# Water Vapor Adsorption and Desorption Isotherms of Biologically Active Proteins

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Using a protein isolated from soy, a dynamic water adsorption method was developed and the data were compared with those obtained from a static gravimetric procedure. Both methods gave comparable results, showing that Type II isotherms with considerable hysteresis were obtained. However, the dynamic procedure was preferred since it provided data rapidly and used significantly less material. Using the dynamic method, water adsorption isotherms at 25°C were also determined for four biologically active proteins: α-amylase, β-glucuronidase, lipase, and urease. BET (Brunauer, Emmet, and Teller) parameters were calculated and the specific surface areas for the native, biologically active proteins were found to be similar, 238.4 ± 20.2 $m^2/g$. On the other hand, the specific surface area for the denatured soy protein isolate was 144.6 $m^2/g$. Nevertheless, the heat of absorbance for all of the proteins examined was similar, suggesting that they have comparable degrees of hydrophilicity.

**KEY WORDS:** urease; lipase; α-amylase; β-glucuronidase; isolated soy protein; static/dynamic water adsorption and desorption.

## INTRODUCTION

In previous studies, we have demonstrated the importance of moisture to the compactional properties of soy protein (1) and to the physical properties of compacts made from wheat germ lipase (2). Preliminary evaluation indicated that direct measurement of water content by Karl Fischer methodology or gravimetric procedures following heating was destructive and did not provide adequate information. Drying over phosphorus pentoxide removed most moisture from the system but not necessarily the final residues that adhere tenaciously to protein structures. Accordingly, we opted to evaluate water adsorption processes in four biologically active enzyme preparations (lipase, urease, α-amylase, and β-glucuronidase) using static and dynamic methods developed when measuring water sorption behavior of soy protein. Static gravimetric procedures have been widely utilized (3–6) and are carried out by exposing the protein powder to water vapor over a range of equilibrium relative humidities until the change in weight is constant. An alternative is to pass air of different relative humidities over the powder and continuously monitor the weight changes using a sensitive microbalance—a dynamic method. We have evaluated both procedures.

## MATERIALS AND METHODS

The following materials were used as received: soy protein isolate, Ardex-R Archer Daniel Midland, Chicago; α-amylase (from *Bacillus* spp), Preparation A6380, β-glucuronidase (from abalone entrails), Preparation G0258, urease (from wheat germ), Preparation L3001, and calcium nitrate tetrahydrate, Sigma Chemical Company, St. Louis, MO; and lithium chloride USP, calcium chloride dehydrate USP, fine granulated, sodium nitrite crystalline ACS grade, ammonium chloride, ACS grade, ammonium phosphate monobasic, ACS grade, and phosphorus pentoxide ACS grade, Fisher Scientific, Fairlawn, NJ. Water was double glass distilled.

### Static Adsorption Method

Approximately 500 mg protein powder was thinly spread on weighed aluminum dishes, 4.5 cm in diameter, and exposed over phosphorus pentoxide in a desiccator for 1 week. After weighing the dishes they were transferred to a second desiccator (containing saturated aqueous lithium chloride [15% relative humidity (RH) (7)] and again allowed to equilibrate for 1 week before reweighing. This procedure was repeated over various RH environments of 0, 15, 31, 51, 66, 80, and 93% RH and, again, a 0% RH. The procedure outlined here was adopted in order to avoid changing the environment during the weighing period which had to be carried out at ambient conditions. One week was found to be sufficient for equilibrium to be obtained, although Tabidi and Hollenbeck (6) previously concluded that, even after 123 days of exposure, true equilibrium of water adsorption was not achieved at any relative humidity. Nevertheless, most adsorption had taken place after 14 days and at least 95% of the apparent final levels were achieved within 7 days.

### Dynamic Adsorption Method

The dynamic system developed for this purpose is shown in Fig. 1, and is centered on the use of a Cahn Model 2000 vacuum electromicrobalance (Cahn Instruments, Inc., Cerritos, CA). Dry air is mixed with saturated air in different proportions by means of valves on the equipment. The device was equipped with three hygrometers (Cole Parmer Thermohygrometer, Model 3309-60, Cole Parmer Instrument Company, Chicago) and a flow meter (Calcuflow flow meter, Model 36-541-125, Manostat Inc., New York). The hygrometers were individually calibrated on a daily basis against atmospheres of known RH over three saturated salt solutions (15, 51, and 80% RH). The electrobalance was zeroed and calibrated with a 100-mg standard weight (using the strip-chart recorder) under a constant flow rate of 50% RH air at 2300 ml/min in order to correct any buoyant effects by air flow during the experiment. At this point 1–2 mg of the powder sample was spread thinly on the sample pan, and the sample was degassed overnight at 80–190 Pa. The dry air was introduced into the system by allowing the vacuum side arm

