Reports and Letters

Rapid and Precise Measurement of Moisture in Biological Materials

The classical methods for determining water content either by drying to constant weight, or chemically as with the Karl Fischer reagent, are not entirely satisfactory. The most serious disadvantage of the first method is that it is tedious and time consuming, whereas the chemical method is difficult to apply without introducing traces of atmospheric moisture and must be repeatedly standardized against a known amount of water. Moreover, side reactions with some biological materials may occur, and the sample is lost.

This paper (*1*) describes a method, using an instrument similar to that devised by Anderson (*2*), that is fast and precise because the water is measured manometrically and the sample being tested is never exposed, even momentarily, to the atmosphere. Although the apparatus was designed for the specific purpose of determining residual moisture in dried biological material, it is useful for determining the moisture in air, foods, pharmaceuticals, and a wide variety of industrial products.

Figure 1 shows that the apparatus consists of four main parts: the condenser, *A*; manometer, *B*; device for attaching samples, *C* or *D*; and an interchangeable expansion chamber, *E*. The manometer measures the vapor pressure of the water; since the pressure is directly proportional to the amount of water and inversely proportional to the volume of the vapor, the apparatus is calibrated simply by measuring its volume.

For a given pressure the amount, or weight, of water can be calculated by the following formula.

$$W = P[V + PM]\,\frac{273 \times 18 \times 10D_o}{T \times 22.4 \times 760 \times D_{Hg}},$$

where W is the weight of water in milligrams; P is the pressure of oil in centimeters; V is the volume of system at zero pressure in cubic centimeters; M is the amount of oil displaced per centimeter of pressure in milliliters; T is the absolute temperature in degrees; D_o is the density of oil at temperature T; and D_{Hg} is the density of mercury at temperature T.

Figure 2 shows the results obtained with known amounts of water. It is evident that the manometric method is accurate as well as precise, because the individual points deviated only slightly from the theoretical curve based on the equation given in the previous paragraph.

After the unit has been calibrated and completely dried, the manometer is filled to the zero level with a suitable low vapor pressure oil, the density of which has been determined. Octoil S was the most satisfactory of those tested.

To degas the oil, the entire system is evacuated to a pressure of less than 50 μ mercury, with stopcocks *G* and *H* open. Then, while pumping is continued, the left arm of the manometer is heated with a bunsen burner. The oil is rapidly degassed as the oil boils over through the stopcock *H* and down the right arm of the manometer.

Operation of the apparatus is divided into the following six steps.

1) Flame-sealed ampules and rubber stoppered bottles containing the samples are attached to the unit by means of adapters on a 10/30 standard taper connection (Fig. 1, *C* and *D*). The ampules are attached by means of adapter *C*, which consists of a 1-in. length of thin-walled metal tubing inserted within gum pressure tubing so that the latter extends about ¾ in. beyond both ends of the metal. The scored neck of the ampule is inserted so that the tip just enters the metal tube; it is held in this position by the distal portion of the rubber tubing. The bottles are attached by inserting the hypodermic needle of adapter *D* into the rubber stopper only far enough to occlude the bevel.

After the container is attached to the apparatus, stopcock *M* is opened and the air and water vapor are removed from the entire system, including the adapters, by continued evacuation through *F*.

2) The condenser is immersed in a dry ice ethanol bath after all air and water have been removed as indicated by a reading of 50 μ on a Pirani gage or by return of the manometer pressure to virtually zero.

3) After the condenser has cooled, the samples are connected to the system. The ampules are connected by breaking off the tip by bending at the point where the neck of the ampule enters the metal tubing, and the bottles are connected by pushing the needle through the rubber stopper.

4) The water is distilled over into the condenser either at room temperature or by immersing the bottle in a water bath (*L*). Since the amount of water that can be removed from a dried preparation is correlated with the temperature, the latter should be carefully controlled. Although 100°C may not denature dry proteins or kill microorganisms, it is safer to use a lower temperature, such as 50° or 60°C, in order to retain the biological activity of the sample after determining its moisture content.

