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STUDIES ON BOUND WATER IN FISH MEAT MUSCLE (II)

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3. On the molecular theoretical Bound Water in fish meat protein and fish meat by the vapour tension method.

As stated in the previous paper, Sponsler, Bath and Ellis(3d) have discussed the molecular constitution of gelatine-gel on the basis of the relation between analytical results by X-ray diffraction of gelatine and the analytical results of constituent amino acids of gelatine. They have concluded that the so-called Bound Water in the molecule of water binds with hydrogen centers which are capable of forming hydrogen bonds in gelatine molecule, and the amount of the Bound Water depends in chemical equivalency on the number of molecules of gelatine.

Lloyd,(4b, c) Pauling(3b), Buswell(3c) and Higashi et al.(1) have independently been establishing the definition of the so-called molecular theoretical Bound Water on the basis of the hydrogen bond formation as Sponsler et al., stated.

Recently as to the adsorption of gases by catalysers, in proportion to the active engagement in analytical study of the adsorption isotherm by the theory of multi-molecular layers adsorption which was offered by Brunauer et al.(32a), this theory has been applied to the study of the adsorbed water in gelatine and other various protein.(31)(42c, d)

One of the typical investigators, Bull(31) has said that the surface of the solid molecule of protein consists of hydrophilic planes, and that the molecules of water are adsorbed among the planes.

The present author has analyzed the thermal change in the water content "g" — water-activity "a" curve obtained by vapour tension method by using gelatine, fish meat protein and raw fish meat and has analyzed the same curve by the equation of B. E. T. of Brunauer et al. in order to learn whether the molecular theoretical Bound Water of protein is the molecule of water combined with the molecule of protein through the hydrogen bond formation in a definite combining potential.

These experiments have been carried out at the same time with those described in Experiment II, I(3 a) of the previous paper, so there are few overlapping results in regard to the gelatine and raw fish meat; the author has avoided those overlapping results.

(1) Experimental Methods.

(I) Samples.

(A) Gelatine.

The samples of gelatine in these experiments were the same as in Experiment III, 1, of the previous paper, gelatine No. 2 and No. 3. The sample of No. 2 gelatine was purchased on the market (water content 17.95%, ash 0.69%) and the sample of No. 3 was some purified by the Second Faculty of Engineering of Tokyo University (water content 18.66%, ash 0.43%).

In order to clarify the relation between salts and Bound Water, the author has added NaCl (Japanese Pharmacopeia) in proportion of 0.3 and 25% to the dried matter of sample of gelatine No. 2. The author has used sample of gelatine No. 2 to which cobaltous chloride in the proportion of 27% was added for the dried matter of the gelatine according to Hatschek's method.

To 1～3 gm of these samples of gelatine was added distilled water in a definite volume; they were let sufficiently swell in room temperature (18°～20°C), and then dissolved by heat on the water bath. After cooling, the solidified samples become gelatine-gel containing 80～90% of water. The author has used these gelatine gel samples crushed in the agate mortar.

(B) Fish Meat Protein

Two kinds of fish meat protein were used, one of them was prepared from the common Squid (*Ommastrephes sloani pacificus*) meat which was soluble in 0.8% NaCl solution, and the other was prepared from the Cod fish (*Gadus macrocephalus*) meat which was soluble in 0.2% NaOH solution. These proteins were prepared carefully in the laboratory of Prof. Yoshimura in the Faculty of Fisheries (Hakodate), Hokkaido University. The former was prepared by the following method. To the crushed raw fresh Squid meat was added 10 times volume of 0.8% NaCl solution; after 30 minutes shaking, it was placed in ice room for 12 hours, then ammonium sulphate was added to the solution in half saturation. The precipitated protein was separated by the centrifugal separator and then the protein was dialized several times with distilled water, and washed with alcohol and ether. The washed protein was dried at low temperature. The main component of this protein was myosin.

The latter fish meat protein was prepared by the following method. The crushed Cod fish meat was extracted by 10 times volume of 0.8% NaCl solution for 12 hours. The extracted solution was separated by the centrifugal separator, and the insoluble residue was again extracted with five times of 0.2% NaOH solution for 12 hours. After centrifugal separation, 2-N acetic acid was added to the extracted solution to make the pH value 5.5. The precipitated protein was filtered and the residue was washed with alcohol and ether, and then dried. The main component of this protein was myotelin.

These two kinds of fish meat protein were not purified proteins containing only a single component, but they may have several kinds of protein in their components. The author has used these protein for a first preliminary test to compare with the sample of gelatine in order to study Bound Water in the marine animal meat protein. In the present experiment, to 2∼3 gm of the sample of protein was added an adequate volume of distilled water and then the sample was allowed to absorb water sufficiently to make up an 80∼90% water content. At the time of the experiment with the NaCl added protein, to the dried protein was added the calculated amount of NaCl (J. P.) in the percentage of weight as well as the sample of gelatine added with NaCl.

(C) Fish Meat Sample.

In order to compare the results obtained from the fish meat protein with the result from the raw fresh Atka Mackerel meat (*Pleurogrammus azonas JORDAN et METZ*) at 15°C in Experiment Ⅲ, 1, the author has carried out the experiment at 10°C using raw Atka Mackerel meat

(Ⅱ) Experimental Method.

(A) Vapour tension method.

The method of determining vapour tension of water in the sample was the same as that described in the Experiment Ⅲ, 1, in the previous paper.(38a)

The temperature of the determination was 13°C and 8° (±1°C) for the samples of gelatine, and 15° and 10° (±1°C) for the samples of fish meat protein and raw fresh fish meat.

After the determination of the vapour tension of the samples at their every water-content, the weights of the samples were once estimated. After the last determination of the vapour tension of the samples, they were dried in the drier at 105°∼110°C, and the constant weight of every sample was determined by the usual method. From those data, the values of the water-content of every sample were reckoned at each time of the estimation of the vapour tension.

The method of determining the vapour tension employed by the author is supposed the best method for the easily denaturizable and decomposable samples such as the protein or the living matter, because the dehydrating velocity is high and the dehydration is done at comparatively low temperature. The dehydration from the sample is done in a definite time at a definite lower temperature by the rotary oil vacuum pump.

But on the other hand, that there is certain fault in the method yielding some error is supposed in the estimation of the water-content. However, as this method gives values the same as those obtained by the usual vapour tension method using adsorbents (or desiccating agents) such as sulphuric acid(10a, b), calcium chloride or sodium bromide(39) taking a long time, the author has thought this method sufficiently applicable to determination of the amount of water in the samples.

(B) Electrical resistance method.

The values from the method by electrical resistance for the samples of No. 2 and No. 3 gelatine and the samples of No 2 gelatine to which NaCl was added in the proportion of 0.3 and 25% respectively to the dried matter were obtained in the same way as described in Experiment Ⅲ. 1, of the previous paper. The electrodes used in this experiment were made of brass 0.7 cm. in length and 2 mm. in diameter; the distance between the two electrodes was 2.3 cm. Estimates were made at a temperature of 18°C. Electric current was 50 cycles A. C. The type of electrical resistance was Wheatston's bridge.

(Ⅲ) Arrangement of the experimental results.

As in Experiment Ⅲ, 1, described in the preceding paper(38), the water-content "g" – water-activity "a" curve (strictly speaking, water-content "g" – relative vapour pressure "p/p_0" curve) assumes a S-shape as a characteristic.

The part of A of the curve where the range is 0.1~0.7 of the values of "a" (or p/p_0) and the part of C where the range is 0.2~0.0 of the same value, are steep grade. The part of B where the range is 0.7~0.2 of the same value is a gentle grade. In order to examine in detail the properties of water at the parts of B and C, the author has examined the thermodynamic properties of water in the samples and analyzed the curves above obtained by B. E. T. equation.

(A) Calculations of the change of values of differential molal free energy, $\Delta\bar{F}$, differential molal enthalpy, $\Delta\bar{H}$, and differential molal entropy, $\Delta\bar{S}$, at every water-contents of the samples.

The water-content "g" – water-activity "a" curves obtained at the definite temperatures by the vapour tension method in this experiment perhaps indicate the dehydrating process of so-called adsorbing isotherm which shows the relation of adsorbed amount of water in the samples and the water-activities at the time when the values of adsorbed amount were given.

Therefore, when one mol of water molecule is adsorbed to an indefinite amount of samples at various water-contents of the samples, the change of value of differential molal free energy is calculated by the following equation (3)(38, 40):

$$\bar{F}^{\circ}-\bar{F}=\Delta\bar{F}=-RT\ln p/p_0=-RT\ln a \quad - - - - - - - - \quad (3)$$

where, "$\bar{F}^{\circ}$" is a differential molal free energy when one mol of water is present in the pure phase (namely pure water) at the temperature of T°K. "$\bar{F}$" is differential molal free energy when one mol of water is present in the sample at the same temperature. "R" is gas constant, "p_0" and "p" are saturated pressures of pure water and of water in the sample at the temperature of T°K.

If the water in the samples conforms to the rule of true solution, "p/p_0" will be shown as the water-activity "a" in the sample at saturated pressure "p".

The change of the value of differential molal enthalpy $\Delta\bar{H}$ per one mol of water at the adsorption of water is calculated by the following equation (4)(38, 40):

$$\Delta\bar{H}=-T^2\frac{d}{dT}\left(\frac{\Delta\bar{F}}{T}\right)=-\frac{RT_1T_2}{T_1-T_2}\left(\ln\frac{p_2}{p_{02}}-\ln\frac{p_1}{p_{01}}\right)=-\frac{RT_1T_2}{T_1-T_2}\ln\frac{a_2}{a_1} \quad - - \quad (4)$$

where, "p_i" and "p_{0i}" are the saturated pressures of water in the sample and of pure water respectively at the temperature of T°_iK. "a_i" is the water-activity of water in the sample at T°_iK.

If $\Delta\bar{S}$ is the change of value of differential molal entropy per one mol of water as well as $\Delta\bar{F}$ and $\Delta\bar{H}$ at the time of adsorption of water in the sample at T° K, there is surely a thermodynamic functional relation among those values of $\Delta\bar{F}$, $\Delta\bar{H}$ and $\Delta\bar{S}$ as in the following equation (5):

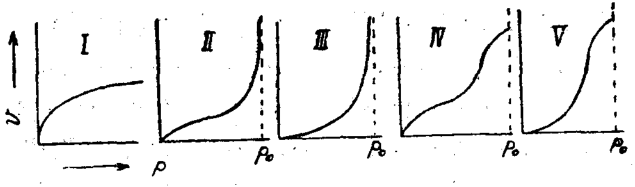
$$\Delta\bar{F}=\Delta\bar{H}-T\Delta\bar{S} \quad - \quad (5)$$

When one wishes to calculate the values of $\Delta\bar{F}$, $\Delta\bar{H}$ and $\Delta\bar{S}$, one must strictly give consideration of hysteresis and capillary condensation which are widely recognized at adsorption and dehydration isotherm. But in this experiment, the author has employed the values obtained under the same dehydrating process. Although the values of $\Delta\bar{F}$, $\Delta\bar{H}$ and $\Delta\bar{S}$ are commonly shown for each and every water-content in the samples, the author has conveniently shown the relation among the values of $\Delta\bar{F}$, $\Delta\bar{H}$ and $\Delta\bar{S}$ for the water-activity "a" in the sample.

(B) The analysis of the water-content "g"—water activity "a" curve by the B. E. T. equation.

There are five types as shown in Fig. 11 for the relation between adsorption capacity "v" and equilibrium pressure "p" at the definite temperature which is generally found in physical adsorption phenomenon(32b).

Fig.11. General forms of isotherm of physical adsorption.



Unimolecular layer's adsorption equation (6) which was offered by Langmuir(41) in 1918 is fitted to the curve of I-type in Fig. 11, but not fitted to the curves of the other four types:

$$v = abp/(1+ap) \text{ - - - - - - - - - - - - - - - - - - - } (6)$$

where "a" and "b" are constants.

However, in 1938 Brunauer, Emmett and Teller(32a) offered an adsorption isotherm, B. E. T. equation fit for the five types in Fig. 11, from dynamic consideration, supposing the multimolecular layer's adsorption. As the detail of the derivation of this B. E. T. equation has been explained by Brunauer et al, Keii(32b), Suga(42a) and Higuch(42b), the author omits details and shows the equation only as follows:

$$p/v\,(p_o - p) = 1/v_m c + \frac{c-1}{v_m c}\,(p/p_o) \text{ - - - - - - - - - - } (\ )$$

where, "p" is equilibrium pressure of the sample, "p_o" is the saturated pressure at the same temperature, "v" is adsorbed amount of water at pressure "p", and "v_m" is adsorbed amount of water when the entire adsorbent surface is covered with a complete unimolecular layer, and "c" is a B. E. T. constant, this constant depends on the kinds of the sample, but it differs somewhat with the estimating temperatures, and it is supposed to be a kind of index for heat of adsorption. The constant, c, is supposed to be shown as equation (8) from the derivation of equation (7):

$$c = (a_1 g/b_1)\ \exp\ E_1 - E_L/RT \text{ - - - - - - - - - - - - - } (8)$$

where, "a_1" and "b_1" are constant, which are concerned in the entrance and the exit of water molecules (that is the condensation and the evaporation of water molecules) at the first layer of the surface of the molecule of the adsorbed substances. "g" is the ratio of "a_i" to "b_i" (a_i/b_i), which is appropriately constant to above the 2nd layer. Here a_i and b_i are constants at 2nd, 3rd, - - - ith - - - i_∞th layer, which are similarly defined as "a_1" and "b_1" respectively. E_1 is the heat of adsorption at the first layer, and E_L is the heat of liquefaction at the saturated pressure, "p_o". In the derivation of equation (8), it was supposed that the heat of adsorption at the 2nd and higher layers (that is E_2, E_3 - - -E_i - - -E_∞) are the same as E_l (E_l is 9.7 kcal/mol in the case of the adsorption of water molecule). This is equivalent to saying that the evaporation—condensation properties of the molecules of water at the second and higher layers are the same as those of the liquid state.

