Study of Water Vapor Sorption by Pharmaceutical Powders

By W. A. STRICKLAND, Jr.

Water vapor sorption by pharmaceutical powders was studied by methods designed to yield data suitable for use in the preparation and stability testing of pharmaceuticals such as capsules, tablets, etc. The study yielded sorption-desorption isotherms near room temperature that permit the estimation of water sorption behavior of these powders in normal atmospheric conditions.

POWDERS may sorb water vapor from the surrounding atmosphere by physical and/or chemical bonding. The tenacity with which water is held by such powders as starch and certain metallic oxides is well known. The affinity of powders for water may be a factor of importance when considering the fabrication and stability of pharmaceutical preparations such as capsules, tablets, powders, and granules.

The literature reveals an abundance of work concerning sorption and diffusion of water and water vapor in such fields as textiles, foods, ceramics, and fuels, most of which has little application to the subject at hand. Leeson and Mattocks (1) recently studied the decomposition of aspirin at various water vapor levels. Babbitt (2) used a quartz spring sorption balance to study the sorption of water vapor by wheat. Dacey, *et al.* (3), used a similar apparatus to study the adsorption of water by saran charcoal. Hoover, *et al.* (4), applied polarization theory to sorption of water vapor by high polymers, principally protein. The surface area of porous solids such as soils, gels, and charcoals were determined from their water adsorption isotherms by Puri and Sharma (5).

The purpose of this work was to study the sorption and desorption of water vapor by representative pharmaceutical powders. This report describes methods used in the study and gives data obtained.

EXPERIMENTAL

Three distinct approaches were employed in this study, each will be discussed separately.

Screening

All powders were initially tested by placing accurately weighed, untreated samples of about 5 Gm., suitably spread in aluminum foil containers, into humidity chambers for a period of approximately 1 week. At the end of the equilibrium period, the samples were weighed a second time and loss or gain in weight noted. The humidity chambers, which consisted of large Pyrex glass desiccators, were kept in a room maintained at 21 ± 2°. The desired relative humidities were obtained by placing appropriate concentrations of sulfuric acid-water solutions (6) or saturated solutions of appropriate salts (7, 8) in the bottom of the desiccators. Corn starch was used as a reference in screening tests because it exhibited a predictable gain or loss of weight at various relative humidities.

Discussion of Results of the Screening Procedure.—Table I gives typical qualitative results of the screening procedure with powders that contain appreciable water or possess marked tendency to sorb water vapor. Powders showing such results were deemed suitable for further study. The amount of water held by the powder at 45% relative humidity was considered as a normal water content.

TABLE I.—RESULTS OF SCREENING TESTS SHOWING WATER VAPOR SORPTION OR DESORPTION ON UNTREATED POWDER SAMPLES

Compound	Gain or Loss of Weight, mg./Gm. Starting Weight, in Humidity Chambers				
	0% RH[a]	20% RH	45% RH	70% RH	90% RH
Casein	−50	−18	0	43	95
Magnesium trisilicate	−82	−20	3	17	30
Thiamine hydrochloride	−40	−6	2	4	30
Magnesium carbonate	−15	−2	0	8	22
p-Aminobenzoic acid	0	0	0	0	33
Methylene blue	−40	−2	0	113	135
Polyglycol E4000 (Dow)	−19	−18	−11	5	125
Polyglycol E20,000 (Dow)	−11	−10	−7	2	44
Sodium diphenylhydantoinate	−10	−2	4	239	291
Corn starch	−99	−24	2	40	88
Sodium phenobarbital	−16	..	6	..	91
Methocel 4000 cps. 90 Hg	−59	−20	10	69	141
Procaine base	0	0	0	0	154

[a] RH, relative humidity.

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If a powder neither gained nor lost weight it was presumed to be essentially free from water vapor sorption characteristics and was not further studied. Table II lists several powders that neither gained nor lost weight in these humidity chambers.

