POLAROGRAPHIC STUDIES ON ADSORPTION FROM SOLUTIONS; DISCONTINUITIES IN ADSORPTION ISOTHERMS AND THE APPLICATION OF THIS PHENOMENON TO THE MOLECULAR-WEIGHT DETERMINATION OF PROTEINS

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In the isothermal adsorption of gas or from solutions by solids, the relation between the pressure or the concentration in equilibrium and the amount adsorbed is, in general, expressed by Freundlich's adsorption isotherm, Langmuir's equation or similar equations, which graphically show a continuous, smooth, curve.

In 1928, however, A. J. Allmand and co-workers (1, 2) found that, in adsorption of gas by porous substances, the adsorption isotherms were discontinuous. Shortly after A. F. Benton and T. A. White (3) found a number of discontinuous isotherms for adsorption of gas on several metals. Thereafter, a number of investigators have studied this phenomenon, but there were some cases where no discontinuities of the postulated type were found (4) though investigated by the same method as has been used by the previous investigators. Hence, the discontinuities can not yet be considered to be a general characteristic.

As to adsorption in solutions, R. Chaplin (1932, (5)) was the first to find the discontinuities. He investigated adsorption of phenol by active charcoal, but his experiments were simple and his methods not indisputable. Ch. Ockrent (6) who investigated the adsorption of organic acids on active charcoal, could not find the discontinuities. Since then investigations on this subject have not been reported, and the discontinuities in adsorption isotherms from solutions remain unknown.

A number of investigators who found the irregularities in adsorption isotherms before Allmand and Benton confirmed the discontinuities, attributed them to experimental errors. As is evident from this, demonstrating the discontinuities is influenced greatly by the sensitiveness and accuracy in the method of analysis employed in the experiments and it is particularly so in adsorption in solutions. It is presumably for this reason that to now investigation of the discontinuities in adsorption from solutions has not been made.

Further, the peculiarity in the substance of adsorption in solutions compared with the case of gas exists in that adsorbents are not always solids, but are in

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many cases colloidal particles. In biology and medicine, the latter case is by far the more frequent and of more significance. With nearly all the usual methods of analysis it is, first of all, necessary to remove colloidal particles as adsorbent in order to determine free ions or molecules not adsorbed. For this purpose dialysis and other methods have been employed, but, with these methods it is difficult to expect accurate results. In this point lies the greatest difficulty in the investigations on adsorption from solutions by colloidal particles.

In order to solve these two difficulties I employed the polarographic method of analysis and demonstrated that in general this excellent accurate micro-analytical method is the most suitable for experiments of adsorption in solutions and it is possible to determine only free ions not adsorbed in presence of proteins. In the present work, the adsorption of lead ions from lead nitrate aqueous solutions by active charcoal and such protein molecules, as hemoglobin, egg albumin and gelatin was investigated by the polarographic method, and all these adsorption isotherms have been found and demonstrated to be discontinuous.

Further, a study of the mechanism of this phenomenon was conducted and the possibility of determining simply and accurately the molecular weight of proteins was demonstrated.

EXPERIMENTAL MATERIALS AND METHOD

Materials. Active charcoal, hemoglobin, egg albumin and gelatin were employed as the adsorbents.

Active charcoal. Vegetable active charcoal was used, well powdered, dried at 105° C. and made into a 0.7% suspension.

Egg albumin. Merck's egg albumin was used, well powdered, dissolved in water to give a 0.2% solution and filtered through accurately weighed filter papers. But, as this albumin powder was not specially dried, the same sample was dried by heat at 105° C. and its water content was measured. The weight of albumin actually dissolved in the aqueous solution used in the experiments was measured by deducting the water content and the amount of undissolved sediment on filter papers.

Hemoglobin. Takeda's ox hemoglobin powder was used. The solution was made up in the same way as in the case of egg albumin aqueous solution.

Gelatin. Pure granular gelatin was used. As the quality of gelatin solution varies according to the condition under which it is made up, a 0.2% solution was always prepared strictly under the same conditions.