Therefore, if it can be supposed that the constants, "a" and "b", are equal at evey layer of $i_1, i_2, ---i_\infty$, the values of "$a_1 g/b_1$" can be represented as 1, then the heat of adsorption $(E_1 - E_L)$ or E_1 will be calculated by the equation (8).

B. E. T. equation (7) as above mentioned was offered to the multimolecular layers adsorption, but if the thickness of the adsorbed layer cannot exceed some definite number, n, the following equation (9) is offered instead of equation (7):

$$v = \frac{v_m c x}{1-x}\left\{\frac{1-(n+1)\,x^n + n x^{n+1}}{1+(c-x)-c x^{n+1}}\right\} \text{ - - - - - - - - - - - - - - } (9)$$

where, "x" is "p/p_o".

In equation (9), if the number of layers, n, is equal to 1 (that is, the adsorption is ended at unimolecular layer), equation (7) is represented as the following equation (10).

$$v = v_m c\,(p/p_o)/\{1 + c\,(p/p_o)\} \text{ - - - - - - - - - - - - - - - } (10)$$

This equation (10) is the same as Langmuir's equation (6) which was offered to the unimolecular layer's adsorption.

The explanation above stated is an outline of B. E. T. equation. It is clear from equation (7) that a plot of "$p/v\,(p_o - p)$" against "p/p_o" should give a straight line, whose intercept is "$1/v_m c$" and whose slope is "$(c-1)/v_m c$". From the slope and intercept, the two constants "v_m" and "c" can be evaluated.

The water-content "g"—water-activity "a" curve obtained by the vapour tension method in this experiment is the Ⅱ-type shown in Fig. 11, that is to say a S-type curve. In this case E_1 is larger than E_L, but when E_1 is smaller than E_L, the curve takes the Ⅲ-type as shown in Fig. 11.

Regarding the theory of B. E. T. equation, many discussions have been presented by many investigators, especially Higuchi[42b] has compared this theory of B. E. T. equation with the capillary condensation theory which he himself had asserted; he has pointed out that the B. E. T. theory is not yet complete, and it is open to discussion.

Next, if the amount of water "v_m" necessary to cover the entire surface of the molecule of the sample with unimolecular water layer is determined, the surface area "s" per unit of adsorbed substance will be calculated from the equation (11)[43]:

$$s = \frac{v_m\, s_m}{M/N} \text{ - } (11)$$

where, "N" is Avogadro number, "M" is the molecular weight of the substance which was adsorbed to the sample (when the substance is water, M is 18), "s_m" is molecular sectional area of the substance which was adsorbed (when the substance is water, "s_m" is 10.45×10^{-16} cm².).

When the amount of the adsorbed substance "v" which was obtained from this experiment (in this experiment it is shown as the amount of water per gm of the dried matter of the sample "g"), the equilibrium pressure of water in the sample, p, and the saturated pressure "p_0" of pure water at the same temperature are substituted respectively into the equation (7), and then the relation between "$p/g(p_0-p)$" and "p/p_0" shows as traight line, the values of "v_m" and "c" will be determined. When the values of "v_m" and "c" are substituted into the terms of "v_m" and "c" in the equation (9), and the curve of the relation between the values of "v" (that is "g") which are the amount of the adsorbed substance (when the adsorbed layer "n" is 1, 2, 3 - - - i - - - i_∞) and "p/p_0" (that is "a"), is compared with the observed curve of water-content "g" — water-activity "a", the approximate number of the adsorbed layer, n, will be determined by the resemblance of the curve to the observed curve.

However, in the case of substituting the values of "g" and "p/p_0" (or "a") in equation (7), Briggs(10b) has said that if the estimated value of "p/p_0" (or "a") is below 0.2, the error of the estimation is too large. So if the author plott the value of "gp_0/p" against the value of "p/p_0" according to Bull(31), the curve is a parabola as shown in Fig. 12. The author extrapolated an end of this parabola from 0.2 of the value of "a" to 0.0, and obtained the values of "gp_0/p" corresponding to several points in this curve which shows below 0.2 of the value of "a", and then calculated the values of "g" multiplying them by the values of "p/p_0" (or "a") corresponding to those points. These values of "g" were substituted in equation (7).

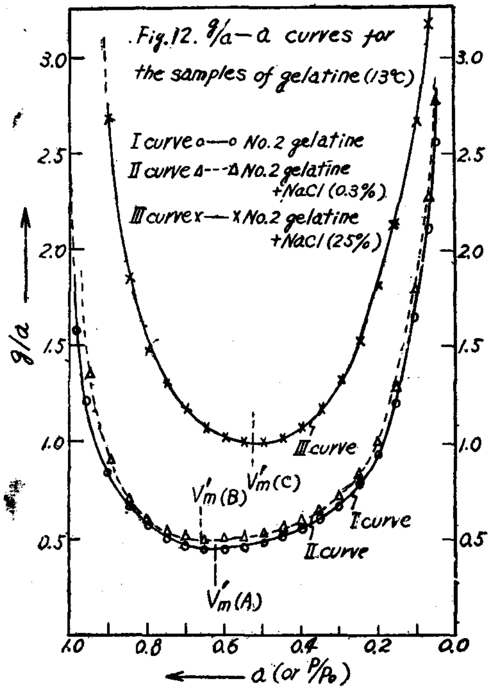


Fig. 12. g/a — a curves for the samples of gelatine (13°C)

(C) The relation between the estimated amount of water and the calculated amount of water corresponding to every water-activity in the sample to which salts are added.

As stated in Experiment Ⅱ, 1[(3a)], from the supposition that the difference between the estimated amount of water $g_{2.obs}$ per gm of the bone dried sample to which salts are added corresponding to a water-activity "a_1" and the estimated amount of water $g_{1.obs}$ per gm of the bone dried sample to which no salt is added, is the amount of water which hydrates with the added salts showing the same water-activity, the author has obtained in similar calculation with Experiment Ⅱ, 1 in the previous paper, the theoretical curve of water-content "g"—water-activity "a" in the sample, to which salts are added, on the base of the curve of "g—a" in the sample to which no salt is added.

The author compared this theoretical curve obtained by calculation with the curve of "g—a" in the sample to which salts are added. Here, the equivalent weights of salt (m/M, "m" is the quantity of salts added per gm of the dried matter of the sample. "M" is the molecular weight of salt) are as follows:

(a) NaCl was added in proportion of 0.3% to the dried matter of the sample of No. 2 gelatine (at 13° and 8°C)

--------- 0.0513×10^{-3} Mol NaCl

(b) NaCl was added in proportion of 25% to the dried matter of the sample of No. 2 gelatine (at 13° and 8°C)

--------- 4.276×10^{-3} Mol NaCl

(c) $CoCl_2$ was added in proportion of 27% to the dried matter of the sample of No. 2 gelatine (at 8°C)

--------- 1.956×10^{-3} Mol $CoCl_2$

(d) NaCl was added in proportion of 12.77% to the dried matter of Squid meat protein (at 13°C)

--------- 2.183×10^{-3} Mol NaCl

(2) Experimental results and Discussion.

(I) Results and Discussion on gelatine.

(A) Experimental results.

The experimental results and the arranged results are shown as in Tables 13～17 and in Figs. 13～18.

The sign A in each Table and Fig. indicates the sample of No. 2 gelatine, B the sample of No. 2 gelatine to which NaCl was added in proportion of 0.3% to the dried matter of the sample, C the sample of No. 2 gelatine to which NaCl was

Table 13. Relation between the values of water content "g" and water-activity "a" of the samples of gelatine.

Sample A		Sample B				Sample C				Sample D		Sample E			
(No. 2 gelatine)		(NaCl was added in the proportion of 0.3% per gm of bone dried No.2 gelatine)				(NaCl was added in the proportion of 25% per gm of bone dried No. 2 gelatine)				(CoCl$_2$ was added in the proportion of 27% per gm of bone dried No.2 gelatine)		(No. 3 gelatine)			
8°C (p_0 = 0.8045 cm. Hg)		8°C		13°C (p_0 = 1.1231 cm. Hg)		8°C		13°C		8°C		8°C		13°C	
g (gm of water per gm of bone dried gelatine)	a (or p/p_0) (water-activity of water in the sample)	g	a	g	a	g	a	g	a	g	a	g	a	g	a
2.70	0.979	2.69	0.977	9.87	0.994	2.92	0.916	8.38	0.930	3.23	0.974	4.02	0.987	3.10	0.966
1.63	0.956	0.92	0.850	4.00	0.994	1.38	0.821	4.05	0.922	1.72	0.844	2.71	0.937	1.93	0.950
0.96	0.914	0.60	0.787	1.82	0.983	1.11	0.731	2.26	0.890	1.19	0.731	2.02	0.892	0.98	0.935
0.52	0.792	0.346	0.677	1.24	0.934	0.795	0.602	1.29	0.800	0.86	0.620	1.23	0.908	0.556	0.909
0.333	0.674	0.250	0.438	0.70	0.874	0.646	0.556	0.855	0.681	0.646	0.572	0.829	0.874	0.334	0.789
0.258	0.468	0.181	0.105	0.37	0.724	0.583	0.554	0.738	0.673	0.589	0.530	0.735	0.866	0.258	0.659
0.256	0.415	—	—	0.275	0.548	0.467	0.385	0.644	0.639	0.556	0.495	0.476	0.816	0.205	0.409
0.215	0.324	—	—	0.223	0.330	0.435	0.407	0.533	0.530	0.555	0.495	0·388	0.754	0.158	0.114
0.212	0.260	—	—	0.210	0.181	0.362	0.287	0.403	0.353	—	—	0.223	0.451	—	—
0.192	0.164	—	—	—	—	—	—	0.376	0.223	—	—	0.146	0.128	—	—
—	—	—	—	—	—	—	—	—	—	—	—	0.126	0.074	—	—

Table 14. Relation between the values of water content "g", and of water-activity "a" with the values of $\Delta \overline{F}$, $\Delta \overline{H}$ and $\Delta \overline{S}$ of the samples of gelatine at 13°C.

a (or p/p_0)	g					$\Delta \overline{F}$ (kcal/mol)	$\Delta \overline{H}$ (kcal/mol) 8°~13°C					$\Delta \overline{S}$ (cal/mol)				
	A	B	C	D	E	Sample A, B, C, D and E.	A	B	C	D	E	A	B	C	D	E
0.1	0.165	0.180	0.267	0.280	0.140	1.30	12.5	4.10	—	—	1.4	39.2	9.8	—	—	44.5
0.2	0.189	0.201	0.363	0.325	0.178	0.92	7.3	3.35	—	—	8.77	22.3	8.5	—	—	27.4
0.3	0.206	0.219	0.400	0.410	0.199	0.70	7.12	2.73	1.07	—	6.60	22.4	7.1	1.3	—	20.3
0.4	0.221	0.239	0.435	0.465	0.210	0.52	7.12	2.20	2.04	1.8	7.11	23.1	5.9	5.3	4.5	23.0
0.5	0.243	0.268	0.502	0.523	0.219	0.40	6.35	2.04	2.14	3.22	6.35	20.8	5.7	6.1	9.8	20.8
0.6	0.270	0.304	0.613	0.586	0.232	0.30	6.49	2.60	2.66	4.85	5.80	21.6	8.0	8.3	15.9	19.2
0.7	0.330	0.354	0.820	0.700	0.274	0.20	3.12	2.18	2.25	5.45	3.40	10.2	6.9	7.2	18.3	11.2
0.8	0.456	0.472	1.185	0.990	0.360	0.13	1.67	1.77	1.60	4.27	2.70	5.5	5.8	5.1	14.4	9.0
0.9	0.760	0.815	2.42	1.80	0.588	0.06	1.47	1.87	1.01	2.49	2.15	4.9	6.3	3.3	8.5	7.3
0.95	1.150	1.275	—	2.30	1.93	0.03	0.80	1.81	—	—	1.24	2.7	6.2	—	—	4.2

Table 15. Relation between the amount of water (%) and the electrical resistance (50 cycles) of the samples of gelatine. (Estimating temp., 18°C)

Sample B		Sample C				Sample E	
T.W. (Total water content in %)	E.R. (Electric resistance in KΩ)	T. W.	E. R.	T. W.	E. R.	T. W.	E. R.
9.83	2.3	98	0.27	25.7	8.5	64.13	1.55
9.08	0.8	88.7	0.09	22.0	230	59.0	2.10
8.17	0:55	67.5	0.05	21.5	160	55.9	1.95
6.85	0.80	59.5	0.065	21.2	380	52.2	2.70
5.40	1.33	47.5	0.115	21.1	290	47.3	2.80
5.23	1.10	36.9	0.200	20.9	700	41.8	4.80
4.80	2.70	35.2	0.250	20.4	1,200~2,000	37.4	5.5
3.96	9.0	33.0	0.85	20.0	1,000	30.2	15.5
3.61	16.0	31.9	1.3	—	—	18.4	470
3.26	75	30.2	2.2	—	—	14.9	8,000
2.84	300	28.7	2.5	—	—	—	—
2.70	450	27.9	4.8	—	—	—	—
2.30	1,000 over	26.9	5.6	—	—	—	—

Table 16. Analytical results of the "g – a" curves of the sample of gelatine by B. E. T. Method.

