[CONTRIBUTION FROM THE EASTERN REGIONAL RESEARCH LABORATORY[1]]

Water Absorption of Proteins. II. Lack of Dependence of Hysteresis in Casein on Free Amino Groups[2]

BY EDWARD F. MELLON, ALFRED H. KORN AND SAM R. HOOVER

Introduction

A series of benzoylated casein samples containing different amounts of free amino groups was used previously[3] to show that the amino group is responsible for the absorption of about one quarter of the total amount of water absorbed by casein, throughout the entire range of relative humidities. Furthermore, it appeared that the water absorbed by the amino group could be subdivided into three distinct portions. Since the amino group accounted for such a large proportion of the total water absorbed, we thought it would be interesting to know whether any of the water absorbed on the amino group is involved in the hysteresis phenomena. Therefore, the same series of benzoylated caseins was used to study the desorption phenomena of casein.

Experimental

The preparation of the benzoylated caseins, their analyses, the manipulations necessary to obtain a reproducible dry weight, and a detailed description of the apparatus and method for measuring the absorption or desorption over saturated salt solutions are given in the earlier paper.[3] The desorption experiments were made only at 30.0 ± 0.1°. The samples were thoroughly dried at 70° and then equilibrated at one of the higher humidities (51, 75, 84 or 93%). The water absorbed at these higher humidities was the same as that absorbed in the previous absorption experiments, where the absorption proceeded gradually from the lower to the higher humidities. The samples were then put into an atmosphere of the next lower relative humidity. It took the desorption samples about eighteen days to reach equilibrium—three times as long as the absorption samples—and there was a greater fluctuation in the equilibrium values obtained for different specimens of the same sample run at different times. When equilibrium was achieved at one humidity, the samples were moved to the next lower humidity until the value at 6% relative humidity was obtained. Then the samples were dried to check the starting weight.

Results and Discussion

Four desorption isotherms were determined for each of the benzoylated caseins. Each began at a different point on the absorption isotherm. From two to four determinations, made at different times, were averaged to determine each point on each isotherm. Figure 1 shows the four desorption isotherms of sample 1 only (purified casein) as compared with the absorption isotherm. The curves for the benzoylated samples are similar. All these curves show clearly that there is a marked hysteresis in the water-sorption phenomenon. It is also apparent that the total water content of the sample approaches a characteristic or limiting desorption curve regardless of the initial water content. Sets of isotherms similar to these have been obtained for wool by Speakman and Cooper[4] and for titania gels by Rao.[5]

Because the desorption isotherms from the higher humidities become identical at lower humidities, we can discuss the main desorption characteristics by discussing the upper limiting curve for each material. The data which fall on this upper curve for each sample at each humidity have been averaged, and the average value for each is reported in Table I. The standard deviation from these average values ranges from 0.002 to 0.004 g./g. of total nitrogen. The average values for the water content on absorption and the hysteresis values are also included in Table I. The water contents are reported on the basis of a gram of total nitrogen because this value relates the water content to the protein content and frees the calculations from the effects of the different weights of the benzoyl groups in the different samples. We have assumed that the benzoyl group does not absorb water.

TABLE I

WATER CONTENT OF BENZOYL CASEINS

Sample	NH_2-N, %[a]	Curve[b]	Water content[c] at relative humidity, %						
			5.9	11.8	31.4	50.9	75.1	83.6	93.3
1	0.93	A	159	227	465	660	981	1149	1441
		D	233	310	584	786	1064	1194	..
		H	74	83	119	126	83	45	
2	0.58	A	140	204	423	603	892	1040	1288
		D	218	290	540	729	989	1106	
		H	78	86	117	126	97	66	
3	0.36	A	126	186	394	559	841	978	1193
		D	204	268	509	690	938	1041	
		H	78	82	115	131	97	63	
4	0.26	A	124	183	386	547	820	943	1146
		D	193	257	490	674	912	1007	
		H	69	74	104	127	92	64	

[a] Moisture-free basis. [b] A = absorption, D = desorption, H = hysteresis. [c] g. H_2O/g. N × 1000.

The absolute magnitude of the hysteresis reaches an observed maximum of about 2.0 g. of water per 100 g. of protein at 51% R.H. This value is relatively large and wholly reproducible. The rapid approach of the 84% R.H. desorption isotherm to that of the 93% R.H. curve argues that the desorption curve would not be displaced

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(1) One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

(2) Presented before the Division of Colloid Chemistry, 112th Meeting of the American Chemical Society, New York City, September, 1947.

(3) Mellon, Korn and Hoover, THIS JOURNAL, **69**, 827 (1947).

(4) Speakman and Cooper, *J. Textile Inst.*, **27**, 183T (1936).

(5) Rao, *J. Phys. Chem.*, **45**, 506 (1941).