TABLE II.—POWDER SAMPLES THAT NEITHER GAINED NOR LOST WEIGHT (LESS THAN ±0.5%) DURING SCREENING TESTS IN HUMIDITY CHAMBERS

Sulfathiazole	Phenothiazine
Phenobarbital	Hydroquinone
Benzocaine	Diphenylhydantoin
Tetracaine base	Chloramphenicol
Talc	Codeine alkaloid
Quinine alkaloid	Holocaine hydrochloride
Cincophen	Prochlorperazine maleate
Niacin	Procaine hydrochloride
Niacinamide	Aminopyrine
dl-Amphetamine sulfate	Aminophylline

Determination of Water Vapor Sorption Isotherms in Humidity Chambers

Accurately weighed samples, approximately 2 Gm. each, of desiccated powders in tared glass weighing bottles of such diameter that the loosely spread powder was less than 0.5 cm. deep, were placed in a humidity chamber which consisted of a large Pyrex glass desiccator, and dried at 110° under reduced pressure for 4 hours, when melting points permitted. The powder samples, some of which were recrystallized, were seived through 100-mesh screens. After the final weighing, conducted in such manner as to avoid sorption of atmospheric water vapor, concentrated sulfuric acid was introduced into the lower portion of the chamber and the top sealed in place. Normal atmospheric pressure and content existed in the humidity chamber at all times during the determination. The assembly was placed in a constant temperature water bath maintained at 25 ± 0.2° and allowed to equilibrate for about 1 week. At the end of this time the powders were weighed swiftly on a projection balance with due caution to avoid sorption of atmospheric water vapor. Subsequently, solutions of sulfuric acid and water were introduced into the chamber bottom to give increases in relative humidity of 10–20% each week. An aliquot portion of this solution was titrated with standard base at each weighing time to determine precisely the equilibrium concentration. The relative humidity corresponding to concentration of sulfuric acid was found by reference to the International Critical Table (6).

The desorption isotherm was obtained by reducing the relative humidity in the chamber by weekly increments similar to those used in the sorption study.

Discussion of the Sorption and Desorption Isotherms Obtained in Humidity Chambers.—The shape of the isotherms obtained in this study (Figs. 1–4) were reproducible; however, precise reproduction of points on the curves was not usually achieved. Perhaps surfaces of the particles were altered during the several weeks of each study, for a second determination with a given sample did not yield precisely the same result as the first. Close agreements were obtained with successive samples of the same powder.

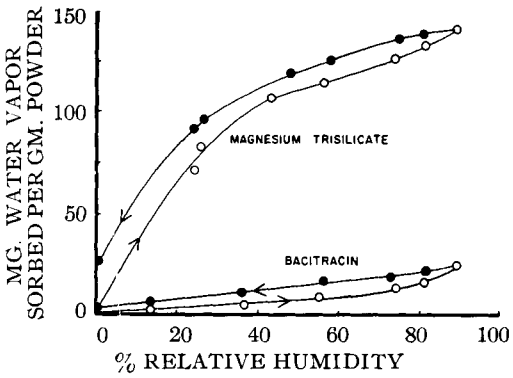

Fig. 1.—Sorption and desorption of water vapor in relative humidity chambers (25°) on 100-mesh powders. ○, Sorption; ●, desorption.

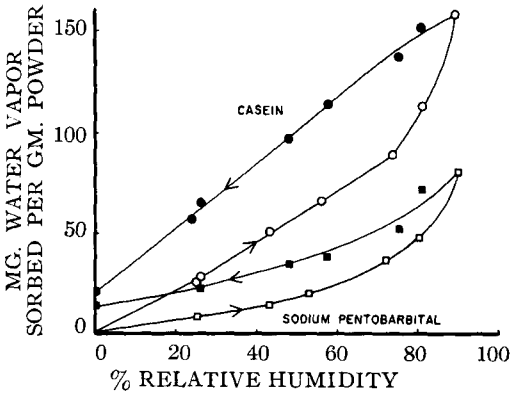

Fig. 2.—Sorption and desorption isotherms (25°) obtained in humidity chambers using 100-mesh powders. The closed points indicate desorption portion of curves.