Sample	Estimating temp. (°C)	v_m	v_m'	c	E_1-E_L (kcal/mol)	E_1 (kcal/mol)	s (m^2/mg)	v_m'/v_m
A	13°C 8°	0.179 0.186	0.280 0.305	42 38.1	3 78 3.88	13.53	0.645	1.56 1.64
B	13° 8°	0.225 0.230	0.326 0.352	28.1 34.1	3.40 3.54	13.17	0.980	1.45 1.53
C and D	13° or 8°C	0.353	0.530	19.0	3.0	12.7	1.240	1.50
E	13° 8°	0.172 0.196	0.250 0.260	24.5 18.0	3.25 2.90	12.8	0.650	1.45 1.33
Katz(10a)	23°	0.170	0.294	7.85	2 20	11.9	0.600	(1.73)
Bull(31)	25°	0.0873	0.141	17.4	3.04	12.74	0.309	(1.62)

Table 17. Ratio of the theoretical amount of water "$g_{calc.}$" to the observed amount of water "$g_{obs.}$" of the samples of gelatine to which salts were added.

Water activity "a" (13°C or 8°C)	Sample B		Sample C		Sample D
	13°C	8°C	13°C	8°C	8°C
0.1	0.922	0.925	0.903	0.921	0.832
0.2	0.946	0.948	0.815	0.840	0.750
0.3	0.950	0.986	0.855	0.888	0.762
0.4	0.934	0.987	0.915	0.934	0.760
0.5	0.918	0.975	0.944	0.930	0.760
0.6	0.901	0.945	0.946	0.872	0.707
0.7	0.945	0.960	0.942	0.864	0.659
0.8	0.981	0.910	0.980	0.888	0.703
0.9	0.954	—	0.922	—	—
0.95	0.931	—		—	—
Average	0.938	0.955	0.904	0.892	0.742

Note: The average value of $g_{cacl.}/g_{obs.}$ of the sample D at 13°C was 0.848 as shown in Table 6 of the previous Exp. Ⅲ, Ⅰ.(38a)

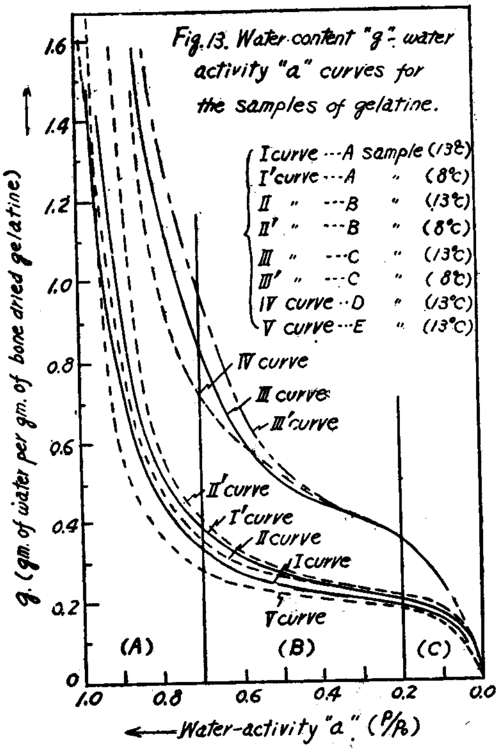


Fig. 13. Water-content "g" - water activity "a" curves for the samples of gelatine.

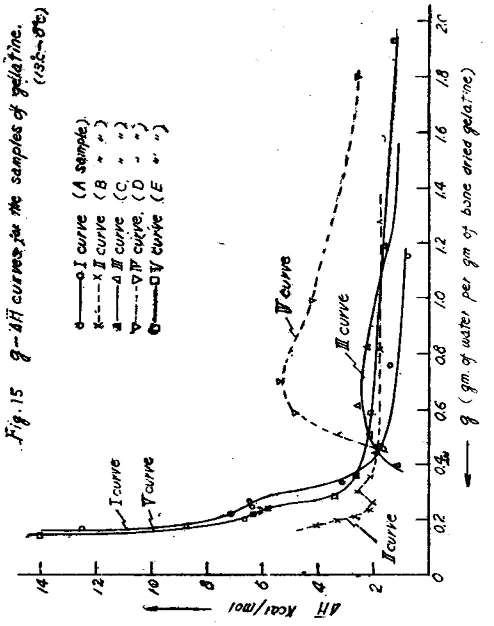


Fig. 15 g - ΔH̄ curves for the samples of gelatine. (13°C~8°C)

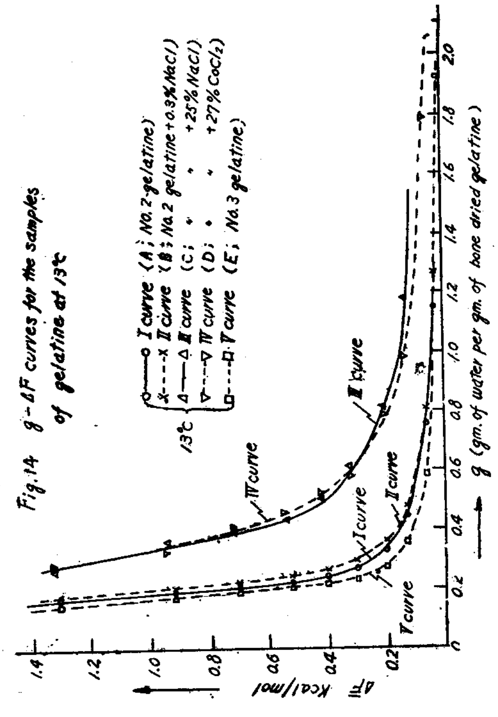


Fig. 14 g - ΔF curves for the samples of gelatine at 13°C

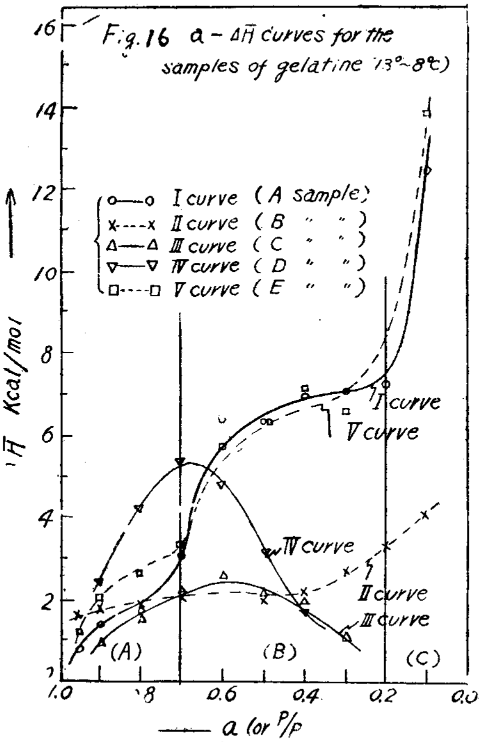


Fig. 16 a - ΔH̄ curves for the samples of gelatine (13°~8°C)

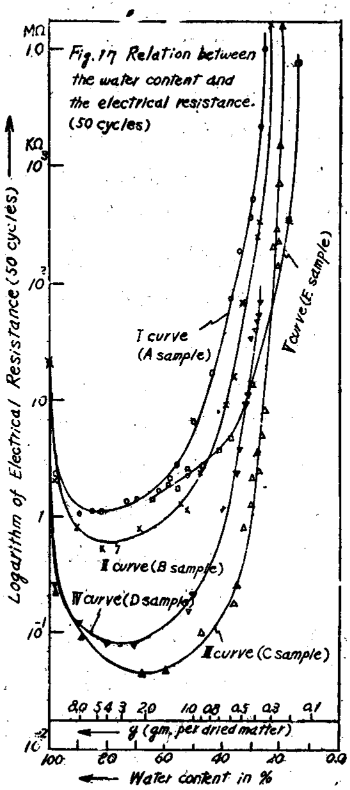


Fig. 17 Relation between the water content and the electrical resistance. (50 cycles)

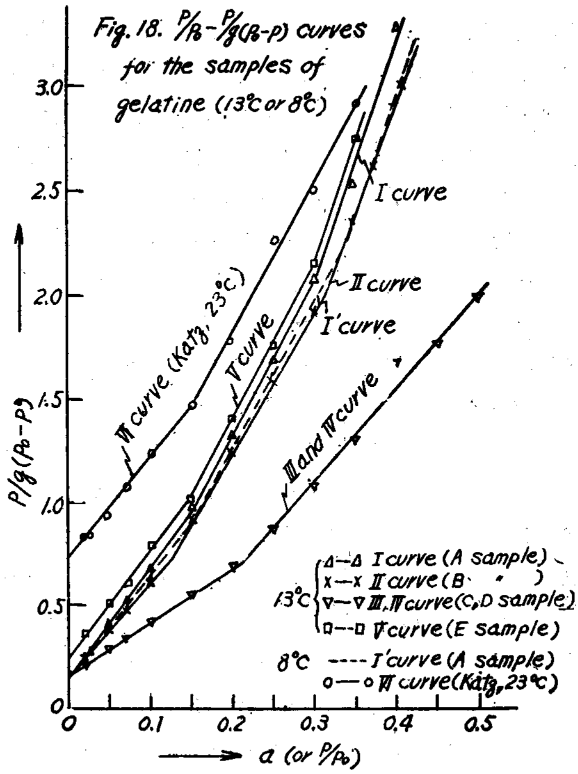


Fig. 18. $P/P_0 - P/g(P_0-P)$ curves for the samples of gelatine (13°C or 8°C)

added in proportion of 25% to the dried matter of sample, D indicates the sample of No. 2 gelatine to which cobaltous chloride was added in proportion of 27% to the dried matter of the sample, E the sample of No. 3 gelatine.

Table 13 shows the relation between the water content "g" (the amount of water per gm of the dried matter of the sample of gelatine, gm) and the water-activity "a" in the sample. As the experimental results for the samples of A and D at 13°C have been previously described in the Experiment III, 1, so they are omitted here. The results shown in Table 13 were plotted as in Fig. 13. The curves I, II, III, IV and V in Fig. 13 are the experimental results for the samples of A, B, C, D and E at 13°C respectively, and the curves I′, II′ and III′ are the results for the sample of A, B, C at 8°C respectively. The results for the sample of D, E at 8°C are omitted in Fig. 13.

Table 14 shows the relation among the values of ΔF (13°C), $\Delta \bar{H}$ (13°~8°C) and $\Delta \bar{S}$ (13°C) which were calculated by equations (3), (4), and (5) from the estimated curves of each sample at 13°C and 8°C shown as in Fig. 13, and the water-content "g" of each sample and the water-activity "a" in each sample respectively. The relation between the water-content "g" and the values of $\Delta\bar{F}$ which are shown in Table 14 is shown as Fig. 14. The relation between the "g" and the value of $\Delta\bar{H}$ is shown as Fig. 15. The relation between the value of $\Delta\bar{H}$ and the water-activity "a" in each sample is shown in Fig. 16, in exchange for "g" shown as in Fig. 15. The relation between the value of $\Delta\bar{S}$ and the water-content "g" is the same as the relation between the value of $\Delta\bar{H}$ and "g", so the Figure to show that relation is omitted here. Table 15 shows the relation between the amount of water in every sample of B, C and E (total amount of water %) and the electrical resistance of 50 cycles(KΩ). The results in Table 15 were plotted as in Fig. 17. In Table 15, the experimental results for the sample of A and D were omitted, because the results for them have previously been shown in Table 4 in the Experiment Ⅲ, 1(33). The curves Ⅰ, Ⅱ, Ⅲ, Ⅳ and Ⅴ in Fig. 17 show the experimental results for each sample of A, B, C, D and E respectively. Table 16 shows the values of B. E. T. constant, c, the amount of the adsorbed water, needed to cover the surface of the gelatine molecule with unimolecular water layer, v_m, the apparent heat of adsorption E_I-E_L, the heat of adsosption at the first layer, E_1, and the adsorbed area of surface, s. The value of "v_m'" which is shown in the 4th column of Table 16 is the value of the water-content "g" at the minimum point of the curve of "g/a−g" as shown in Fig. 12. (In Fig. 12, the value of "v_m'" was shown as v_m'(A), v_m'(B) and v_m'(C) for the curves Ⅰ, Ⅱ and Ⅲ for each sample of A, B and C respectively.) The author has added to Table 16, the anlayzed value by B. E. T. equation from the experimental results which were obtained by the vapour tension method by Katz(10a) on gelatine at 23°C, and the values of experimental results obtained by Bull(31) on gelatine at 25°C. Fig. 18 shows the relation between the values of $p/g\,(p_0-p)$ and p/p_0 of each sample which were obtained from the results shown in Fig. 13. (In the equation of B. E. T. the sign of "g" is shown as "v"). The curves shown in Fig. 18, which show the relation as above stated, are the straight lines in a definite range of the value of "a" (or "p/p_0)". This fact shows that the equation of B. E. T. (7) is certainly applicable to these relations. In Fig. 18, the curve Ⅵ was obtained from the results of Katz. The analyzed results of each sample of B, C, D and E at 8°C were omitted.