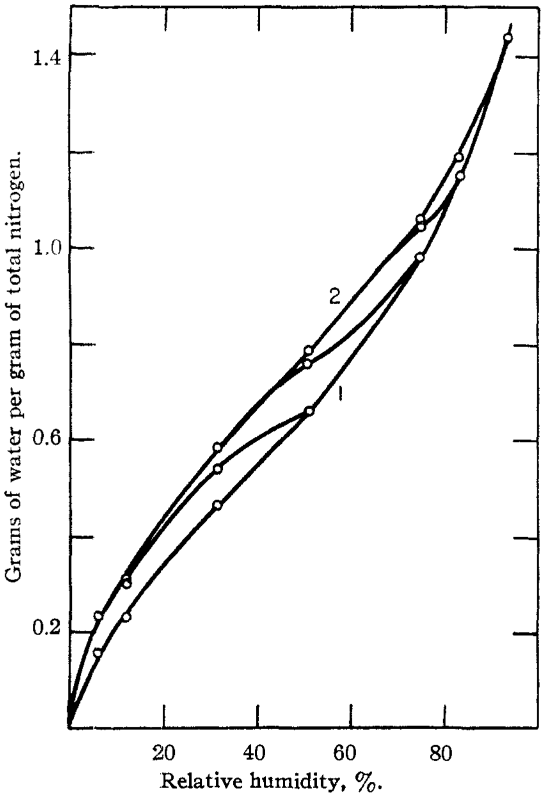

Fig. 1.—Absorption and desorption isotherms for casein: (1) absorption, (2) desorption.

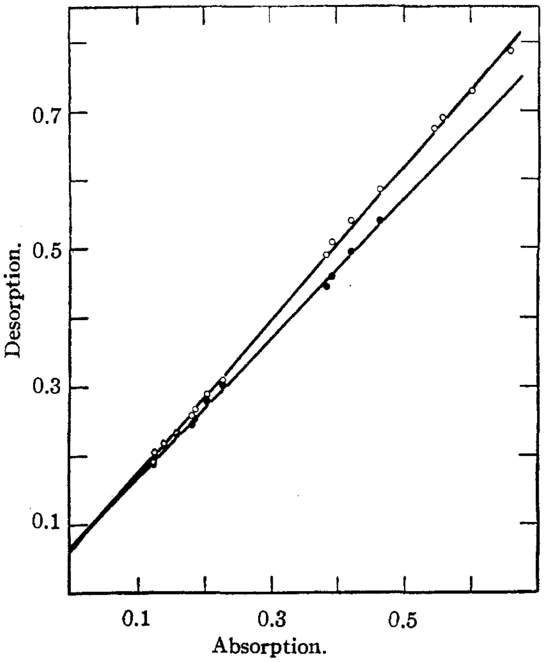

Fig. 2.—Relationship between absorption and desorption water contents: ○, limiting desorption values; ●, desorption values from 51% R.H.

appreciably if desorption data from 100% R.H. were available. In our experience, reliable sorption data in the range 95–100% R.H. were difficult to obtain. The relative magnitude of the hysteresis is a maximum at the lowest humidity studied, where the hysteresis value is one half the amount absorbed. At intermediate humidities, the samples on desorption reach the same water content at relative humidities about 10–12% lower than on absorption. Speakman and Cooper[4] reported a comparable displacement of about 18% R.H. for wool.

The hysteresis values for the four samples are of similar magnitude, and, except for sample 1 at high humidities and sample 4 at low humidities, the variation is well within the experimental error (Table I), indicating that the amount of hysteresis is independent of the amino content of the sample and, therefore, cannot be due to the presence of the amino groups. This is in marked contrast to the high percentage of the water content held by the amino group both on absorption and desorption.

The nature of the hysteresis can be shown more clearly if the limiting desorption data for all four samples at the lower humidities are plotted (Fig. 2) against the absorption data. The data for the limiting curve of all four samples fall on a straight line with a slope of 1.12 and an extrapolated intercept of 0.059 (g. H_2O/g. N). This intercept is about half the magnitude of the maximum hysteresis obtained in these experiments. This would indicate that the water involved in the hysteresis consists of two types—a constant amount and a portion proportional to the amount absorbed.

The desorption data from 51% R.H., which do not approach the upper curve in Fig. 1 as rapidly as the higher humidity data, now become of particular importance. When these desorption values are plotted against the absorption values (Fig. 2), they fall on a straight line with a slope of 1.0 and an extrapolated intercept of 0.067 g. H_2O/g. N. The difference between these two slopes is statistically significant.

The intercept values appear to be associated with a type of water which is bound firmly at relatively low humidities. Its quantity is independent of the humidity from which the desorption is begun. The slope of 1.0 shows that it is the only type of water involved in the hysteresis, if desorptions are started from below about 60% relative humidity. This type of hysteresis has not been treated in any of the previous literature on the phenomenon.

