique quite adequately meets the accuracy requirements for monitoring inert gas flushing efficiency at levels greater than 98%.

Table I—Relationship of Consumed Air to Intact Antioxidant Level

Reaction Condition	Air Remaining per Ampul, μl[a]	Antioxidant Remaining per Ampul, mg.[a]	Antioxidant Reacted per Ampul, mg.	Air Consumed per Ampul, μl. Exptl.	Theoret.
Sodium Metabisulfite					
30 hr., 35°	22	0.89	0.07	41	43[b]
48 hr., 45°	9	0.87	0.09	58	56[b]
48 hr., 60°	4	0.86	0.10	61	62[b]
24 hr., 35°	161	0.43	0.53	332	329[b]
48 hr., 60°	35	0.22	0.74	455	459[b]
Sodium Bisulfite					
30 hr., 35°	22	0.85	0.07	39	37[c]
48 hr., 45°	6	0.83	0.09	53	51[c]
24 hr., 35°	192	0.39	0.53	297	300[c]
48 hr., 60°	48	0.14	0.78	443	442[c]

[a] Average assay value on five individual ampuls (air analyses $\sigma_{av.} = \pm 3.2\%$; antioxidant analyses $\sigma_{av.} = \pm 1.9\%$). [b] Calculated from the relationship that 1.62 mg. of $Na_2S_2O_5$ is equivalent to 1.00 ml. air at 25°, 760 mm. [c] Calculated from the relationship that 1.77 mg. of $NaHSO_3$ is equivalent to 1.00 ml. air at 25°, 760 mm.

Vial Procedure—*Precision and Accuracy*—Both the precision and accuracy of this technique were obtained by performing replicate assays on known volumes of air injected into nitrogen-flushed vials with a butyl rubber stopper-aluminum cap closure and measuring the percentage recovery. This was accomplished by flushing and crimping empty vials, with accurately measured void volumes of approximately 15 ml., under an absolute nitrogen atmosphere. Following the removal and analysis of a portion of the headspace gas from the individual vials to correct for residual air in the flushing process, 0.15 and 1.50-ml. levels of air were injected into the vials, producing synthetic air contamination levels at 1.0 and 10%, respectively. Data collected on replicate assays of 15 individual vials at the 1.0% contamination level indicated recoveries of 97 to 103% of the theoretical amount present with a relative standard deviation of 4.2%. Recoveries of 99 to 101% of theory were obtained on 15 individual vials at the 10% air contamination level with a relative standard deviation of 2.1%. In this phase of the study, the data were calculated as described by Eq. 1.

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NOTES

Muscarinic Agents: The Isomeric 6-Acetoxy-2-methylisoquinuclidine Methiodides

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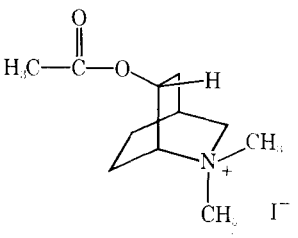
Abstract □ Preparation of 6-*endo* and 6-*exo*-acetoxy-2-methylisoquinuclidine methiodides are described. Muscarinic assay data are reported. Neither of the compounds showed activity when compared to acetylcholine and 3-acetoxyquinuclidine methiodide.

Keyphrases □ 6-Acetoxy-2-methylisoquinuclidine methiodides—synthesis □ Pharmacological screening—6-acetoxy-2-methylisoquinuclidine methiodides □ IR spectrophotometry—identity, structure □ NMR spectroscopy—structure

Hypotheses delineating the architectural features of cholinergic receptors have been based on observations of pharmacological activity of various substituted derivatives of the neurohormone, acetylcholine. Differences in the activity of these analogs of acetylcholine have long been explained on the basis of molecular steric and electronic effects in the drug-receptor interaction (1, 2). Spectral data concerned with conformational aspects of acetylcholine have also been studied and developed in recent attempts to describe receptor site architecture (3–7).

In further studies to determine the conformational requirements of the drug-receptor complex, in which the authors' assume a large degree of complementarity of the drug and receptor in this interaction, a number of conformationally rigid or semirigid analogs of acetylcholine have been prepared (8–13). Each, although incorporating the essential features for cholinergic activity, also inherently must be constructed of an additional number of carbons to maintain the desired conformational rigidity. Comparison of activities of agents structurally similar to each other seems valid.



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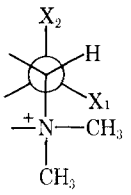

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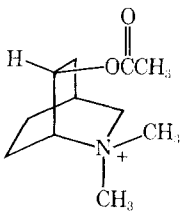
IIA, $X_1 = OAc, X_2 = H$



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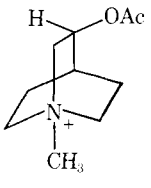

VIII

PREPARATION OF ANALOGS

In a program of preparation of agents to further determine the geometric requirements of the muscarinic agents, it was decided to prepare cholinergic analogs in the isoquinuclidine system in which