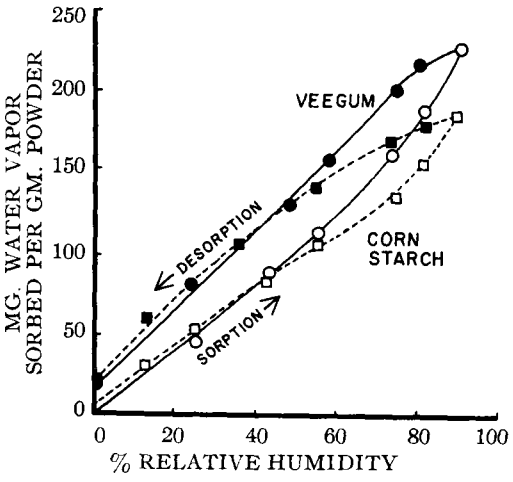

Fig. 3.—The sorption and desorption of water vapor observed in humidity chambers (25°). The Veegum (R. T. Vanderbilt Co., colloidal magnesium aluminum silicate) samples, used without sieving, consisted of particle sizes ranging from small flakes to fine powder.

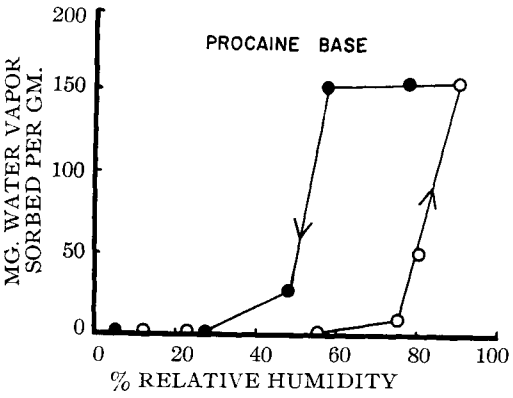

Fig. 4.—The sorption-desorption isotherm of procaine base powder, 100 mesh, in humidity chambers (25°) was influenced by formation of a dihydrate. This behavior was elucidated by further study in the quartz spring sorption apparatus. O, Sorption; ●, desorption.

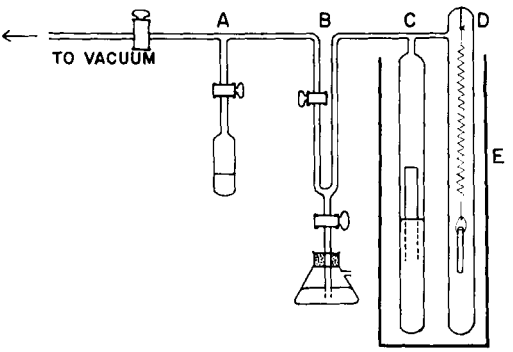

Fig. 5.—Diagram of the vapor sorption apparatus. A, Water vapor reservoir; B, mercury trap; C, Dubrovin gauge; D, sample chamber; E, water bath.

The pronounced hysteresis loop of the desorption curve found in many of these isotherms may be due in large measure to failure to attain equilibrium on the sorption curve and/or on the desorption curve. Hysteresis has been encountered in other systems and can be accounted for in different ways aside from lack of equilibrium, such as the "ink bottle" theory, but the hysteresis shown here was reproducible and it existed in the same magnitude even though extended periods were allowed for equilibration.

Determination of Water Vapor Sorption Isotherm with Quartz Spring Sorption Apparatus

The isotherm for procaine base (Fig. 4) was substantially different than that for most powders. Procaine base is known to form a dihydrate when recrystallized from ethanol-water solutions (9) and has been described as a hygroscopic powder (10); however, literature search did not reveal studies on the nature of this water vapor sorption. Consequently, an apparatus was constructed (Fig. 5) which permitted study of the mode of water vapor sorption and was capable of yielding data pertaining to the energy of hydration. The essential difference in the data obtained in this apparatus and that previously described was that this hydration takes place in the presence of water vapor alone and was not subject to the ambiguities introduced by the presence of atmospheric gases.