Adsorbate. As adsorbate lead nitrate was used. From a 5.000×10^{-2} M/L stock solution, original solutions of different concentration were made by dilution with water two times. The ranges and differences in concentration were decided according to the ranges and intervals of experimental points suitable for the experiments. The original solutions were prepared immediately before each experiment.

Experimental method. The experiments were carried out according to that usual for experiments of isothermal adsorption in solutions. 10 ml of suspension

or solution of adsorbent was introduced into each of a series of 50 ml conical flasks, the number depending upon the experimental need, from about 20 to 30. To each 10 ml of lead nitrate aqueous solution of different concentration, was added and well stirred. In consequence the concentration of both adsorbent and lead nitrate is reduced to half. This is the concentration of lead before adsorption begins, namely, an initial concentration. These flasks which contain the mixture of adsorbent and lead solution, were plugged with rubber stoppers, and immersed up to the neck in a thermostat kept at $20.0^{\circ}\text{C} \pm 0.1^{\circ}$. During the first 12 hours the flasks were shaken every 20 minutes. Hereafter they were allowed to stand and when, from 20 to 30 hours after the commencement of the procedure, the equilibrium of the system was attained, the supernatant in each flask was withdrawn, and free lead ions in the supernatant fluid were determined polarographically. The number of hours the system requires before attaining perfect equilibrium was experimentally decided to be 20 hours both in the case of active charcoal and proteins. Each experiment was carried out in a neutral medium.

The calculation of the adsorbed quantity of lead ions was made as follows: the lead ion concentration in the supernatant liquid determined by the polarographic method corresponds to the equilibrium concentration, c , and the difference between the initial and the equilibrium concentration corresponds to the adsorbed amount, x/L (10^{-4} g-atom/L). As x/L represents the adsorbed amount when the volume of the mixture of the adsorbent and the adsorbate is assumed to be one litre, the amount adsorbed by one gram of adsorbent, x/m (10^{-4} g-atom/1 gram) is calculated from this and the adsorption isotherm is, consequently, obtained from the data of c and x/m .

Method of analysis. The analysis for lead was carried out by the polarographic method, whose quantitative-determination error is 2%, taking into consideration three times the value of probable error.

The half-wave potential of lead in presence of proteins. A lead nitrate solution is added to a protein solution, and the mixture is stirred sufficiently for 24 hours, and, to the supernatant liquid is added KNO_3 to the extent of 0.1 N as the supporting electrolyte, it is then electrolyzed, and a polarogram is obtained showing a remarkable reduction wave. Whether or not this reduction wave is due to free lead ions not adsorbed is then examined. In this case what is reduced at the cathode is considered to be as follows: (1) free lead ions which are not adsorbed by proteins, (2) lead complex ions with a certain radical of protein molecules, (3) lead ions already adsorbed on protein molecules may be liberated and reduced as free ions when free ions at the surface of the cathode have been completely exhausted and supplied by diffusion, (4) though it is universally known that such protein molecules, as are employed in the present work, cannot be reduced, they may change their quality by adsorbing lead ions and may become reducible and thus a reduction wave for the latter may result. These four can be estimated, and the half-wave potential of these reduction waves in the presence of proteins can be determined experimentally. The results are shown in table 1. In each case it is -0.44 volt vs. N.C.E. and corresponds to that of a pure lead nitrate solution, and moreover, the shape of the

Table 1
Half-wave potential of lead ion in presence of proteins

Solution	volt vs. N.C.E.
In presence of 0.02% hemoglobin	-0.442
In presence of 0.09% egg albumin	-0.443
In presence of 0.1% gelatin	-0.436
Standard solution 0.500-10.00 $\times 10^{-4}$ M/L Pb (NO ₃) ₂ in 0.1 N KNO ₃	-0.440

reduction wave is also exactly the same as with pure lead nitrate solution. In consequence, the reduction wave obtained from the mixture of protein and lead nitrate solution can be taken to be that of free lead ions, for, if it be the case of (2) or (4), it must be different entirely from each other both in potential and in shape. Adsorption and desorption of metal ions from solutions cannot be accomplished instantaneously, but requires a relatively long time. On the other hand the time during which one drop of mercury grows and then falls at the cathode, is 3.5 seconds according to my capillary, and by the fall of a drop of mercury a new mercury-solution interface is formed. In consequence, if the lead ions are liberated from protein molecules at the cathode, they must occur in as short a time as 3.5 seconds. This seems to be improbable. Even if it occurs, the amount liberated in such a short time is extraordinarily small and may be negligible. Consequently, by the polarographic method, only free lead ions which are not adsorbed by protein molecules can be determined precisely and simply in presence of proteins.