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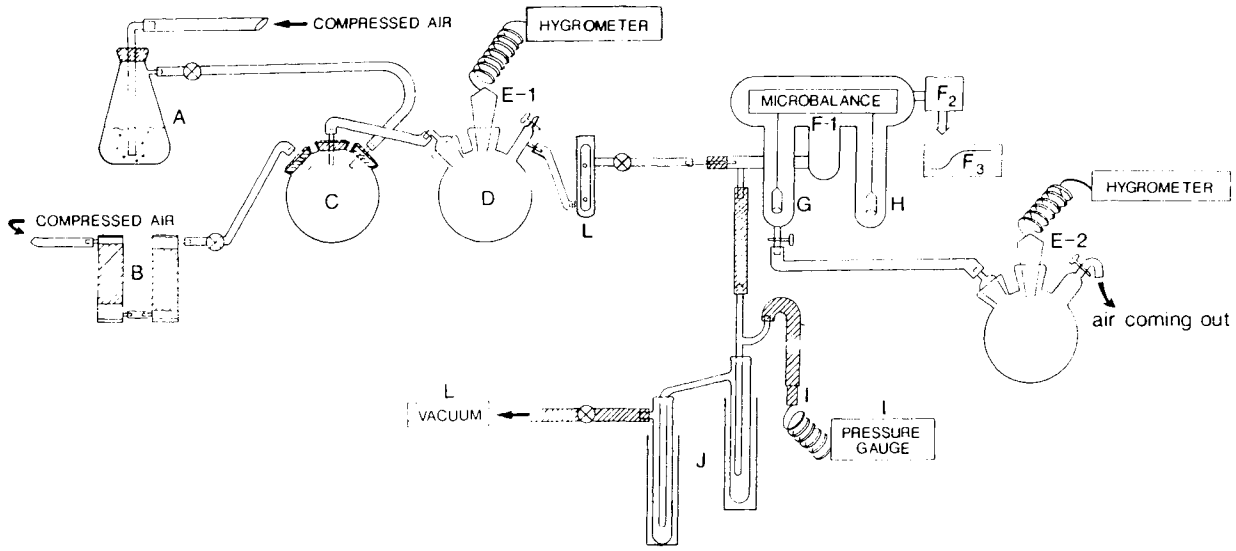


Fig. 1. Dynamic adsorption method. (A) Water reservoir filled with double-distilled water. (B) Drierite, column for drying. (C) Primary mixing tank. (D) Gas reservoir. (E-1, E-2) Hygrometers. (F-1) Electrobalance with a vacuum bottle system. (F-2) Electrobalance control unit.(F-3) Strip-chart recorder. (G) Sample pan. (H) Tare-weight pan. (I) Pressure gauge. (J) Condenser. (L) Vacuum pump.

to leak slowly until the sample chamber had reached 1 atm ($10^5$ Pa). Dry air was then allowed to pass over the sample at a flow rate of 2300 ml/min, and the sample weight was recorded until there was no further change, indicating the "dry" sample weight. The saturated water vapor supply was slowly adjusted to provide a small increment in RH and the weight change was observed until there was no additional change before proceeding to the next incremental change. After allowing the saturated air supply to pass over the sample overnight, the desorption process was initiated by increasing the proportion of dry air introduced into the mixture. All measurements were made at the same flow rate (2300 ml/min) and at ambient room temperature (24 ± 2°C). Three replicate runs were carried out, using new samples for each run.

## RESULTS

Water vapor adsorption and desorption isotherms for isolated soy protein are shown by both dynamic and static methods in Fig. 2. Although the results of the two methods differ, the evident area of hysteresis is broadly similar. The isotherms themselves separate beyond RH 30%, differences becoming more pronounced as the humidity was increased. This suggested that more water was retained by the solid material in the static method. However, there is a hundredfold increase in the time scale between the two methods since the dynamic method took 1–2 hr and the static method took 1 week to arrive at an apparent equilibrium. This longer time frame allows water vapor to diffuse into deeper levels of the solid. During the desorption process the converse situation applies in that it takes longer to diffuse out. Furthermore, as suggested by Okhamafe and York (8), it is unlikely that unbound (free) water exists on the sample surface under the same humidity conditions represented by the dynamic method. It is likely that both mechanisms are operating simultaneously.