Equipment.—The apparatus (Fig. 5) consisted of two large connected tubes, one of which contained a quartz spiral spring from which the sample was suspended. The quantity of water sorbed by the powder was obtained gravimetrically by measuring the extension of the quartz spring, which had the following characteristics: unloaded length approximately 6 cm., extension of 1 cm. per 51 mg. added weight, and a maximum load of 2 Gm. The second tube contained a Dubrovin gauge (11) having a multiplication factor of 4.91. Both the extension of the quartz spring and the position of the Dubrovin tube were determined with a cathetometer which could be read to ±0.01 cm. The water bath, in which the assembly was immersed was maintained at 23.6 ± 0.1° by a temperature regulator-stirrer.

Procedure.—Recrystallized samples of procaine base were sieved through 100-mesh screen and dried under vacuum. An accurately weighed sample of approximately 1 Gm. was suspended, in a cylindrical waxed-paper container, from the lower hook of the quartz spring. After the sample and spring were sealed in place, the apparatus was evacuated at less than 0.1 cm. mercury pressure for approximately 3 hours, after which time the system was isolated by closing the stopcock and raising the mercury seal (Fig. 5). After initial readings of pressure and spring extension with the cathetometer, water vapor was introduced into the assembly from the water reservoir. Sorption appeared to be complete in about 8 hours, however, 24 hours was the usual time allowed for equilibration, after which pressure and spring extension were determined. Increments of 10 to 20 mg. of water vapor were admitted daily to the sample chamber. When the water vapor rose to about 2.10 cm. mercury corresponding to $p/p_0 = 0.96$ (p_0 for water at 23.6° is 2.185 cm. Hg) (12) the determination of the sorption isotherm was considered complete. The desorption isotherm was subsequently determined by removing daily increments of water vapor with the vacuum pump until the water vapor pressure was reduced to about 0.1 cm. of mercury.

Discussion of Results.—The sorption and desorption isotherms obtained in the quartz spring sorption apparatus were essentially identical and did not exhibit the hysteresis observed in results from humidity chambers. Since desorption data were somewhat more precise than that obtained on initial sorption, it was utilized in this report. The isotherm seen in Fig. 6 (data in Table III) indicated the formation of a dihydrate without evidence of a monohydrate, at an equilibrium water vapor pressure of 1.26 cm. Hg ($p/p_0 = 0.57$).

Equilibrium pressures obtained at other temperatures, shown in Table IV and plotted in Fig. 7 yielded a net ΔH (thermodynamic enthalpy of the reaction) of -29.6 ± 3 Kcal. per mole of procaine for the following reaction

$$\text{Procaine}_{(\text{solid})} + 2H_2O_{(\text{gas})} \rightarrow \text{Procaine}\cdot 2H_2O_{(\text{solid})}$$

The order of the reaction between water vapor and procaine base powder was investigated by

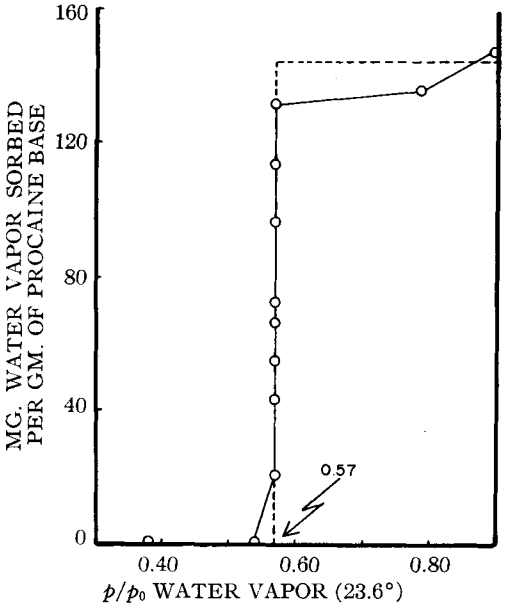

Fig. 6.—This isotherm shows the hydration of procaine base as it occurs in the presence of water vapor alone. The departure from theory at the upper portion of the isotherm may be due to failure to achieve equilibrium occasioned by the decreasing amount of unhydrated procaine base and the accompanying increase in difficulty of water vapor diffusion through the crystals as hydration nears completion. ---, Theory; ——, actual.