The hysteresis indicated by the difference of the slope (Fig. 2) from 1.0 is associated with water absorbed above the upper break in the absorption curve, but it persists at the lower humidities, where it is proportional to the amount absorbed. Therefore, it might be attributed either to the capillary phenomena of Zsigmondy[6] and McBain[7] or to the swelling of the gel according to Urqu-

(6) Zsigmondy, *Z. anorg. Chem.*, 71, 356 (1910).

(7) McBain, THIS JOURNAL, 57, 699 (1935).

hart.[8] Smith[9] has recently proposed an extension of Urquhart's treatment, and applied it to cellulose, nylon and wool. However, our absorption and desorption data do not give the linear extrapolation characteristic of the approximate method of Smith. The excellent data of Wiegerink[10] on the sorption of wool give a curve of similar shape to that obtained from our data when plotted according to Smith; and it is quite possible that the protein gels require a more complicated swelling function than the simple weight function used by Smith. Moreover, several observations have been made which seem to indicate that the structure of casein is fairly open and most of the groups should be freely available to water vapor without swelling. The first of these is that even though the large benzoyl group has been introduced to modify the amino group there is apparently little disruption of the remainder of the molecule, as shown by the strict linearity of the absorption *versus* amino-content plots of the earlier paper.[3] This situation does not prevail in the cellulose–cellulose acetate system, where opening of the structure by intermediate degrees of acetylation is clearly demonstrable.[11] Secondly the lack of hysteresis in the absorption of the amino group indicates that no new amino groups are made available by swelling.

Summary

Desorption isotherms have been obtained for a number of benzoylated casein samples with various amounts of free amino groups.

A definite hysteresis in the sorption phenomena of about one-half the absorption value at 6% R.H. and about one-fifth at 50% R.H. was observed.

The hysteresis was independent of the content of free amino groups in the samples, although the amino group is responsible for one-fourth the water absorption in casein.

The hysteresis appears to be of two types: a constant hysteresis independent of the humidity from which desorption was started, and a hysteresis proportional to the amount absorbed and due only to absorption above the upper break in the absorption curve. This appears to be the first demonstration of the two-fold nature of the hysteresis phenomena.

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[8] Urquhart, *J. Textile Inst.*, **20**, 125T (1929).

[9] Smith, THIS JOURNAL, **69**, 646 (1947).

[10] Wiegerink, *Textile Research*, **10**, 357 (1940).

[11] Hoover and Mellon, *Textile Research J.*, **17**, 714 (1947).

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Aminomethylation of Thiophene. I. The Reaction of Thiophene with Formaldehyde and Ammonium Salts

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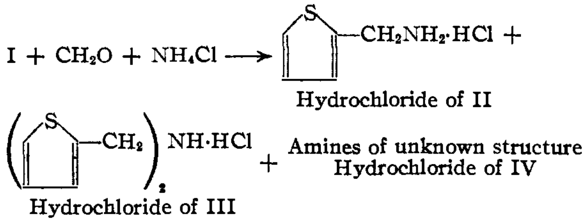
Recently the authors reported that thiophene possessed alpha hydrogens of sufficient reactivity to undergo a type of Mannich reaction.[2] At the same time Holdren and Hixon reported that the Mannich reaction had been extended to 2-methylfuran.[3] They also reported that only resinous products were obtained from the use of ammonium chloride with formaldehyde. Bachman and Heisey reported that pyrroles, azoles and their benzalogs which contain the —NH— group in the heterocyclic ring will undergo the Mannich reaction.[4]

The reaction of thiophene, I, with ammonium chloride and formaldehyde has been investigated and found to give a variety of products. Besides the formation of complex amines of unknown structure, IV, 2-thenylamine (2-aminomethylthiophene), II, and di-(2-thenyl)-amine, III, have been isolated.



The yields of II and III leave much to be desired and many unsuccessful attempts were made to improve the yields over those previously reported.[2] Lower yields were obtained when the reaction quantities were increased five-fold. Yields obtained from various molar ratios of reactants are listed in Table I.

TABLE I

EFFECT OF MOLAR CONCENTRATION ON YIELDS OF AMINES II, III AND IV

Moles of reactants				Weight % of total product		
C_4H_4S	CH_2O (Aqueous)	NH_4Cl	Mole ratio	II	III	IV
5	10	5	1:2:1	17.6	5.3	77
2	2	2	1:1:1	15.6	3.1	80
4	2	2	2:1:1	17.7	20.3	62
5	4	4	1.25:1:1	22.2		77
1	1	3	1:1:3	36.8	15.2	48

Hexamethylenetetramine in the presence of concentrated hydrochloric acid was found to react

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[1] Present address: Owens-Illinois Glass Co., Glassboro, N. J.

[2] Hartough, *et al.*, THIS JOURNAL, **68**, 1389 (1946).

[3] Holdren and Hixon, *ibid.*, **68**, 1198 (1946).

[4] Bachman and Heisey, *ibid.*, **68**, 2496 (1946).