Results of the dynamic measurements on the four enzymes are shown in Figs. 3–6. Water sorption isotherm measurements for all of the proteins were broadly similar in form to the Type II isotherm described by Brunauer *et al.* (9,10). This classical investigation made the fundamental assumption that the evaporation/condensation properties of the second and subsequent layers of adsorbed water have the same properties as liquid water and that adsorption occurs on the free surface under isothermal conditions. This is not likely to be the situation with water adsorbing onto an anhydrous protein where, as noted, energetic considerations would tend to rule out isothermal conditions. Nevertheless, it is of interest to note that the Brunauer, Emmett, and Teller [BET (9,10)] approach appears to apply to these proteins, suggest-

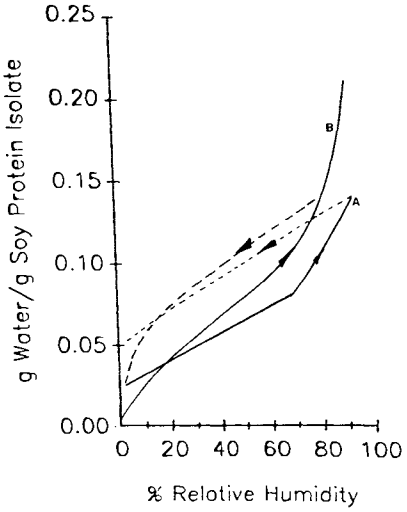


Fig. 2. Water vapor adsorption–desorption isotherms of soy protein isolate from dynamic (A) and static (B) studies. For clarity data points are omitted, arrows indicate direction of adsorption (→) or desorption (←).

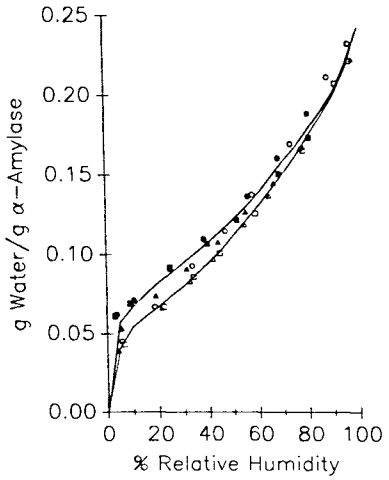


Fig. 3. Water vapor adsorption–desorption isotherms of α-amylase obtained from the dynamic method. Open symbols, adsorption; filled symbols, desorption. Circles, first experiment; triangles, second experiment; squares, third experiment.

ing that, at least beyond the initial adsorption stages, these materials are behaving in the same way as any other adsorbing/desorbing surface. All proteins tested are noncrystalline and appear to be amorphous by scanning electron microscopy (11). The fit of the BET model may be readily demonstrated by application of the BET equation (9,10):

$$P/[V(P_o - P)] = 1/(V_mC) + [(C - 1)/(V_mC)]\,(P/P_o) \quad (1)$$

where $V$ is the volume of gas adsorbed at pressure $P$, $P_o$ is the saturated vapor pressure at constant temperature, $C$ is the BET constant [approximately equal to $\epsilon^{(E_1 - E_L/RT)}$, $E_1$ being the heat of adsorption of the first layer, $E_L$ the heat of liquefaction, $R$ the gas content, and $T$ the absolute temperature], and $V_m$ is the volume of gas required to form a complete unimolecular adsorbed layer.

$P/P_o \times 100$ is equivalent to the RH and Eq. (1) converts to

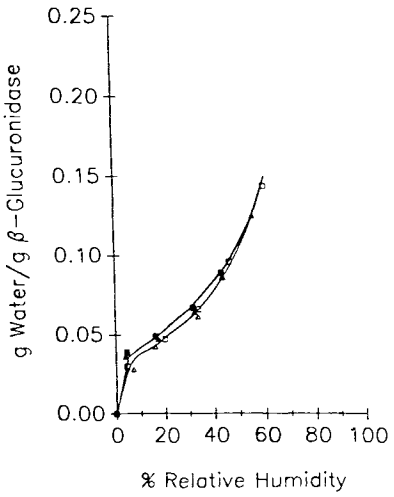


Fig. 4. Water vapor adsorption–desorption isotherms of β-glucuronidase obtained from the dynamic method. For key, see the legend to Fig. 3.