Table 17 shows the ratio of the theoretically calculated water content "$g_{calc.}$" as above stated to the observed water content "$g_{obs.}$" at each water activity of each sample of B, C and D to which the salts were added. The result for the sample of D at 13°C was already previously shown in Table 6 in Experiment Ⅲ, 1(33), so the results are omitted from Table 17.

(B) Discussion on the experimental results on gelatine.

From the experimental results obtained, the author will discuss separatlely the following themes : (a) On the samples of gelatine No. 2 and No. 3 to which no salt is added (samples A and E), (b) On the samples of gelatine No. 2 to which salts are added (samples B, C and D).

(a) Discussion on the samples of gelatine No. 2 and No. 3 (samples A and E).

(i) The relation between the water-content "g" and the values of ΔF and ΔH :-

When the "g-a" curves for the same sample which were obtained at 13°C and 8°C were compared with each other, a little difference is seen as shown between the curves I (or II, III) and curve I′ (or II′, III′) in Fig. 13. That is to say, the "g-a" curves which were obtained at lower temperature slipped down to the right hand of the "g-a" curves which were obtained at higher temperature (when we compare the curves at the same water-activity, the curves at lower temperature are situated above the position of the curves at higher temperature).

However, when the water-activity "a" is more than 0.9, the water-content "g" of the curves obtained at higher temperatures is sometimes larger than that of the curves obtained at lower temperatures at the same water-activity.

This fact will be because when the temperature is higher, the thermokinetics of water molecules becomes energetic and the active surface of adsorption becomes newly larger at high moisture.

From that fact, it may be supposed that these thermal phenomena are due to the change of the thermodynamic function.

In the relation between the water-content "g" and the value of ΔF as seen from the curves I and V for each sample of A and E respectively in Table 14, as the water-content "g" is less, the value of ΔF becomes gradually larger. When the value of ΔF shows 0.1~0.2 kcal/mol, and when the "g" is less than 0.3~0.4, the value of ΔF increases rapidly. At the time of decreasing of the amount of water in the sample, the difference, "ΔF", between the differential molal free energy "F°" of pure water and the differential molal free energy of water in the sample F, is supposed to be small, while the water in the sample has properties of Free Water. But when the water in the sample loses properties of Free Water, ΔF is supposed to be naturally larger. From those facts, the water, of which the water-content "g" is less than 0.3~0.4, certainly has no property of Free Water.

When the water-content "g" is 0.3~0.4, the electrical resistance of 50 cycles is on the rapid increase as shown in the curves I and V for each sample of A and E in Fig. 17.

In the relation between the water-content "g" of the samples and the value of $\Delta\bar{H}$ in Fig. 15, with the decreasing of the water-content "g", the value of $\Delta\bar{H}$ increases gradually as shown in the curves I and V. When the water-content "g" is less than 0.3～0.4, the value of ΔH shows 2～3 kcal/mol. And when the water-content "g" is less than 0.3～0.4, the value of $\Delta\bar{H}$ increases suddenly, as well as the change of the value of $\Delta\bar{F}$.

It is a noteworthy fact that when the water-content "g" is in the neighbourhood of 0.2～0.3, a step is recognized in the curve of "$\Delta\bar{H}-g$". This fact was also recognized in the samples of both No. 2 and No. 3 gelatine. From the relation between the water-activity "a" and the value of ΔH which was shown as curves I and V in Fig. 16, the value of ΔH is observed to be a definite value of 3～7 kcal/mol at the part of B of which range of the water-activity "a" is 0.7～0.2. But the value of ΔH is observed to be 0～3 kcal/mol at the part of A of which the range of the water-activity "a" is 0.7～1.0. On the contrary, the value of $\Delta\bar{H}$ is observed to increase suddenly at the part of C, of which the water-activity "a" is less than 0.2, for example the value of $\Delta\bar{H}$ is 12～14 kcal/mol in the neighbourhood of 0.1 of the water-activity "a". Those facts will be discussed by comparing with the analyzed results by B. E. T. equation in the following separate article.

Next, the author will compare the experimental results as above stated with the results obtained by other investigators. Katz(40) has compared the values of ΔF and ΔH in the various high molecular substances, and he has concluded that the values of ΔF and ΔH are almost the same, and there is no change of entropy of that substances at the time of the adsorption of water molecules. But Fricke(41) et al. have made it clear that the entropy of the substances increased with the decreasing of the water-content for the samples of casein and keratin. In the experimental results of the author for the sample of gelatine, it has been recognized that the entropy of the substance (the differential molal entropy), increased with the decreasing of the water-content. Rosenbohm(45) has estimated directly the value of ΔH of gelatine by the ice-calorimetric method, and has said that the limit value of ΔH in the extreme drying is 4 kcal/mol. Rosenbohm's value is not a differential molal enthalpy as the author's results, but 4 kcal/mol of the value of ΔH corresponds nearly to 0.3 of the water-content "g" (23% of the total amount of water). This fact shows that in Rosenbohm's experiment, Bound Water which is strongly combined with the sample perhaps was not completely dehydrated from the sample, because the sample was dried in room temperature.

According to Bull[31], the value of ΔF is 1.537 kcal/mol, and the value of ΔH is 3.8 kcal (at 25°C) in gelatine. But the values of ΔF and ΔH vary with the change of the water-content, and there was no detailed result concerning this point, so it is difficult to compare these results with each other. However Bull's consideration was concerning the water in gelatine of which the water-activity is less than 0.7, so the value of ΔH of water in the part of B on the "g–a" curve of which the water-activity is 0.7～0.2 is supposed about 4 kcal. Then the value of ΔH which was 3～7 kcal/mol in the range of 0.7～0.2 of "a" in the author's result is nearly in agreement with Bull's result.

(ii) Consideration of analyzed results of the "g–a" curve by B. E. T. equation.

In the relation between the water-activity "a" and "$p/g(p_0-p)$" as shown in Fig. 18, if the water-activity "a" is within the range 0.05～0.2 (or 0.3), each sample shows a straight line, but if the water-activity "a" is less than 0.05 or is larger than 0.2～0.3, the angle of inclination of the lines increases rapidly. This fact is recognized as follows: when the molecules of the protein hydrate slowly from the bone dried stage, the hydration follows a path to adsorbing process forming many new active surfaces of adsorption.

The author has shown the curve which was analyzed from Katz's experimental results at 25° by the B. E. T. equation (7) in Fig. 18. Supposing from Table 16 and Fig. 18, as shown in the curves of 25°C, 13°C, one recognizes that there are differences among the values of B. E. T. constant "c", the values of heat of adsorption "E_1-E_L" or "E_1" according to estimating temperatures.

As shown in Table 16, v_m, which is adsorbed amount of water when the entire adsorbent surface is covered with a complete unimolecular water layer, was average 0.183 gm per gm of the dried matter of No. 2 gelatine, 0.184 gm per gm of dried matter of No. 3 gelatine.

The values of apparent heat of adsorption, "E_1-E_L" of No. 2 gelatine and No. 3 gelatine were 3～4 kcal/mol, the value of heat of adsorption at the first layer of the adsorption, E_1, was 12～14 kcal/mol. When those value obtained by the author are compared with the values obtained by Katz and Bull, regarding to the value of "v_m", Katz's value was 0.170 gm per gm of the dried matter of gelatine, which agrees with the author's result; but Bull's value was 0.0873 gm per gm of the dried matter of gelatine, which corresponds to a half of the values of Katz and the author. Regarding Bull's result, the present author can not enter into detailed discussion only from his paper. Regarding values of "E_1-E_L", the results obtained by Bull and by the author are almost the same.

The values of "v_m'" which are recorded in the 4th column of Table 16 are the values of the water-content "g" which are corresponding to the minimum value of "g/a" for the curve of "g/a—a" in Fig. 12. The minimum points of the curves "g/a—a" for all the samples are situated in the range of 0.6~0.7 of "a". The ratio of "v_m'" to "v_m" is about 1.5 on the average.

According to Bull, at the time of the adsorption of water molecules by the protein molecules, the amount of water needed to form a layer of one molecule of water as the first step of the adsorption to the hydrophilic surfaces which are linked cohering with each other in the molecule of the protein, is shown as v_m. At that time, one molecule of water links with those hydrophilic surfaces with hydrogen bonds. With the advance of hydration, as the second step, while the amount of water which corresponds to "v_m'" (it is about twice "v_m") is adsorbed, it is believed that the two molecules of water enter between the hydrophilic surfaces which are situated among the molecules of protein, and are linked with the protein forming so-called water-water bridge.

According to Bull's results, the ratio of "v_m'" which is obtained from the minimum point of the "g/a—a" curve, to "v_m" which is obtained from B. E. T. equation is 1.62 for the sample of gelatine. The author has also obtained about 1.5 on the average for the gelatine, so this value agrees with those of Bull and Katz.

Katz(39) and Sponsler(3d) have recognized from the result of diffraction by x-ray on the gelatine, that when 0.18 gm of water per gm of the dried matter of gelatine is adsorbed, one molecule of water enters among the side chains of the molecules of gelatine and it links with hydrophilic radicals in both the side chains of the molecules of gelatine by hydrogen bonds; and when the hydration advanced and 0.5 gm of water per gm of the dried matter of gelatine is adsorbed, one molecule of water enters among the main chains of the molecule of gelatine, and two molecules of water enter among side chains of the molecules of gelatine; and when the adsorbed amount of water is larger than 0.5 gm, the image of the diffraction by X-ray shows already no clear ring and the arrangement of molecules in gelatine is abnormal. Considering the facts as above stated and the results obtained by the author and by Katz that the value of "v_m" is about 0.17~0.19 as the water-content "g", the value of E_1 is 12~14 kcal/mol, and the energy of one hydrogen bond is about 5 kcal/mol, one molecule of water is supposed to link with both molecules of gelatine by two hydrogen bonds in the part of C in Fig. 13. In the range of 0.17~0.3 gm of the water-content "g" of Fig. 13, that is to say, in the range from "v_m" to "v_m'" (the part of B whose water-activity is 0.2~0.6 of "a"), two molecules of water are supposed to enter in the molecules of gelatine to form so-called water-water bridge, having one hydrogen bond (This is supposed from the value of "E_1-E_L" is 3~4 kcal/mol.) This conclusion is also obtained from fact that the the fact that the change of the value of differential molal enthalpy, $\Delta\bar{H}$, is 3~7 kcal/mol in the range of 0.2~0.7 of "a". It is reasonable that water which is situated in the part of B and C of the "g-a" curve which was obtained by the vapour tension method [the value of "a" (or "p/p_0") is less than 0.7] is so-called molecular theoretical Bound Water from the idea of hydrogen binding.

The adsorbed area per unit of gelatine "s" is 0.6~0.65 m^2/mg as shown in Table 16 by the author and by Katz, and about 0.3 m^2/mg by Bull. The difference of these values and reasonableness of these values are reserved for further studies.

(b) Discussion on the sample of gelatine to which salts were added.

(i) Relation of the water-content "g" to $\Delta\bar{F}$ and $\Delta\bar{H}$.

It is clear, from the 1st to 7th column of Table 14, and the curves II, III and IV in Fig 14, that the value of $\Delta\bar{F}$ increases with decreasing of the water-content "g" in both cases of the samples of gelatine to which salts are added or not added.

For example, when the water-content "g" of sample B (to which 0.3% NaCl was added) is 0.3~0.4, the value of $\Delta\bar{F}$ shows 0.1~0.2 kcal/mol, and when the water-content "g" of sample C (to which 25% NaCl was added) and the sample D (to which 27% $CoCl_2$ was added) is 0.6-0.8, the value of $\Delta\bar{F}$ is 0.2~0.4 kcal/mol, and when the water-content "g" is less than 0.3~0.4 or 0.6~0.8 respectively, the value of $\Delta\bar{F}$ increases rapidly.

In comparing the values of $\Delta\bar{F}$ with the same value of "g", the larger the amount of the added NaCl is, the greater the value of $\Delta\bar{F}$ becomes for the samples of B and C. This is due to the fact that a part of water which was present initially in the samples of gelatine hydrates with the salts (NaCl or $CoCl_2$) which were added.

In the relation between the value of $\Delta\bar{H}$ and the water-content "g" of Table 14 and the curves II, III and IV in Fig. 15, it is observed that the values of $\Delta\bar{H}$ increase gradually with initial decreasing of the water-content "g" and afterwards the value of $\Delta\bar{H}$ decreases for the samples of C and D. For example, when the water-content "g" is 0.6~0.8 (total amount of water is about 32~44%), the value of $\Delta\bar{H}$ shows temporarily the maximum value, and then the value of $\Delta\bar{H}$ decreases with the decreasing of the water-content "g", and when the water-content "g" is

0.3～0.4 (the total amount of water is about 23～28%), the value of $\Delta\bar{H}$ shows negative values. The maximum value of $\Delta\bar{H}$ is about 2.5 kcal/mol for the sample of C and is about 5.4 kcal/mol for the sample of D. The decreasing of the water-content "g" below the value of "g" which corresponds to the maximum value of $\Delta\bar{H}$, is due to the heat of combination of NaCl with water. That is to say, as the heat of dissolution and the heat of dilution of NaCl in water are minus, the NaCl adsorbs the heat which was given out from the gelatine. Therefore the value of $\Delta\bar{H}$ of the sample to which NaCl was added is less than that of the sample to which no NaCl was added.