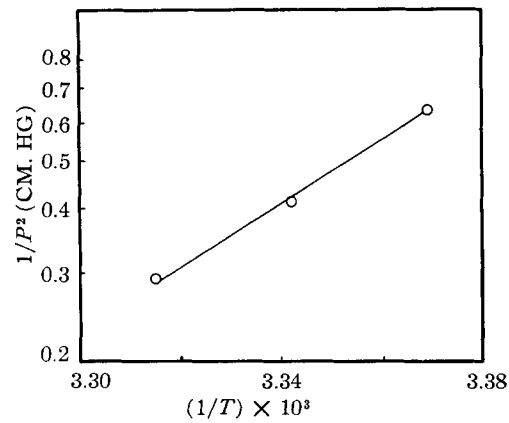

Fig. 7.—A Clausius-Clapeyron plot of temperature *vs.* equilibrium pressure between water vapor, procaine base, and procaine base dihydrate to obtain the energy evolved in this hydration (see Table IV).

studying the rate of hydration. This was accomplished by measuring the weight gain of the sample as a function of time while maintaining a relatively constant water vapor pressure of about 2.0 cm. Hg (corresponding to a p/p_0 of 0.90 to 0.95). The result, seen in Fig. 8, indicated a zero-order reaction. Perhaps the rate limiting factor was the diffusion of water molecules from the surface to the interior of crystals.

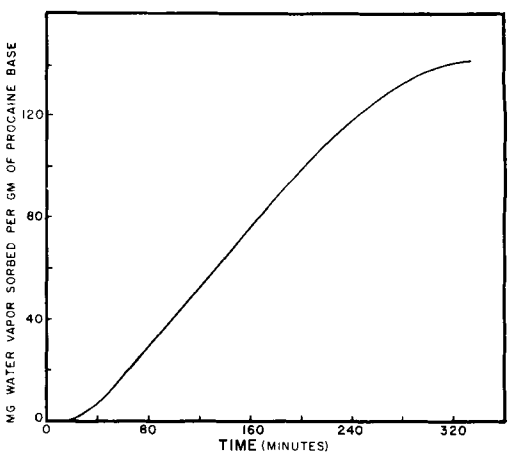

Fig. 8.—The rate of hydration of a sample of procaine base, 100-mesh powder, in water vapor (p/p_0 of 0.90-0.95) at 23.6°. The straight line portion of the curve indicated a zero-order reaction.

TABLE III.—EQUILIBRIUM PRESSURES OF WATER VAPOR WITH PROCAINE BASE OBTAINED IN THE QUARTZ SPRING SORPTION APPARATUS

Mg. of Water Sorbed per Gm. of Procaine Base	Water Vapor Pressure at Equilibrium, cm. Hg	p/p_0 at 23.6° (p_0 = 2.185 cm. Hg)
147	2.04	0.93
136	1.71	0.78
131	1.25	0.57
114	1.24	0.57
96	1.25	0.57
72	1.26	0.58
66	1.26	0.58
55	1.26	0.58
43	1.23	0.57
21	1.24	0.57
0	1.15	0.53
0	0.80	0.37
0	0.48	0.22

TABLE IV.—EQUILIBRIUM PRESSURES OF PROCAINE DIHYDRATE AND WATER VAPOR AT DIFFERENT TEMPERATURES

Temperature, °C.	$1/T \times 10^3$	Pressure, cm. Hg	$1/p^2$
23.6	3.369	1.26	0.630
26.0	3.342	1.56	0.411
28.5	3.315	1.85	0.292

CONCLUSIONS

The hygroscopic nature of several medicinal powders has been studied. The methods employed were designed to yield data suitable for use in the fabrication and study of pharmaceutical systems such as tablets, capsules, granules, and powders. The study of hygroscopic powders in relative humidity chambers yielded isotherms that exhibited hysteresis, indicating that sorption and desorption of water vapor by common

pharmaceutical powders was not a freely reversible process. Procaine base was cited as an example of powder exhibiting an unusual sorption of water vapor which can be accounted for by formation of a crystalline dihydrate.