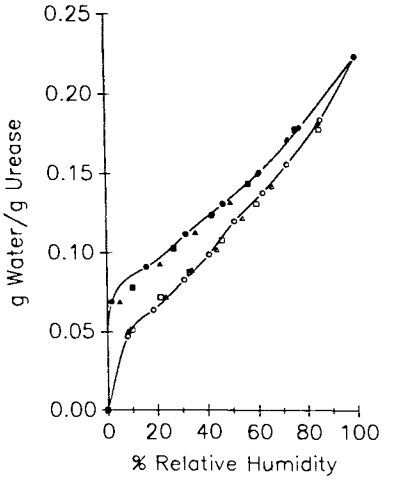


Fig. 5. Water vapor adsorption–desorption isotherms of urease obtained from the dynamic method. For key, see the legend to Fig. 3.

$$RH/[W(100 - RH)] = 1/(W_mC)\,[(C - 1)/(100\,W_mC)]\,RH \quad (2)$$

However, gravimetrically, instead of the volume $V$ of water adsorbed, the total weight of water adsorbed, $W$, is measured. A plot of $RH/[W(100 - RH)]$ vs RH yields a straight line of slope $(C - 1)/(100\,W_mC)$ and an intercept of $1/W_mC$, where $W_m$ is the weight of adsorbate forming a monolayer. Thus, $W_m$ and $C$ can be calculated. The former provides a measure of the specific surface area of the sample if the cross-sectional area of the adsorbing water molecule is known ($=1.25 \times 10^{-19}$ m$^2$).

The specific area, m$^2$/g, $= (W_m/18)\,(1.25 \times 10^{-19} \times N)$, where $N$ = the Avogadro number ($=6.022 \times 10^{23}$ mol$^{-1}$). Summarized gravimetric results for soy protein isolate are shown in Fig. 7. Above approximately RH 66% the deviation from the BET equation becomes progressively more pronounced by both methods. This is similar to data reported by Brausse *et al.* (4), who found a deviation above 50% RH for lyophilized hemoglobin. This limitation has been reported for other materials (12,13). In these situations, thought to

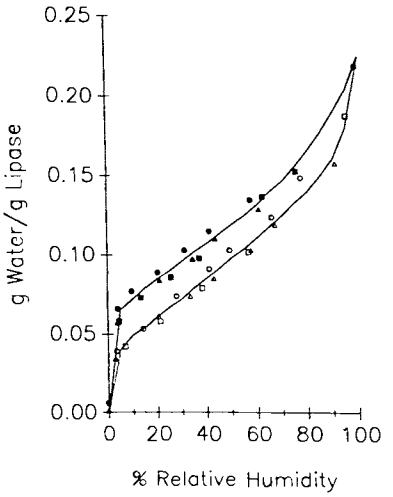


Fig. 6. Water vapor adsorption–desorption isotherms of lipase obtained from the dynamic method. For key, see the legend to Fig. 3.

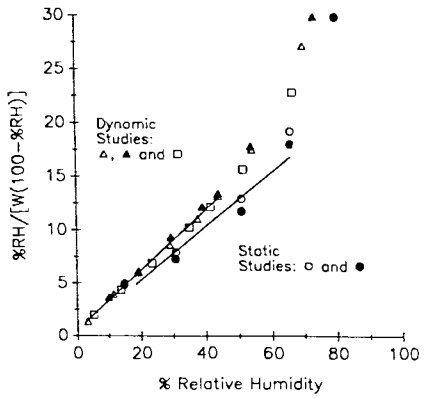


Fig. 7. BET plots of soy protein isolate powder from both static and dynamic studies. The lines indicate the predicted adsorption based on the BET equation and data points illustrate deviation from the prediction above an RH of 50%.

arise because of a limited, finite thickness of the adsorbed layer, a simplified version of Eq. (1) is applied (9).

$$W = \frac{W_m C_x [1 - (b - 1)x^b + bx^{(b+1)}]}{(1 - x)[1 + (C - 1)x - Cx^{(b+1)}]} \quad (3)$$

with $W$, $W_m$, and $C$ as before, $x = P/P_o$, and $b$ = maximum number of layers that build up. By trial and error an optimum value of $b = 11$ was determined and the fit of the data is shown in Fig. 8 for the static method. Determination from Eq. (1) of the relative humidity at which a monolayer forms ($RH_m$) is as follows:

$$100W_m C \cdot RH_m = W_m (100 - RH_m)[100 + (C - 1) RH_m]$$

$$W_m (C - 1) RH_m^2 = 0$$
$$+ 200W_m RH_m - 10^4 W_m$$

$$\text{or} \quad (C - 1) RH_m^2 = 0$$
$$+ 200 RH_m - 10^4$$

$$\text{for } RH_m > 0 = \frac{100C^{0.5} - 100}{(C - 1)}$$

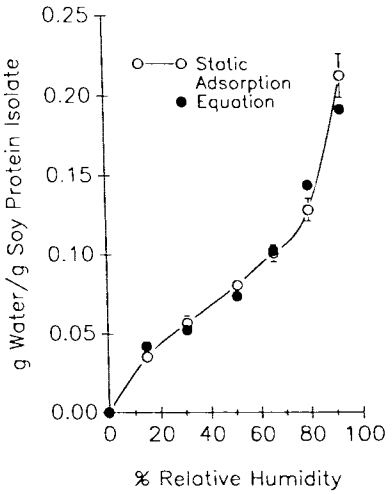


Fig. 8. Comparison of data points obtained by the static adsorption method and the theoretical values predicted by Eq. (3) for soy protein isolate powder. Static data points (○); predictions (●).