When the value of $\Delta\bar{H}$ decreases further with decreasing of the water-content "g", the value of ΔH becomes minus, and the sample to which NaCl was added shows the endothermic reaction. When the water-content "g" of gelatine is in the neighbourhood of 0.3～0.4, the gelatine to which NaCl was added attains already a solid state, and the electrical resistance of the gelatine is on the way to rapid increase with the decreasing of the water-content "g", as shown in Fig. 17. Therefore the dehydrating ability of NaCl for water in gelatine becomes stronger in the neighbourhood of 0.3～0.4 of "g", and NaCl perhaps scrambles for water which is hydrated with gelatine. The value of $\Delta\bar{H}$ of B sample of No. 2 gelatine to which NaCl was added in proportion of 0.3% to the dried matter of the sample, as shown in curve II in Fig. 15, is larger than the value of $\Delta\bar{H}$ of curve I of A sample of No. 2 gelatine to which no NaCl was added, when these samples are compared at the same water-content "g". This is perhaps because a part of water in gelatine hydrates with NaCl, and the amount of the water to be bound with gelatine decreases, therefore the water-content "g" decreases and the value of $\Delta\bar{H}$ increases relatively.

Similar conclusion is based on curve IV of the sample of No. 2 gelatine to which $CoCl_2$ was added. That is to say, the value of $\Delta\bar{H}$ of the sample to which $CoCl_2$ was added is larger than the value of $\Delta\bar{H}$ at the same water-content of the sample to which no $CoCl_2$ was added, even after the addition of $CoCl_2$. It was not clear for the sample of No. 2 gelatine to which NaCl was added in the proportion of 25%, whether the same conclusion can be given.

From Fig. 16 which was shown as the relation between the water-activity "a" and the value of $\Delta\bar{H}$ changing from the relation between the water-content "g" and the value of $\Delta\bar{H}$, the value of $\Delta\bar{H}$ is 2～3 kcal/mol for the sample of B at the part B shown in Fig. 13, that is to say, the water-activity is less than 0.7. This value of $\Delta\bar{H}$ is less than the value of $\Delta\bar{H}$ for the sample of A (curve I) to which no NaCl was added and for the sample of E (No. 3 gelatine) (curve V). This result is in agreement with Lloyd's(4c) opinion that the presence of electrolyte such as NaCl in the protein weakens the electrostatic attraction among the side chains of the molecules of the protein.

(ii) Discussion on the analyzed result of the curve of "g−a" by B. E. T. method.

As shown on the curves Ⅱ, Ⅲ and Ⅳ in Fig. 18, when NaCl was added to No. 2 gelatine in the proportion of 0.3% (in the case of sample B) and 25% (sample C), or $CoCl_2$ was added in the proportion of 27% (sample D), the relation between "$p/g(p_0-p)$" and "p/p_0" shows the linear function when the range of the water-activity "a" is 0.05∼0.1 (or ∼0.21) as well as the samples to which no salt was added. But the greater the amount of added salts, the smaller the angle of inclination of the line is. Therefore the curves Ⅱ, Ⅲ and Ⅳ are located under curve Ⅰ which shows for the sample to which no salt was added. Therefore the value of B. E. T. constant "c" which was calculated from the B. E. T. equation from the relation of lines, becomes smaller as shown in Table 16, and each value of the heat of adscrption of "E_1-E_L" and E_1 becomes smaller.

On the other hand, the larger the amount of the added salts is, the greater the value of "v_m" becomes, e. g. the value of "v_m" was 0.18∼0.19 for the sample of No. 2 gelatine (A) to which no salt was added; it was 0.22∼0.23 for the sample (B) to which NaCl was added and it was about 0.35 for the sample of C, D, to which NaCl or $CoCl_2$ were added in the proportion of 25% or 27% to the dried matters of the samples respectively. The value of "v_m" perhaps includes the amount of water hydrated with salts. The amounts of water hydrated with the added salts were calculated from equations (10) and (11), supposing that all the added salts are dissociating (in 100%) in the samples: 0.0018 gm for the sample of B, 0.07∼0.1 gm for the samples of C and D. Even if the amounts of water hydrated with added salts are subtracted from the values of "v_m", the remainders are larger than the values of "v_m" of the samples to which no salt is added, perhaps due to the abnormal decreasing of vapour tension by the addition of salts, that is to say due to the abnormal decreasing of water-activity "a". However, the increasing of the value of "v_m" is quite probably due to the crystallisation of a part of added salt for the reason that all the amount of the added salts was not dissolved by water in the gelatine, but a part of the added salts are present in a solid state, and there is the layer of water molecule on the surfsce of solid salts as discussed in Experiment Ⅲ. 1(38a) Such a crystallization out of the solid of salts appears in clear, when large influence of salts is exerted on Bound Water of which the water-content "g" is less than 0.5, (that is to say, the water-activity "a" is less than 0.7).

Considering a system of the gelatine molecule, salt molecule and water molecule, the number of layer of adsorption "n", or adsorbed area per unit amount of the sample, "s", perhaps increases. These facts are clearly shown in the value of "s" in Table 16 or the value of "n". On the value of the number of layer of adsorption "n" will be discussed later.

(iii) Discussion on the relation between the observed value of the water-content "$g_{obs.}$" and the calculated value of the water-content "$g_{calc.}$".

As shown in Table 17, the ratio of the water content calculated "$g_{calc.}$" to the water-content observed "$g_{obs.}$" at every water-activity of the samples B and C which are No. 2 gelatine to which NaCl was added in the proportion of 0.3 and 25% to the dried matter of the samples respectively, and of the sample D which is No. 2 gelatine to which $CoCl_2$ was added in the proportion of 27% to the dried matter of the sample, is 0.946 both at 13° and 8°C for the sample to which smaller quantities of salt were added such as sample B and it is 0.898 for the sample to which larger quantities of salt were added such as sample C. That is to say, the ratio decreases with the increasing of the amount of adding salt.

The ratio of "$g_{calc.}/g_{obs.}$" for the sample D to which $CoCl_2$ was added is 0.742 at 8°C, 0.848 at 13°C, and 0.795 on the average. This ratio is less than the ratio of the sample C.

As the values of the ratio of "$g_{calc.}/g_{obs.}$" are less than 1, if these values are considered on the curve of "g—a" as shown in Fig. 13, the curve of "$g_{calc.}$ —a" situates under the curve of "$g_{obs.}$—a" at the same water-activity "a", because the "$g_{obs.}$" is larger than "$g_{calc.}$" (The curve of "$g_{calc.}$—a" is located to the left of the curve of "$g_{obs.}$—a" at the same water-content). Therefore, for three kinds of samples, even if all the added salts are dissociated (to 100%), the abnormal depression of vapour tension, that is to say the abnormal depression of the water-activity naturally still happens. But, as in Rudorff's(34) results discussed in the previous Experiment II, 1(38a), the electrolyte solution always shows such an abnormal depression of the vapour tension. Therefore it is unreasonable to calculate theoretically the value of "$g_{calc.}$" from equations (10) and (11), because this value of "$g_{calc.}$" is widely different from the practical value of water-content "g". The abnormal depression is rightly due to the presence of the electolyte. Therefore, from the fact that the curve of "$g_{obs.}$—a" is situated above the curve of "$g_{calc.}$—a", such conclusion that water regarding Bound Water (corresponding to water of the difference of "$g_{obs.}$" and "$g_{calc.}$") increase by the addition of salts, does not follow. As discussed in the previous Experiment of Ⅲ, 1, the curve of "$g_{obs.}-a$" is considered to be situated under the practical curve of "$g-a$" at the same water-activity, since the greater the quantities of added salts (NaCl or $CoCl_2$) is, the stronger the force of taking water in gelatine will be.

However, the entire amount of added salts is not considered to hydrate with water in gelatine, but a part of the salt will remain as solid. The theoretical base on which the above conclusion was based, will be stated later on the problem of the number of adsorption "n".

(Ⅳ) Results and Discussion on the Fish meat protein and Fish meat.

(A) Experimental results.

Tables 18~20 and Figs. 19~22 show the experimental results on the Cod (*Gadus macrocephalus*) meat protein (this protein consists mainly of 0.2% NaOH soluble protein that is myotelin.), the common Squid (*Ommastrephes sloani pacificus*) meat protein (this protein consists mainly of 0.8% NaCl soluble protein that is myosin) and fresh raw Atka Mackerel (*Pleurogrammus azonas*) meat.

Table 18 and Fig. 19 show the relation between the water-content "g" and water-activity "a" for every sample, which was obtained by the vapour tension method at 15°C and 10°C.

The curves Ⅰ (or Ⅱ) and Ⅰ′ (or Ⅱ′) in Fig. 19 show the experimental results on the Cod meat protein (or Squid meat protein) at 15° and 10°C respectively. The curves Ⅲ and Ⅲ′ show the results on the Atka Mackerel meat at 15° and 10°C respectively. The curve Ⅱ″ shows the water-content "g"—water activity "a" for the Squid meat protein to which NaCl was added in the proportion of 12.77% of the dried matter of the protein. The curve Ⅱ‴ shows the water-content "g"—water activity "a" which was theoretically obtained for the same sample containing salts after the calculation of the amount of hydrated water from every salt solution at each water-activity as a basis on curve Ⅰ which was obtained at 15°C for samples to which no NaCl was added.

Table 19 shows the relation among the water-content "g", water-activity "a", and the values of $\Delta\bar{F}$ and ΔH. Fig. 20 shows the relation between the water-content "g" and the value of $\Delta\bar{H}$. Fig. 21 shows the linear function between "$p/g(p_0-p)$" ("g" is the same as "v" in B. E. T. equation) and "p/p_0" (or "a") which was obtained from the curves of water-content—water-activity for every sample which

Table 18. Relation between the values of water content "g" and the water-activity "a" of Cod fish meat protein, a Squid meat protein and the raw Atka Mackerel meat at 15° or 10°C.

Sample A※				Sample B※		C※		Sample D※				Sample E※	
15°C(p_0 = 1.2788cm.Hg)		10°C(p_0 = 0.9209cm.Hg)		15°C		15°C		15°C		10°C		10°C	
g_1	a_1	g_2	a_2	$g_{3\,(obs.)}$	a_3	$g_3'_{(calc.)}$	g_3'/g_3	g_4	a_4	g_5	a_5	g_6	a_6
4.99	0.968	6.1	0.947	5.6	0.948	—	—	2.90	0.971	6.14	0.947	2.08	0.998
1.20	0.864	2.2	0.953	1.00	0.748	—	—	1.185	0.919	2.20	0.953	1.18	0.914
0.75	0.850	0.419	0.874	0.435	0.679	0.436	1.001	0.751	0.879	0.42	0.874	0.725	0.824
0.402	0.851	0.215	0.683	0.253	0.543	0.310	1.225	0.325	0.804	0.215	0.683	0.413	0.668
0.251	0.740	0.146	0.325	0.184	0.327	0.219	1.190	0.133	0.405	0.146	0.325	0.246	0.548
0.135	0.299	0.108	0.123	0.166	0.246	0.194	1 168	0.104	0.187	0.108	0.081	0.198	0.436
0.095	0.065	0.097	0.075	0.148	0.195	0.180	1,217	—	—	0.098	0.081	0.169	0.319
—	—	—	—	0.147	0.113	0.146	0.986	--	—	–	—	0.161	0.222

Note: (1) The average value of $g_{calc.}/g_{obs.}$ of Squid fish meat protein was 1.153 in the range of 0·7~0 05 of "a".
(2) The estimating curve for the Atka Mackerel meat at 15°C shown as Ⅲ curve in Fig. 19.

Table 19. Relation between the values of water content "g" and of water-activity "a" with the values of ΔF and ΔH of the samples of Cod fish meat protein and the raw Atka Mackerel meat at 15°C.

a (or p/p_0)	g		ΔF (kcal/mol)	ΔH (kcal/mol) 10°~15°C	
	Sample D※	Sample E※	Sample D and E	Sample D	Sample E
0.1	0.078	0.097	1.25	12.9	—
0.2	0.107	0.124	0.87	13.9	—
0.3	0.120	0.140	0 65	16.5	16.9
0.4	0.135	0.157	0.50	13.4	16.5
0.5	0.151	0.183	0.38	8.00	10.6
0.6	0.179	0.227	0.28	2.98	5.96
0.7	0.230	0.304	0.19	0.45	4.86
0.8	0.325	0.480	0.12	—	3.14
0.9	0.900	1.220	0.07	—	—

Table 20. Analytical results of "g－a" curves of the samples of fish meat protein and the raw fish meat by B.E.T. method.

Samples※	Temp.	v_m	v_m'	c	$E_l - E_L$ (kcal/mol)	E_l (kcal/mol)	s (m^2/mg)	v_m'/v_m
D	15°C	0.136	0.173	11.1	1.39	11.16	0.478	1.27
A and D	{A…10°C {D…15° or 10°C	0.111	0.192	30.3	1.91~1.95	11.61~11.65	0.391	1.73
E	15°C	0.111	0.183	30.3	1.95	11.65	0.391	1.65
	10°C	0.146	0.228	16.8	1.59	11.29	0.514	1.56
B	15°C	0.164	0.220	17.33	1.63	11.33		1.34

※in Tables 18~20; Sample A…Squid fish meat protein (0.8% NaCl soln. soluble), Sample B…Squid fish meat protein to which NaCl was added in the proportion of 12.77% per gm of bone dried sample, C…Rotio of "$g_{calc.}$" to "$g_{obs.}$" of Squid fish meat protein at each water-activity, Sample D…Cod fish meat protein (0.2% NaOH soln. soluble), E…Raw Atka Mackerel meat.