It was observed that practically all of the moisture in our lyophilized preparations was removed in 10 minutes and that 95 percent was often distilled over in 3 minutes, but the temperature and time required should be determined for each type of material.

5) After stopcocks *F* and *M* have been closed, the condensed water is vaporized by removing the condenser from the cooling bath and placing it in water at room temperature. Although small variations in temperature produce only negligible effects on the pressure, the use of a constant temperature is recommended.

6) When stopcock *G* is left closed, 1 cm of oil pressure is equivalent to approximately 0.1 mg of water. Should the amount of water be sufficient to produce a pressure in excess of 25 cm of oil (about 3 mg of water) the volume of the system must be increased by opening stopcock *G*. If a 1000-ml flask is used as the reser-

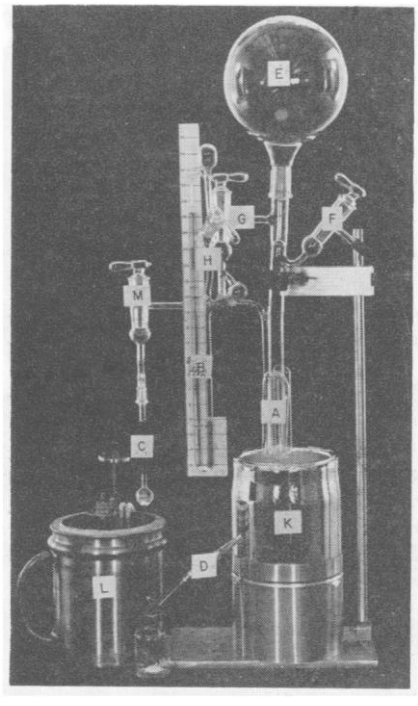

Fig. 1. Apparatus for measuring small amounts of water manometrically.

voir E, 1 cm of oil will be equivalent to about 1 mg of water. When the temperature of the apparatus has reached equilibrium, as indicated by a constant pressure, the manometer reading is recorded. The apparatus is readied for the next determination by opening stopcock *F* and trapping the water in a condenser placed in the vacuum line to the pump.

This apparatus can also be used to determine the pressure within the container before it is opened and can constitute the first step in the moisture determination. To do this, stopcock *F* is closed before the connection between the sample and the apparatus is completed (step 2). The total pressure within the sealed bottle is directly related to the observed pressure and can be obtained from an appropriate conversion graph similar to that used to obtain the amount of water.

Some time after the method had been in use in this laboratory, Beckett (*3*) described an apparatus for measuring moisture that is similar in function and principle to that described in this report. However, the unit described here differs in design and permits easier and more reliable operation. One important feature is the design of the manometer, which makes it convenient to degas the oil *in situ*. Another advantage in the construction is that, even though an error is made in manipulating the stopcocks, there is no possibility of sucking oil either into the pump or into other portions of the apparatus. A third advantage is that if the stopcock *H* is left closed the manometer can serve to check the operation of the instrument, obviating the use of a separate vacuum gage.

By exposing the bottle or ampule directly, temperature equilibrium between the sample and water bath can be achieved rapidly. Most of the procedures described in the literature use temperatures above 60°C but at relatively high pressures, that is, 50 to 100 mm of mercury. Because of the higher vacuum employed in this apparatus, comparable results may be obtained at lower temperatures. Since the temperature of the sample can be easily controlled, procedures are readily standardized to yield results comparable to those obtained by any other method.

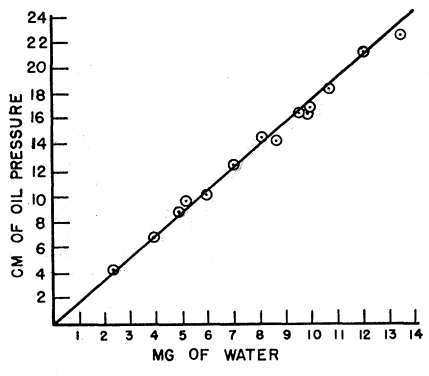

Fig. 2. Observed pressure of known amounts of water. The solid line represents the theoretical curve that is based on the volume of the apparatus; the points represent individual observations.

