214. *The Adsorption of Water Vapour on Insulin and Plasma Albumin.*

By R. A. Robinson.

(1) By slight modifications of the isopiestic method of determining the vapour pressures of aqueous solutions, adsorption isotherms of water vapour on proteins can be determined in a short time.
(2) The adsorption of water vapour on insulin and on a plasma albumin has been measured.
(3) The Brunauer–Emmett–Teller adsorption equation can be used to express the results.
(4) The first, firmly bound, adsorbed layer corresponds to one molecule of water to each polar group in the protein molecule.

In a recent paper Bull (*J. Amer. Chem. Soc.*, 1944, **66**, 1499) described the adsorption isotherms of water vapour on a number of proteins. Although his technique required twelve or more days for equilibrium to be reached between the vapour and the solid phase, the value of his results suggested that any method of accelerating the attainment of equilibrium would be of considerable help in expanding our knowledge in this field. Using two proteins, crystalline insulin and a crystalline bovine plasma albumin as adsorbents, it has now been found, by means of Robinson and Sinclair's isopiestic method (*ibid.*, 1934, **56**, 1830), that equilibrium could be reached in one or, at the most, two days.

Experimental.

Samples of the proteins, each about 1 g., were contained in flat-bottomed silver dishes along with another silver dish which contained a saturated salt solution to determine the humidity or water activity, a_w, and a platinum dish containing standardised sulphuric acid. From the gain or loss in weight of the acid the concentration at equilibrium could be calculated and hence the water activity from the tables of Shankman and Gordon (*ibid.*, 1939, **61**, 2370). The dishes rested on a thick copper block in a desiccator which could be evacuated and rocked in a thermostat. The heavy mass of metal served to smooth out small variations in the temperature of the thermostat and to facilitate the transfer of heat from one dish to another during the distillation. Except at the highest humidities, equilibrium was reached in 24

hours. From the change in weight of the protein sample its water content in equilibrium with sulphuric acid of known a_w could be calculated provided its original water content was known (see below). The insulin was obtained from the Fine Chemical Department of Messrs. Boots Pure Drug Co., Ltd.; it was listed as Batch Z11356, with a moisture content of 9·66% and ash 1·66%, identical with the specimen used by Chibnall, Rees, and Williams (*Biochem. J.*, 1943, **37**, 354). When received, however, its water content had risen to 10·85%. The bovine plasma albumin was a product of Armour and Co., Lot 46, with a moisture content of 4·23% and ash 0·15%; an analysis on a similar preparation is reported by Brand, Kassell, and Saidel (*J. Clin. Invest.*, 1944, **23**, 437). On receipt, however, its moisture content was 16·81%.

To determine the moisture content of the proteins they were rocked in the desiccator along with phosphoric oxide, the loss in weight being determined each day and the oxide renewed. This gave the moisture content on drying in a vacuum over phosphoric oxide at 25°. The following results were obtained :

Time (days)	1	2	3	4	5
Loss, % : insulin	10·24	10·46	10·55	10·82	10·85
,, albumin	14·35	14·67	16·44	16·81	16·81

The final weight was taken as the " dry weight " of the protein and all subsequent water contents will be expressed as g. of water per 100 g. of this dry protein.

To determine the time required to attain equilibrium, these dried samples of insulin and albumin were equilibrated with saturated calcium chloride ($a_w = 0·2963$), the approach to equilibrium being measured on successive days. The samples were then allowed to take up large quantities of water by distillation from a dish containing water, and then brought back to equilibrium with saturated calcium chloride solution. In this way the same point was approached by adsorption and desorption, with the results shown in Table I. This experiment shows that equilibrium is reached very rapidly by adsorption but slowly by desorption, and that hysteresis occurs.

TABLE I.

	G. of H_2O per 100 g. of protein.			
	Insulin.		Albumin.	
Time (days).	Adsorption.	Desorption.	Adsorption.	Desorption.
0	0	21·45	0	25·42
1	6·63	8·92	8·11	10·06
2	6·57	8·92	8·10	10·00
3	6·58	8·87	8·17	9·87
4	6·58	8·75	8·16	9·80

To obtain the adsorption isotherms, the samples were again dried over phosphoric oxide and allowed to adsorb water vapour in small increments. The results are given in Table II. After these measurements, the proteins were allowed to take up water vapour at $a_w = 1$ and this was then slowly

TABLE II.

Adsorption isotherms of insulin and albumin at 25°.

Insulin. G. H_2O/100 g. protein :						Albumin. G. H_2O/100 g. protein :					
a_w.	Obs.	Calc.	a_w.	Obs.	Calc.	a_w.	Obs.	Calc.	a_w.	Obs.	Calc.
0·0310	1·88	1·38	0·3542	7·52	7·50	0·0310	2·15	1·85	0·3219	8·16	8·01
0·0960	3·31	3·20	0·4252	8·73	8·74	0·0590	2·89	2·94	0·3765	8·78	8·96
0·1115	3·65	3·53	0·4949	10·08	10·09	0·0705	3·42	3·30	0·4236	9·79	9·86
0·1695	4·47	4·59	0·5115	10·29	10·44	0·0946	4·04	3·95	0·4921	10·93	11·28
0·1766	4·70	4·71	0·6460	13·29	13·56	0·1147	4·45	4·41	0·5787	13·08	13·33
0·2213	5·38	5·42	0·6712	13·74	14·22	0·1695	5·36	5·48	0·6801	16·08	16·06
0·2263	5·53	5·50	0·7097	14·91	15·23	0·1766	5·47	5·60	0·7528	18·62	18·15
0·2595	6·07	6·01	0·7323	15·77	15·82	0·2627	7·05	7·03	0·8371	23·17	20·65
0·2627	6·05	6·06	0·8300	19·60	18·47	0·2963	7·67	7·59	0·8727	25·78	21·60
0·2963	6·58	6·58									