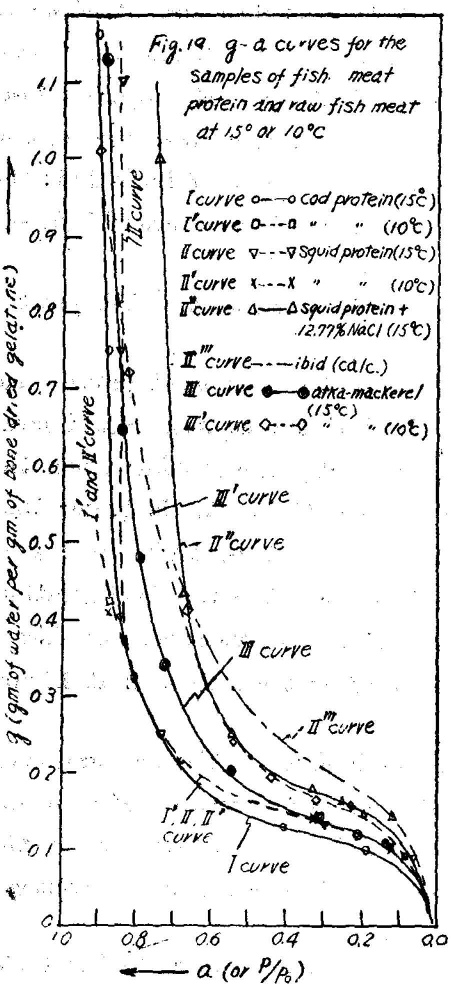


Fig. 19. g - a curves for the samples of fish meat protein and raw fish meat at 15° or 10°C

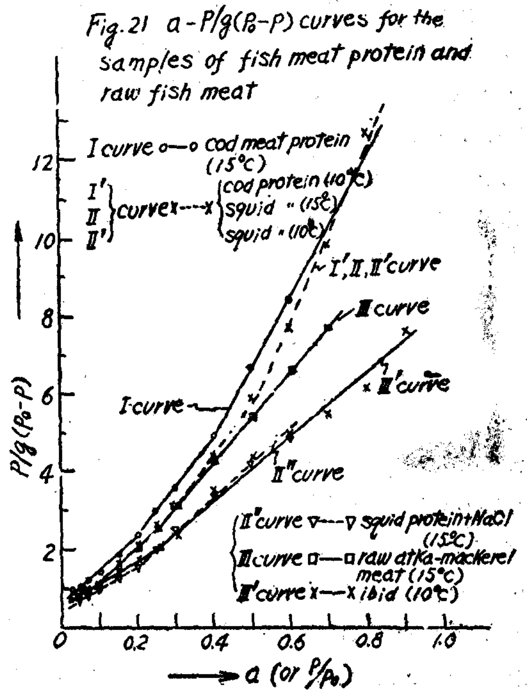


Fig. 21 a - P/g(P₀-P) curves for the samples of fish meat protein and raw fish meat

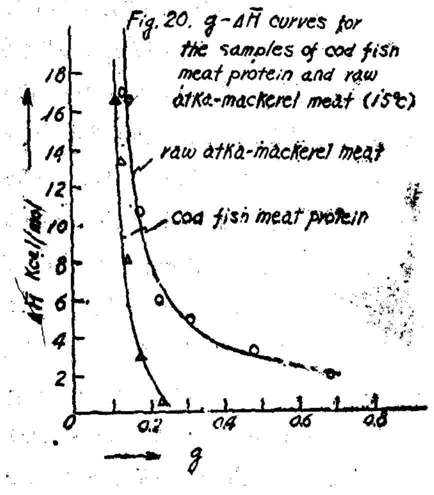


Fig. 20. g - ΔH curves for the samples of cod fish meat protein and raw atka-mackerel meat (15°C)

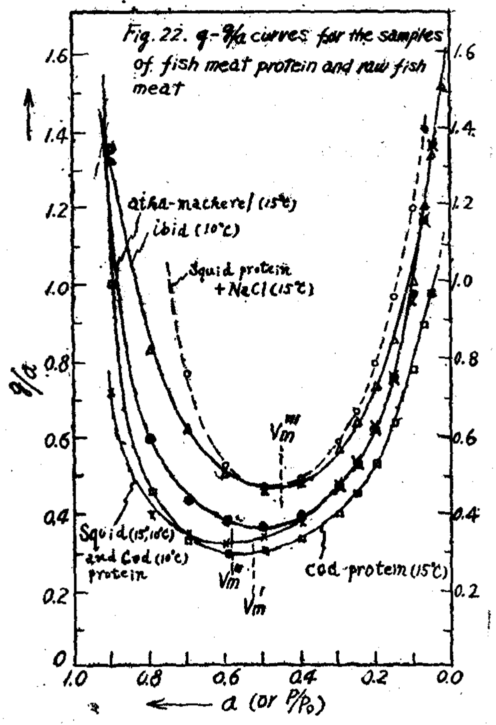


Fig. 22. g - g/a curves for the samples of fish meat protein and raw fish meat

is shown in Fig. 19. Table 20 shows the values of the adsorbed amount of water when the entire adsorbent surface is covered with a complete unimolecular water layer, v_m, B. E. T. constant "c", heat of adsorption at first layer, E_1, apparent heat of adsorption, E_1-E_L, at indefinite adsorption, and adsorbed area per unit of adsorbed substance, s. "v_m'" which is shown in the 4th column of Table 20 is the value of the water-content "g" at the point of curvature of the curve of "g/a−a" which is shown in Fig. 22.

(B) Discussion on the experimental results obtained on the fish meat protein and raw fish meat.

In Fig. 19, curve I′ shows the results at 10°C on the Cod meat protein and curves II and II′ show the results at 15° and 10°C on the common Squid meat protein respectively. These curves I, II and II′ overlap one another when the water-activity "a" is below 0.8. When the water-activity "a" is larger than 0.8, the water-content "g" becomes larger at the same water-activity at higher temperatures. This fact was also recognized in the case of the sample of gelatine; this is perhaps due to the increasing of the area of the active surface of adsorption according to the increasing thermokinetics of water molecules at higher temperatures.

When curve III which shows the results on the fresh raw Atka Mackerel meat is compared with curves I and II which were obtained on the protein of each fish meat, the water-activity "a" of the sample of only fish meat is less than in the samples of fish meat protein at the same water-content according to the existence of salts in the sample.

(a) Discussion on the relation between the water-content "g" and the value of $\Delta\bar{H}$.

In the relation between the water-content "g" and the values of $\Delta\bar{H}$ for Cod meat protein and raw Atka Mackerel meat which is shown in Table 19 and Fig. 20, the value of $\Delta\bar{H}$ increased gradually with the decreasing of the water-content "g" as well as for gelatine. For example, for the raw Atka Mackerel fish meat, the value of $\Delta\bar{H}$ is 4~5 kcal/mol at the neighbourhood of 0.3 of the water-content "g" (about 23% of the amount of water in the sample), and is 16 kcal/mol at the neighbourhood of 0.15 of "g" (about 13% of the amount of water); for the Cod meat protein, as well as for the raw Atka Mackerel meat, the value of $\Delta\bar{H}$ is less than 0.2~0.5 kcal/mol at the neighbourhood of 0.2 of "g" (about 20% of the amount of water), and the value of $\Delta\bar{H}$ increased rapidly with the decreasing of the water-content "g". The water-activity "a" corresponding to the water-content "g" at the rapid increasing of the value of $\Delta\bar{H}$ is about 0.7 for the raw

Atka Mackerel meat, and is about 0.74 for the Cod meat protein according to Fig. 19. The value of $\Delta \bar{H}$ increases rapidly when the water-activity "a" is less than those values. The properties of water in the parts of B and C of which the water-activity "a" is less than 0.7, are different from the properties of water in the part of A of which the water-activity "a" is above 0.7, that is to say, the former perhaps combines with the molecules of the fish meat protein by the hydrogen bonds. This conclusion holds as in the case of gelatine.

(b) Discussion on the analyzed result of the curve of "g—a" by B. E. T. equation.

As shown in Fig. 21, the relation between the water-activity "a" (the relative vapour pressure "p/p_0") and "$p/g(p_0-p)$" is a linear function when the water-activity "a" is in the range of 0.05～0.2 in all the samples, but the angle of the inclination becomes larger with several portions by new increasing of the area of the adsorption surface, when the water-activity "a" is more than 0.2 just as in the case of gelatine.

The values of B. E. T. constant "c", the values of "v_m" which are adsorbed amount of water, when the entire adsorbent surface is covered with a complete unimolecular water layer, the values of heat of adsorption at the first layer of the adsorption, E_1, the values of apparent heat of adsorption "$E_1 - E_L$", where the adsorption comes to the state of saturation and the values of the surface area per unit of adsorbed substances, s, were as shown in Table 20. Here, the values of "c" and "v_m" were calculated from the B. E. T. equation (7) after substituting every value of "p/p_0" and "$p/g(p_0-p)$" to the terms of that equation. The values of "$E_1 - E_L$" were calculated from equation (8).

The values of "v_m" is 0.10～0.14 for the Cod meat protein and the common Squid meat protein, and range 0.1～0.15 for the raw Atka Mackerel meat. The values of "v_m" of gelatine (0.18～0.19) are larger than the "v_m" of the fish meat protein. The values of "s" are 0.4～0.5 for all the samples, those values are the same for the gelatine molecule. The discussion on the values of "s" is left for future experiment. The values of "v_m'" which are the value of the water-content "g" corresponding to the minimum value of "g/a" for the curve of "g/a—a" for fish meat protein and raw fish meat in Fig. 22, are shown in Table 20. The ratio of "v_m'" to "v_m" is 1.51 on the average, almost the same as for gelatine. The values of "a" corresponding to every "v_m'" is in the range of 0.5～0.7.

From those facts, water in the fish meat protein and raw fish meat at the parts of B and C of which the water-activity "a" is less than 0.7 is molecular theoretical Bound Water on the basis of the idea of hydrogen bond, as well as in the case of gelatine.

(c) Discussion on the protein of the common Squid meat to which salts are added.

The relation between "$p/g(p_o-p)$" and "p/p_o" shows linear form (shown as II″ curve in Fig. 21) when the water-activity "a" is in the range of 0.05~0.2 for the Squid meat protein to which NaCl was added in the proportion of 12.77% to the dried matter of the sample. When the II″ curve was compared with II-curve which pertains to the sample to which no salt was added, the angle of inclination of curve II″ is smaller than that of the II curve. Consequently, the values of "c" which were calculated from the B. E. T. equation (7), E_1-E_L, and "E_1" become samller, on the other hand, the values of "v_m" and "s" become large. These facts were also recognized in the case of the addition of salts to gelatine. This is generally clarified that the heat of adsorption decreases by the addition of salts.

The ratio of "$g_{calc.}$" which was calculated from equations (10) and (11) for the Squid meat protein to which salts were added at every water-activity "a", to "$g_{obs.}$" was shown in the 12th column in Table 18. The ratio was 1.153 on the average in the range of water-activity 0.7~0.05. The curve of "$g_{calc.}-a$" is situated above the curve "$g_{obs.}-a$" at the same water-activity "a". (The former is situated to the right of the latter at the same water-content "g").

Therefore, as in Experiment III, I(33) discussed on the relation between the sample No. 2 gelatine and the salt, a part of the added salt hydrated with water in gelatine, and the rest crystallized out as solid. When to the Squid meat protein NaCl was added, the same result is obtained in the case of gelatine.

(III) Discussion on the number of adsorption layers, "n".

Brunauer et al(32a) have offered to equation of adsorption of definite layer (9) besides equation (7) of adsorption of indefinite layer. If the values of adsorbed water-content "g" and the water-activity "a" which were practically estimated are substitued to the terms of equation (7) and the relation of "$p/g(p_o-p)$" and "p/p_o" is obtained, then the values of "v_m" and B. E. T. constant "c" will be calculated. If these values of "v_m" and "c" are substitued to the terms of equaton (9), the values of the amount of adsorbent "v" (the same as the water-content "g") will be calculated in the case of the numbers of adsorption layers "n" as 1, 2, 3, 4, • • • corresponding to the values of "x" (this is equal to the water-activity "a" or "p/p_o)". In this case, the values of "c" and "v_m" are difinite, and various curves of "$g-a$"

corresponding to the numbers of adsorption layers of the samples are obtained.

Figs. 23, 24, 25, and 26 show the curves of "g−a" which were plotted for the samples of No. 2 gelatine, No. 3 gelatine, No. 2 gelatine to which NaCl was added in the proportion of 0.3% to the dried matter, No. 2 gelatine to which NaCl or $CoCl_2$ was added in the previous proportions of the dried matter, respectively, in the case of various numbers of adsorption layers "n" (n=1∼6) at 13°C. Fig. 27. shows the curves of "g−a" which were obtained for the numbers of the adsorption layer "n" (n=1∼6) according to equation (9) on the basis of the values of "v_m" and "c" of Katz at 23°C. The values of "v_m" and "c" of those samples were used from Table 16. The dotted lines in those Figures show the curves of "g−a" which were observed for every sample.