The various BET parameters for the five proteins evaluated by the dynamic method are provided in Table I.

## DISCUSSION

The classical BET equation has been extensively criticized for its assumption of energetically homogeneous surfaces, whereas it may be assumed, especially in the case of proteins, that some sites have higher energy levels than others. D'Arcy and Watt (12) derived a general equation that was applicable to a wider range of water vapor adsorption systems. Three terms were proposed to account for the formation of a so-called Langmuir monolayer on highly reactive sites, monolayers on weakly reactive sites, and multilayers on primary adsorbed sites. The BET assumption that adsorbing molecules in the second and subsequent layer were equal was also questioned. Guggenhein, Anderson, and de Boer (14–16), (GAB), attempted to take the latter consideration into account, and Zographi (17) successfully applied the GAB equation to microcrystalline cellulose.

However, proteins examined in the present study did not necessarily require this qualification; the data in Table I indicate a reasonable agreement among the proteins. The heats of adsorption, $\Delta E$, are similar (6–10 kJ/mol), and the relative humidity producing a monolayer ranges from 11 to 21% RH. Thus, they all have broadly similar degrees of hydrophilicity at their primary surfaces. Interestingly, the native biologically active enzymes all have similar surface areas as determined by water adsorption (mean, 238.4 ± 20.2 $m^2/g$), but the specific surface area of the treated and processed soy protein was slightly less than half this area (144.6 $m^2/g$). Presumably the structure of the native soy protein becomes collapsed and degraded during the extraction from the soy bean.

The hysteresis observed for all the proteins has also been observed for other materials that are morphologically porous or nonporous (14), although the details of the mechanism underlying the hysteresis in the case of proteins remains less than certain. For lyophilized hemoglobin (4) it was suggested that the observed hysteresis was due to hy-

Table I. Summarized BET Parameters of Five Proteins by the Dynamic Sorption Method ($n$ = 3)

| Protein | SSA[a] | $W_m$[b] | $C$[c] | $\Delta E$[d] | $RH_m$[e] |
|---|---|---|---|---|---|
| Isolated soy | 144.6 | 0.0346 | 55.3 | 9.94 | 11.9 |
| | (4.0)[f] | (0.0010) | (4.2) | (0.22) | |
| α-Amylase | 252.6 | 0.0604 | 38.2 | 8.98 | 13.9 |
| | (5.7) | (0.0013) | (10.7) | (0.64) | |
| β-Glucuronidase | 228.2 | 0.0546 | 14.2 | 6.52 | 21.0 |
| | (20.9) | (0.0045) | (1.9) | (0.33) | |
| Lipase | 215.0 | 0.0514 | 61.0 | 10.09 | 14.7 |
| | (9.9) | (0.0024) | (22.8) | (0.87) | |
| Urease | 257.4 | 0.0609 | 33.8 | 8.63 | 11.4 |
| | (6.1) | (0.0010) | (7.3) | (0.52) | |

[a] Mean specific surface area, $m^2/g$.
[b] Weight of water adsorbed for monolayer coverage, g/g.
[c] BET constant.
[d] $\Delta E = RT \ln C$, kJ/mol.
[e] Relative humidity for monolayer coverage (at 25°C), %.
[f] Standard deviation in parentheses.

dration of a quasi-denatured protein molecule to the native state, with a delay in the time frame when water was removed. Arguments about the state of "dry" proteins continue to the present day; Bello (18) recently suggested that minor conformational differences exist between "dry" and completely hydrated proteins that are detected as a hysteresis.

From the present investigation we conclude that the dynamic method of measurement has a number of attractions. It uses only a few milligrams of material, and a complete adsorption–desorption curve can be generated in 2 days, as opposed to the several weeks required for the static method. Neither method is likely to provide an absolute indication of the true water surface area since it is unlikely that the final traces of water can be removed from a protein molecule without causing a collapse or degradation of the complex three-dimensional "native" structure.

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