desorbed, with the results given in Table III. Hysteresis, as measured by the difference in the adsorption and the desorption curves, occurs at all water activities except the very lowest and, presumably, at $a_w = 1$. Over most of the a_w range it amounts to a difference of about 2·3 g. of water per 100 g. of either protein.

In addition, the author has determined a number of isotherms, starting, not with dried proteins, but with material dehydrated to different values of a_w. Such adsorption isotherms are always higher than those of the thoroughly dried material. As the proteins are prepared by partial dehydration, the isotherms naturally start at a point on the higher desorption isotherm and with increasing a_w tend towards the lower adsorption isotherm of the fully dried protein. This trend toward the lower curve is more marked, the lower the initial a_w. These isotherms would seem to have little significance; it is the adsorption isotherm of the properly dried protein which is important, and, perhaps, the desorption isotherm, although the latter must suffer from some inaccuracy because of the time required to attain equilibrium. Indeed, it is possible that no desorption process does correspond to true equilibrium.

TABLE III.

Desorption isotherms of insulin and albumin at 25°

Insulin. (G. of water per 100 g. of protein.)						Albumin. (G. of water per 100 g. of protein.)					
a_w.	Loss.	a_w.	Loss.	a_w.	Loss.	a_w.	Loss.	a_w.	Loss.	a_w.	Loss.
0·0265	2·35	0·1909	6·58	0·5020	12·37	0·0185	2·44	0·2132	7·75	0·5020	13·60
0·0300	2·50	0·2728	8·06	0·6851	16·23	0·0644	4·09	0·2728	9·15	0·6851	18·27
0·0470	3·21	0·2963	8·75	0·7402	17·95	0·0948	4·64	0·2963	9·80	0·7402	20·37
0·0947	4·48	0·3526	9·85	0·8417	21·40	0·1461	6·14	0·3526	10·80	0·8417	24·85

DISCUSSION.

According to the adsorption theory of Brunauer, Emmett, and Teller (*J. Amer. Chem. Soc.*, 1938, **60**, 309), if an infinite number of layers are available for filling as the water activity increases, the amount of water adsorbed (x) is related to the water activity (a_w) by

$$a_w/(1 - a_w)x = 1/X_mC + (C - 1)a_w/X_mC$$

where X_m is the number of g. of water (per 100 g. of protein) in the first unimolecular layer and $C \simeq \exp.(E_1 - E_L)/\boldsymbol{R}T$, $(E_1 - E_L)$ being the difference of the energy of adsorption in the first layer and the latent heat of evaporation. If, however, adsorption is limited to n layers the formula becomes

$$a_w/(1 - a_w)X = (1 - a_w + Ca_w - Ca_w^{n+1})/[1 - (n + 1)a_w^n + na_w^{n+1}]X_mC$$

which reduces to the simpler equation for small values of a_w.

A plot of $a_w/(1 - a_w)x$ against a_w for both proteins is linear up to $a_w = 0{\cdot}3$, so the constants X_m and C can be evaluated. By trial, a value of n is then found which fits the adsorption isotherm at higher values of a_w. By the method of least squares, the data for insulin gave $X_m = 5{\cdot}81$ g. of water per 100 g. of protein, $C = 9{\cdot}35$, and $(E_1 - E_L) = 1320$ cals./mol. For the albumin, $X_m = 6{\cdot}38$, $C = 12{\cdot}18$, $(E_1 - E_L) = 1480$. In each case $n = 7$, although a value of 6 would fit almost as well. The values of x calculated with the aid of these constants are given in Table II.

The desorption isotherms can be fitted to Brunauer, Emmett, and Teller's equation by the values $X_m = 6{\cdot}88$, $C = 16{\cdot}25$ for insulin, and $X_m = 7{\cdot}75$, $C = 14{\cdot}67$ for the albumin.

The constants for the adsorption isotherm may now be compared with the number of polar side-chain groups along the lines suggested by Pauling (*ibid.*, 1945, **67**, 555). If we assume from Chibnall's data (*J. Intern. Soc. Leather Trades Chem.*, 1946, **30**, 1) that a submolecular unit of insulin of weight 12,000 contains the following polar constituents: arginine, 2; histidine, 4; lysine, 2; glutamic acid, 15; aspartic acid, 5; tyrosine, 9; serine, 6; threonine, 2; proline, 3, and that the twelve amide nitrogen atoms are attached to the carboxyls of glutamic and aspartic acid, we are left with 36 polar groups. If each of these can take up one water molecule, then the amount of water in the unimolecular layer should be 5·4 g. per 100 g. of protein (obs. 5·8). For the bovine albumin Branch, Kassell, and Saidel's analysis (*loc. cit.*) corresponds to 6·6 g. per 100 g. of protein (obs. 6·4).

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