Fig. 28, 29, and 30 show the curves of "g−a" which were plotted for the samples of Cod meat protein, common Squid meat protein, raw Atka Mackerel meat and the common Squid meat protein to which NaCl was added in the proportion of 12.77% to the dried matter, respectively, in the case of various numbers of adsorption layers "n" (n=1∼6) at 15° or 10°C. The dotted lines in those figures show the curves of "g−a" which were observed for every sample. The values of "v_m" and "c" of those samples were used from Table 20.

Brunauer et al. said that equation (7) becomes a very good approximation to equation (9), when the value of "a" is small and the numbers of adsorption layers "n" are as large as 4∼5. So the observed curve of "g−a" should become a very good approximation to the curves of "g−a" which are obtained by the B. E. T. equation (9).

However, as in Fig. 23 which shows the curves for the sample of No. 2 gelatine, the observed curve of "g−a" (curve I) agreed with the curve of "g−a" (curve I′) which is obtained when the number of adsorbed layers "n" is 2, when the values of the water-activity "a" are in the range of 0.40∼0.0, but when the water-activity "a" is more than 0.4, the observed curve I slips rapidly away to one side of curve I′. And when the water-activity "a" is more than 0.85, the observed curve I keeps away in its upper part from the curve of "g−a" at which the number of adsorption layers "n" is 6. These facts are perhaps due to the increasing of numbers of adsorption layers by yielding many new surfaces of adsorption with the increasing of the amount of adsorbed water "g" in the process of the adsorption.

Table 21 shows the values of "n" which are adequate to the initial process of adsorption from the relation between the curves I and I′ as shown in Figs.

23~30, and the values of "a" corresponding to the values of "n" in the applicable range of the curves of I and I′.

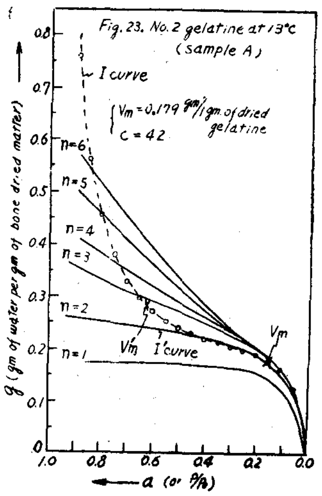


Fig. 23. No. 2 gelatine at 13°C (sample A)

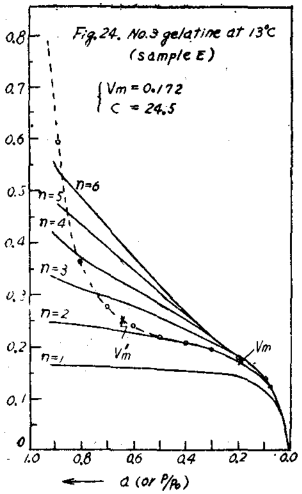


Fig. 24. No. 3 gelatine at 13°C (sample E)

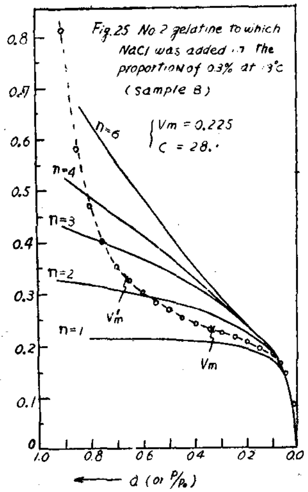


Fig. 25 No 2 gelatine to which NaCl was added in the proportion of 0.3% at 13°C (sample B)

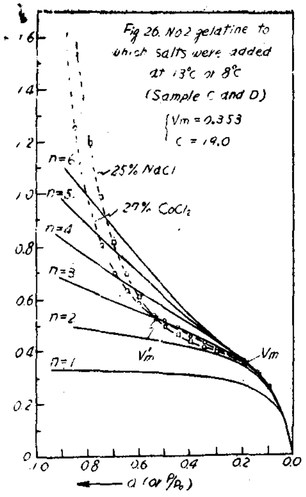


Fig 26. No2 gelatine to which salts were added at 13°C or 8°C (Sample C and D)

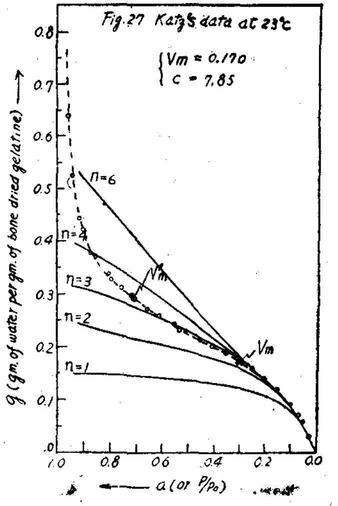


Fig. 27 Katz's data at 23°C

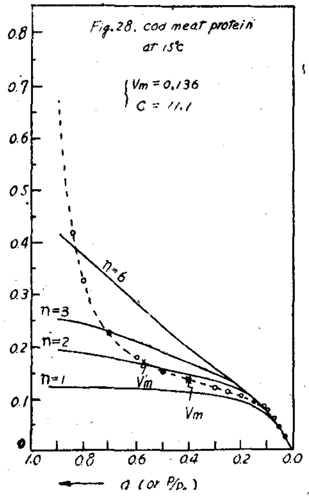


Fig. 28. cod meat protein at 15°C

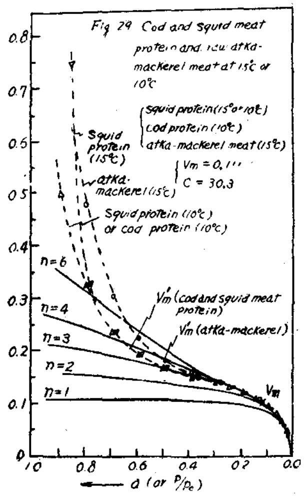


Fig 29 Cod and squid meat protein and raw atka-mackerel meat at 15°C or 10°C

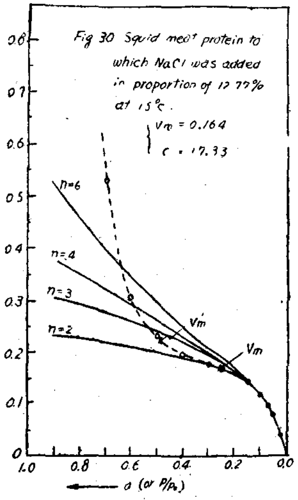


Fig 30 Squid meat protein to which NaCl was added in proportion of 12.77% at 15°C.

Table 21. Relation between the number of adsorption layers "n" and the water-activity "a" corresponding to the values of "n" in the applicable range of the curves I and I′ in Figs. 20~30.

Samples	No. 2 gelatine		No. 3 gelatine		No. 2 gelatine to which NaCl (0.3%) was added.		No. 2 gelatine to which NaCl (25%) or CoCl$_2$ (27%) was added.	Katz(10a)
Temp.	13°C	8°C	13°C	8°C	13°C	8°C	13° and 8°C	23°C
Number of adsorption layers "n"	2	2~3	2	2	1~2	1~2	3	3
Applicable range of water-activity "a"	0.0~0.45	0.0~0.6	0.0~0.55	0.0~0.58	0.0~0.6	0.0~0.55	0.0~0.55	0.0~0.6

(Table 21. Continued)

Samples	Cod meat protein.		Squid meat protein.	Squid meat protein to which NaCl (12.77%) was added.	Raw Atka Mackerel meat.	
Temp.	15°C	10°C	15° and 10°C	10°C	15°C	10°C
Number of adsorption layers "n"	2	3	3	2~3	3~4	3
Applicable range of water-activity "a"	0.0~0.55	0.0~0.5	0.0~0.5	0.0~0.53 ("n" was 2 in the range of 0.0~0.4)	0.0~0.53	0.0~0.45

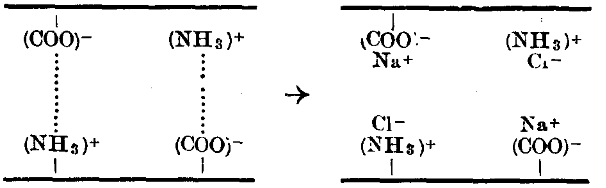
According to Figs. 23~30 and Table 21, the value of "n" in the initial process of adsorption was 2 or 3, and the applicable range of the values "a" to the observed curve is 0.0~0.45 (or 0.6).

The author can explain that the value of "n" is 2 under the same consideration as Bull(31). That is to say, protein molecules in the solid state are linked together to form coherent planes whose exposed surfaces are hydrophile. Water is adsorbed between these planes. Therefore the number of adsorption layers is naturally 2. With the advance of hydration, when the so-called water-water bridge was formed, the value of "n" is perhaps 3. With the further advance of hydrating, the protein tends to dissolve in water and proceeds to indefinite adsorption. The states of binding of hydrophilic plane with water molecule are different with the kinds of protein, e. g., with the molecules of fibre protein such as keratine, collagen or sphero-protein such as myosin, myogen, myotelin, and others The fibre protein has chain-bindings which linked together in parallel and the sphero-protein has some cyclic-bindings in a part of its structure.

However, according to Table 21 obtained by the author, various sphero-proteins used will also have chain-binding in the parallel state with hydrophilic plane.

Considering from the influence of the addition of salts, according to Table 21, Figs. 25 and 26, the number of adsorption layers "n" is 3 or 4, except in case of the addition of salts in small proportion to the dried matter, e. g., the sample to which NaCl was added in the proportion of 0.3%. In general, by addition of salts, "n" will increase by one.

In comparing with the results of the common Squid meat protein, the same sample to which salts were added and the raw Atka Mackerel meat which were shown in Figs. 29 and 30 respectively, "n" of the sample to which salts were already added in large quantity is recognized to be 3 from the initial process of adsorption. The fact that "n" is increased by one by means of the addition of salts is perhaps because in the protein to which salts were added, the salts link with hydrophilic radical in the side chain of the protein as follows:



This state of binding was offered by Lloyd(4b). Therefore, three of the adsorption layers were formed in the chain-bindings of the protein, as well as the linkage of two molecules of water in the chain-bindings of the protein. Consequently the values of "v_m" and "s" should naturally increase.

As shown in Figs. 23—30, it is commonly recognized that the adsorption process follows rapidly to the adsorption of indefinite layers more than the water-content "g" corresponding to the point of "v_m'" in figures. The values of the water-activity "a" corresponding to the values of "v_m'" is less than 0.6—0.7 (in the part of B). This is due to the fact that water whose water-activity "a" is less than 0.7 in the refined sample to which no salt was added, is the so-called molecular theoretical Bound Water.

(3) Conclusion

The author has discussed the thermodynamic properties of water below 0.7 of "a" in the curves of the water-content "g"—water-activity "a" for the common Squid meat protein, Cod meat protein, raw Atka Mackerel meat and gelatine, which were determined by the vapour tension method at two different temperatures, and discussed also the analyzed result by B. E. T. method; the author is certain that water below 0.7 of "a" in the curve of "g—a" should be the so-called molecular theoretical Bound Water on the basis of the idea of hydrogen bond formation.

4. Comparison of vapour tension method with other thermodynamic method.

As in the Experiment III, I(38a) stated, Briggs(10b, c) has said that the amount of Bound Water in the sample varies with the water-activity "a", e. g, "a" corresponding to the concentration of solute in the solvent method, and "a" corresponding to the temperature to freeze the sample in the freezing method, and he has examined the relation of the water-activity "a" and the water-content "g" (gm of water per gm of the dried matter) by isotenoscope method (vapour tension method) and by the method of estimating the depression of the freezing point using the arabian gum, Na-casein; then he has ascertained that the observed values which were obtained by these methods are present on the same contineuous curve of "g—a". According to Briggs' result, water having string binding were estimated in the range of small value of "a" on the curve, but in the range of large value of "a", water containing weak binding, were also estimated at the same time with those water having strong binding.

Recently Higashi and Nukazawa(1c) have arranged the results which had previously been obtained by Moran(7), Lloyd and Moran(4b) or Katz(10a) by various thermodynamic methods using gelatine, and when the values of water-content "g" are plotted in the ordinate, and the values of water-activity "a" are plotted in the abscissa, the values obtained by the freezing method, the swelling tension method, colorimetric method (except the vapour tension method by Katz) are observed to be present on the same curve of "g—a". But the results obtained by Katz by means of the vapour tension method were observed to be much below the above mentioned curve, when the value of "a" is less than 0.95.

Higashi et al. have said that the results obtained by Katz were estimated at 23°C and the curve of the water-content "g"—water activity "a", obtained by vapour tension method, varies generally more or less with temperatures. Therefore, when the results obtained by Katz are to be compared with results obtained by other methods, the results by vapour tension method should be obtained at 0°C.

Here, the present author will describe his results of comparing of No. 2 geltine and No. 3 gelatine at 13° and 8°C by means of vapour tension method with the curves of "g—a" obtained by various other thermodynamic methods for the sample of gelatine.

(1) Examintion method.

(I) Calculating of the values of the water activity "a" in the sample by every thermodynamic method.

In the case of vapour tension method, the value of "a" is manifested approximately by "p/p_0" in equation (3) written in Exp. Ⅱ, 3, (1), (iii), (A) which shows the water activity.

In the freezing method, the value of water-activity "a" is manifested by equation (1) neglecting the heat of dissolution while the values of water-content are large.