REFERENCES

(1) Leeson, L. G., and Mattocks, A. M., THIS JOURNAL, **47**, 329(1958).
(2) Babbitt, J. D., *Can. J. Research*, **27F**, 55(1949).
(3) Dacey, J. R., Clunie, J. C., and Thomas, D. G., *Trans. Faraday Soc.*, **54**, 250(1958).
(4) Hoover, S. R., and Mellon, E. F., *J. Am. Chem. Soc.*, **72**, 2562(1950).
(5) Puri, B. R., and Sharma, L. R., *J. Sci. Ind. Research*, **15B**, 178(1956).
(6) National Research Council, "International Critical Tables," Vol. 3, 1st ed., McGraw-Hill Book Co., New York, N. Y., 1928, p. 303.
(7) "Handbook of Chemistry and Physics," 40th ed., Chemical Rubber Publishing Co., Cleveland, Ohio, 1958, p. 2497.
(8) Carr, D. S., and Harris, B. L., *Ind. Eng. Chem.*, **41**, 2014(1949).
(9) Beilstein, F. K., "Beilsteins Handbuch der Organischen Chemie," Bd. 14, 4 Aufl., Springer, Berlin, Germany, 1931, p. 424.
(10) "Merck Index," 6th ed., Merck and Co., Rahway, N. J., 1952, p. 789.
(11) Germann, F. E. E., and Gagos, K. A., *Ind. Eng. Chem., Anal. Ed.*, **15**, 285(1943).
(12) "Handbook of Chemistry and Physics," 40th ed., Chemical Rubber Publishing Co., Cleveland, Ohio, 1958, p. 2326.

Standardization of Fibrinolytic Preparations

Measurement of Proteolytic and Activator Activity of Streptokinase-Activated Human Plasminogen

By WERNER BAUMGARTEN, JOSEPH L. CIMINERA, and SUZANNE M. VAN PELT

Methods for the determination of proteolytic and activator activity of streptokinase-activated human plasminogen are presented. These assay procedures extend previously published methods by the incorporation of a suitable standard and improved experimental design. The experimental bias is minimized and, by test of validity, differences in preparations can be realized.

IN THE LAST few years fibrinolytic agents have been developed which can assist in the clinical dissolution of venous and arterial clots. Several of these agents have become commercially available and, consequently, standardization of these preparations has become of considerable interest.

A fibrinolytic preparation[1] has been made commercially available by our laboratory. This is a streptokinase-activated human plasminogen preparation. The activity of fibrinolysin has been determined by a fibrinolytic assay procedure (1) which measures the total activity of the preparation. It has been established, however, that fibrinolysin possesses two distinct properties which contribute to its enzymatic activity (2–4). Streptokinase reacts with a proactivator present in human plasminogen producing an activator. The resulting activator then converts the proteolytic precursor which is also present in the human plasminogen into the active enzyme.

In the fibrinolytic assay the total activity is measured; however, it is possible to determine the proteolytic and activator activity separately.

Several procedures (5, 7–9) have been described in the literature; however, they are lacking in statistical treatment and omit the use of an appropriate standard. In our laboratory a self-contained slope ratio assay for the caseinolytic and activator activity of fibrinolysin preparations has been developed. A self-contained assay includes a reference standard and provides statistical procedures for testing the validity of the assay and methods for estimating the relative potency and error of the assay.

Over a limited range of concentration, fibrinolysin preparations give a linear response and in the assay, concentrations are chosen which fall on this linear portion of the curve.

Measurement of the proteolytic activity is done by the direct caseinolytic assay in which the degree of hydrolysis of the casein substrate is determined. In the activator assay added plasminogen is first converted into the active proteolytic enzyme by the activator present in fibrinolysin. The amount of activator which is required for the enzymatic conversion of the

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[1] Thrombolysin is the registered trademark of Merck & Co., Inc., for fibrinolysin.