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References and Notes

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2. A. W. Anderson, personal communication, 1950.
3. L. G. Beckett, in *Biological Applications of Freezing and Drying*, Ed. R. J. C. Harris (Academic Press, New York, 1954), p. 285.

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Desiccator Cover Remover and Sleeve Wrench

The removal of desiccator covers (particularly glass desiccators) that have become tightly adhered has always been a problem. Use of desiccators in low temperatures, for long periods under vacuum, and with improper grades of seal grease, and so forth, are some of the common reasons for the tightly adhered covers. The ordinary means of removing the sticking covers can be hazardous because of the possibility of breaking the lid or desiccator jar; there is also the chance of spilling the samples in the desiccator during the operation. The use of glass desiccators that have a ground glass external collar with turbulature presents a similar problem. Occasionally these sleeves or collars are very difficult to turn, particularly if the desiccator is being used in low temperatures. When grasping the sleeve in an effort to turn it, and using the tabulature or side arm as a means of leverage, one is apt to break the tabulature; this often results in a serious injury to the operator. The following paragraphs describe a desiccator cover remover and a sleeve wrench that have been designed in our laboratory for performing the afore-mentioned operations in a safe and easy manner.

The cover remover is shown at bottom of Fig. 1. On the bottom jaw of the hand lever *A*, two tapered hard rubber rollers *B* are mounted on a ¼-inch pin through the jaw. A slot *C* is cut at an angle in the top jaw to receive the cross pull links *D*. The pull links are fastened through the openings of opposite sections of parallel brass sash chain *E*. The chain is drawn through rubber tubing that keeps it from becoming tangled and still allows flexibility. The cross pull links are made of ⅛-inch stainless steel rod threaded on both ends. Nuts on both sides of each chain section provide proper spacing between the chains and secure the pull links to the chain. The pull links are set at intervals along the chain to accommodate each size of desiccator cover. The ends of the chains are fastened to a hook that consists of an approximately 2-inch square brass plate *F* that is bent slightly to conform somewhat to the curvature of the cover. A ¼-inch square brass rod *G* bent slightly to conform to the outer circumference of the largest cover is soldered to the bottom along one end of the brass plate, forming the hook. Rubber tubing is placed over the handle of the lever to provide a better grip.

In operation, the proper pull link is engaged in the jaw slot and the end plate is placed so that the square rod is over the edge of the lip of the cover. The lever is then placed so that the chains pass over each side of the top of the cover and the rollers are under the flange of the desiccator proper. If the pull links are spaced properly, the handle of the lever should now be in a position slightly below horizontal. By holding down on the brass plate hook with one hand and pressing down on the lever handle with the other, the cover may be drawn across the desiccator far enough for easy removal.

The sleeve wrench is illustrated at top of Fig. 1. It consists of a pair of pivoted levers *A* the handles of which are bent slightly to bring them closer to parallel when they are in use. Rubber tubing *B* is placed over the handles to provide a better grip. One end of a piece of brass sash chain *C* is securely attached to the end of one of the levers. Pure gum rubber tubing is placed over the sash chain allowing a lip *D* of the tubing to cover the point where the chain is attached to the lever. Single extra chain links *E* are attached to the chain at proper intervals through holes cut in the rubber tubing. The extra links are attached in such a way that the loop end may be slipped over a pin *F* that is fastened in the end of the opposite lever. The locations of the extra links are determined by the diameters of the glass sleeves of the desiccators in use. The ex-

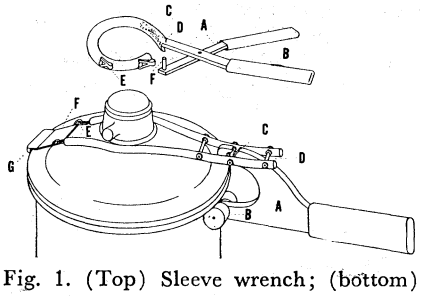

Fig. 1. (Top) Sleeve wrench; (bottom) cover remover.