With the decreasing of the amount of water-content, (that is to say, with becoming high in the concentration of the sample), the heat of dissolution becomes large, and can not be neglected. If the freezing point of a sample is T′°K, the water-activity "a" at T″°K can be calculated from equations (12) and (13).

$$\log a+0.004211\,(273.1-T')+0.0000022\,(273.1-T')^2=x \text{ - - - - - - - - } (12)$$

$$x=-\bar{L}\frac{T''-T'}{2.303RT'T''}+(\bar{C}_p-C_p^\circ)\left(\frac{T''-T'}{2.303RT'}-\frac{1}{R}\log\frac{T''}{T'}\right) \text{ - - - - - - - - } (13,$$

where $\bar{L}$ is the difference between the differential heat content of water in the sample and the pure water at T″°K, $\bar{C}_p$ and C_p° is the heat capacity at constant pressure of water in the sample and the pure water respectively.

If 273.1°K (0°C) is substituted to the term T″°K in equations (12) and (13), the curve of "g−a" at 0°C is calculated from the relation between the water-content "g" and freezing point T′°K.

In the swelling tension method, the relation between the swelling tension of the sample "p" and the water-activity "a" is calculated approximately from equation (14) by Katz.

$$-p=\frac{RT}{v_0}\ln a \text{ - } (14)$$

where "v_0" is molal volume of water in the sample. The greater the value of "p" (the smaller the water-contnet of the sample is), the smaller the value of "a" is. With approaching of the value of "p" to 0, the value of "a" approaches to 1.

As above stated, every relation between "g" and "a" is obtained from various thermodynamic estimating methods of Bound Water.

(II) Calculating of the values of the water activity "a" from the author's results by the vapour tension method.

To compare with each other the relations of "g" and "a" obtained by various thermodynamic methods, the relation between "g" and "a" should be compared from the results obtained at 0°C.

However, the author is very sorry to be unable to carry out the vapour tension method at 0° using gelatine or fish meat.

Accordingly the relation of "g−a" was calculated by the equation of Clausius-Clapeyron (written in the Experiment, equation (4), II, 3) from the results obtained at 15° and 8°C for the samples of No. 2 gelatine and No. 3 gelatine, and then the author obtained the values of water-content "g_1", and differential molal enthalpy $\Delta\bar{H}$.

$$\Delta\bar{H} = -\frac{RT_1T_2}{T_1 - T_2}(\ln a_2/a_1)$$

where "a_2" and "a_1" are the water-activities at $T°_1$ and $T°_2$K corresponding to the definite water content "g_1".

Supposing that the values of $\Delta\bar{H}$ at 0°~13°C or 13°~23°C are constants respectively, the water-activity "a_2" at 0° or 23°C was reckoned from the same water-content "g_1". The results at 23°C were calculated to be compared with the results obtained by Katz.

If the values of $\Delta\bar{H}$, a_1 and T_1 are known in the above equation, the value of "a_2" can be calculated. But the values of the water-content "g_1" corresponding to the known values of "a_1" varies with the estimating temperatures T_1. If there are two different temperatures, A and B, and if $(T_1)_A > (T_1)_B$, then it becomes $(g_1)_A < (g_1)_B$. That is to say, it is clear that in the case of the same water-activity "a_1," the lower the estimating temperature is, the greater the value of "g_1" becomes, as also in the Experiment Ⅱ, 3. Therefore, the values of "a_2" vary with the values of "g_1" which should be as a standard. Therefore, the values of the water-activity "a_2" of which T_2 is 0°C and 23°C respectively are calculated from the values of the water-content "$(g_1)_{13°C}$" and "$(g_1)_{8°C}$" of which A and B of estimating temperatures are 13°C and 8°C respectively. The interpolate lines at every point which are on the relation between the value of "a_2" and "$(g_1)_{13°C}$" or "$(g_1)_{8°C}$" plotted were regarded as the curve of "g−a" at 0° or 23°C.

The values of "g", "a" and $\Delta\bar{H}$ were employed from Table 14 as given in Experiment Ⅲ, 3.

The curves of "g−a" which were obtained by the mothod as above stated at 0°C and 23°C by author were compared with the curve of "g−a" which was calculated from equations (12), (13) and (14) by Higashi (Moran, Lloyd and Moran and Katz by means of freezing method, calorimetric method and swelling tension method using gelatine), and with the curves of "g−a" which were obtained by means of vapour tension at 23°C by Katz.

(3) Results and Consideration.

The curves of "g−a" at 0° and 23°C which were reckoned from the results obtained at 13° and 8°C for the sample of No. 2 gelatine are shown in Fig. 31 (Table 22). The similar curves for the sample of No. 3 gelatine are shown as in Fig. 32 (Table 23).

In Figs. 31 and 32, the curves I and Ⅱ are the observed curve of "g−a" at 13° and 8°C respectively, the curves Ⅲ and Ⅳ are the calculated curves at 0°

Table 22. Relation between the values of water content "g" and the water-activity "a" of the sample of No. 2 gelatine at 23,° 13°, 8° and 0°C.

Water-activity $(a_1)_{t1=13^\circ C}$ or $(a_2)_{t2=8^\circ C}$	No.2 gelatine Water-content $(g_1)_{t1=13^\circ C}$	$\Delta\overline{H}$(kcal/mol) (13°~8°C)	Water-activity $(a_0)_{t0=0^\circ C}$	Water-activity $(a_3)_{t3=23^\circ C}$	No.2 gelatine Water-content $(g_2)_{t2=8^\circ C}$	Water-activity $(a_4)_{t4=0^\circ C}$	Water-activity $(a_5)_{t5=25^\circ C}$
0.1	0.165	12.5	0.035	0.210	0.170	0.052	0.310
0.2	0.189	7.3	0.108	0.324	0.207	0.136	0.387
0.3	0.206	7.12	0.165	0.458	0.212	0.207	0.572
0.4	0.221	7.12	0.223	0.611	0.240	0.276	0.762
0.5	0.243	6.35	0.294	0.729	0.272	0.358	0.889
0.6	0.270	6.49	0.394	(0.882)	0.310	0.427	(1.08)
0.7	0.330	3.12	0.539	0.843	0.380	0.590	0.929
0.8	0.456	1.67	0.696	0.884	0.524	0.733	0.931
0.9	0.760	1.47	0.796	0.982	0.980	0.833	(1.03)
0.95	1.150	0.8	0.888	0.996	1.40	0.911	(1.02)

Table 23. Relation between the values of water content "g" and the water-activity "a" of the sample of No. 3 gelatine at 23,° 13°, 8° and 0°C.

Water-activity $(a_1)_{t1=13^\circ C}$ or $(a_2)_{t2=8^\circ C}$	No.3 gelatine Water-content $(g_1)_{t1=13^\circ C}$	$\Delta\overline{H}$(kcal/mol) (13°~8°C)	Water-activity $(a_0)_{t0=0^\circ C}$	Water-activity $(a_3)_{t3=23^\circ C}$	No.3 gelatine Water-content $(g_2)_{t2=8^\circ C}$	Water-activity $(a_4)_{t4=0^\circ C}$	Water-activity $(a_5)_{t5=23^\circ C}$
0.1	0.140	1.4	0.031	0.230	0.140	0.021	0.358
0.2	0.178	8.77	0.096	0.331 •	0.178	0.126	0.443
0.3	0.199	6 60	0.173	0.444	0.202	0.212	0.646
0.4	0.210	7.11	0.220	0.611	0.220	0.276	0.762
0.5	0.219	6.35	0.204	0.729	0.237	0.358	0.889
0.6	0.232	5.80	0.369	0.847	0.270	0.443	(1.01)
0.7	0.274	3.40	0.527	0.856	0.333	0.586	0.953
0.8	0.360	2.70	0.638	0.939	0.450	0.695	(1.02)
0.9	0.588	2.15	0.752	(1.02)	(1.120)	0.848	(1.09)
0.95	1.93	1.24	0.856	(1.02)	—	—	(1.06)

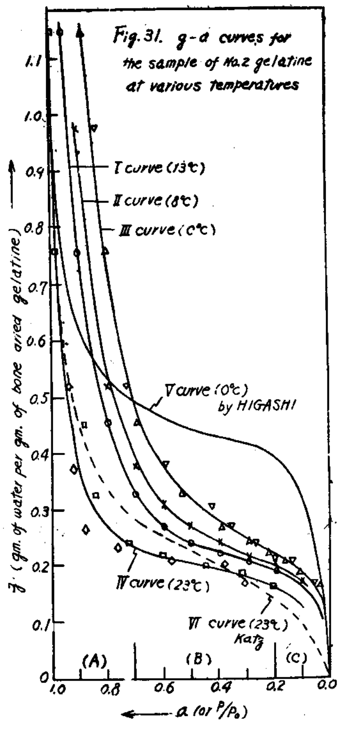


Fig. 31. g-a curves for the sample of No. 2 gelatine at various temperatures

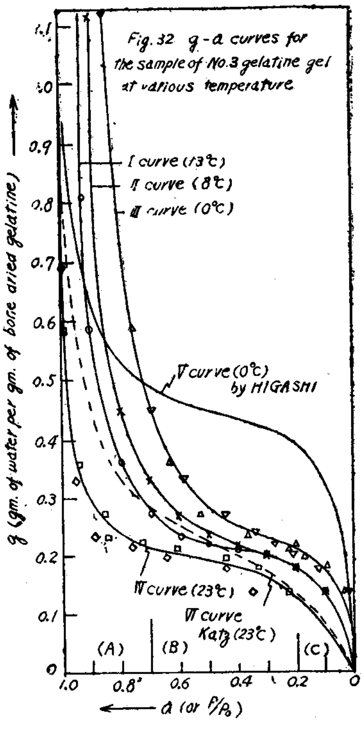


Fig. 32 g-a curves for the sample of No. 3 gelatine gel at various temperature

and 25°C respectively. Curve V was calculated by Higashi et al., from the results obtained by freezing method and other thermodynamic methods at 0°C. Curve VI was plotted the results by Katz by means of vapour tension measurement at 23°C.

From Fig. 31 and Fig. 32, the following facts are commonly recognized.

(i) When curve III of "g−a" at 0°C, calculated from the author's results, was compared with curve V, calculated by Higashi et al., the two curves crossed at the neighbourhood of 0.7 of the value of "a", curve V being situated on the left of curve III at the same water-content "g" above 0.7 of value of "a". Curve V is situated on the right of curve III at the same water-content "g" below 0.7 of the value of "a". As to curve V, when the value of "g" is below 0.5, the value of "a" decreases rapidly with the water-content "g", therefore the angle of inclination of curve V is very gentle in the part of B (0.7〜0.2 of "a").

(ii) When curve IV of "g−a" at 23°C, which was calculated from the author's results, was compared with curve VI, which was obtained by Katz, curve IV agreed with curve VI at above 0.95〜0.98 of the value of "a", and curve of Katz turned away to the right of curve IV at below 0.98 of the value of "a" as seen in every figure.

Considering the fact of (i), in the case of estimation by the freezing method, it is getting difficult to obtain the relation between "g" and "a" at equilibrium state, with depressing of freezing point of the sample according to the decreasing of the water-content "g" due to the non-uniformity of the freezing process of the sample. Therefore, the curve of "g−a" which was obtained by vapour tension method is to be considered perhaps more nearly real than the curve of "g−a" which was obtained by the freezing point determination so far as the range of the value of "a" is small.

If the phenomenon of capillary condensation is taken into consideration respecting curve "g−a" which was obtained by the vapour tension method, the curve of "g−a" which was practically observed will give the smaller value of "a" than the real value of "a" which is shown in the curve of "g−a" of the sample. Therefore, the difference between the curves of "g−a" which were obtained by the vapour tension and the freezing method respectively will become greater.

Briggs(10b) also said that when attempting to determine the water-activity "a" in a system, it is, of course, seldom possible to obtain the accurate values from vapour tension measurements that can be gained from freezing point determination, especially when the activity of the water in the system under consideration, "a", is above 0.9〜0.95, that is not greatly different from that of pure water. However, with the differing of properties of water from that of pure water, the calculated water-activity by freezing point determination is much less accurate than is to be obtained by vapour pressure measurement. The facts in (i) are really recognized.

Next considering the fact of (ii), the difference of curves IV and VI at below 0.98 of water-activity is due to the different mechanism of dehydration or adsorption of water by samples especially by the dehydrating in the author's experiments, and by both the dehydration and adsorption in the Katz's experiments.

In general, the curve of "g−a" which is obtained by the vapour tension method varies with the difference of the components or hydrogen ion concentration of the protein, or with the different mechanisms of dehydration, or with the adsorption of water, even though the protein is the same. Therefore, curves IV and VI are properly not in agreement. However, when the water-activity is above 0.98, that is to say, as far as the thermodynamic properties of water are not so greatly different from those of pure water, it is recognized that there is no great difference of the observed value with the kinds of the sample. Therefore, curves IV and VI are almost in agreement with each other.

(3) Conclusion

Considering the curves of "g−a" for the same sample of gelatine at the same temperature (0°C) which were obtained by vapour tension determination and by the freezing point measurement, both curves cross at the neighbourhood of 0.7 of the water-activity of the sample, but the value of the water-activity "a", which is obtained by the freezing point measurement, is much higher than that by the vapour tension determination at the same water-content "g", when the value of "a" is above 0.7, and "a" of the former is conversely less than that of the latter when the value of "a" is below 0.7. The disagreement of the two curves obtained by the two different measurement due to the non-attainment of equilibrium state by the freezing point measurement when the value of "a" is below 0.7.

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