RESULTS

1. Adsorption of lead ions by active charcoal from lead nitrate solution.

In the way described above the adsorption experiments at 20° C. were made repeatedly for four times, taking 17 experimental points at concentrations lying between 0.116 and 1.091×10^{-4} M/L. The adsorption isotherms obtained are in each experiment exactly the same in shape, but as a whole there is some displacement of isotherms, which seems to be due to errors in weighing well-dried charcoal powder. Hence, as it is not proper to average the data obtained in each experiment, one series of experiments is shown in table 2, figs. 1 and 2. The isotherm, as shown in fig. 1, is markedly step-like in shape, and is composed of three distinct steps. When the logarithms of c and x/m are plotted, this becomes more obvious; all the experimental points which belong to a step are in a straight line and the location and inclination of each straight line is entirely different from each other. Hence, it is evident that these steps are separated from each other. Next, in order to examine the conformability of curved portions in the steps to Freundlich's equation, the constants of the equation were calculated by the method of least squares, and are shown in column 7 of table 2. Since the differences between x/m observed and that calculated are

Table 2. Adsorption of lead ions by active charcoal at 20° C.
Active charcoal 0.35%

Experimental point No.	Initial concentration 10^{-4} M/L	Equilibrium concentration 10^{-4} M/L	x/L 10^{-4} g-atom/L	x/m 10^{-4} g-atom/1 g-charcoal	Ratio of adsorption %	Constants of $\frac{x}{m} = \frac{\alpha c^{1/n}}{m}$	x/m calculated
1	0.750	0.116	0.634	0.181	84.5	$\frac{1}{n} = 3.0857$ $\log \alpha = 1.7496$ $r = 0.0119$	0.224
2	0.875	0.167	0.708	0.202	80.9		
3	1.000	0.167	0.833	0.238	83.3		
4	1.125	0.176	0.949	0.271	84.4		
5	1.250	0.182	1.068	0.305	85.4		
6	1.375	0.192	1.183	0.338	86.0		
7	1.500	0.247	1.253	0.358	83.5	$\frac{1}{n} = 0.4507$ $\log \alpha = -0.1646$ $r = 0.0061$	0.365
8	1.625	0.269	1.358	0.388	83.5		
9	1.750	0.325	1.425	0.407	81.4		
10	1.875	0.359	1.516	0.433	80.9		
11	2.000	0.490	1.510	0.431	75.5	$\frac{1}{n} = 1.1562$ $\log \alpha = -0.0078$ $r = 0.0047$	0.431
12	2.125	0.522	1.603	0.458	75.4		
13	2.250	0.594	1.656	0.473	73.6		
14	2.375	0.568	1.807	0.516	76.1		
15	2.500	0.647	1.853	0.529	74.1		0.463
16	2.750	0.842	1.908	0.545	69.4		
17	3.000	1.091	1.909	0.545	63.6		

r represents probable error. Experimental point No. 13 is excepted in the calculation of the formula.

Fig. 1. Adsorption of lead ions by active charcoal at 20° C.

within experimental error, the three steps conform to the equation. However, on the first and third curves the values of $1/n$ are more than 1.0, so it may be stated that in detail these two curves are somewhat different from Freund-

Fig. 2. Adsorption of lead ions by active charcoal at 20° C.

lich's equation. It is necessary to examine whether a step-wise (discontinuous) curve is due to experimental error, instead of being essentially a single smooth (continuous) curve. For this, assuming that all the experimental points belong to a single smooth curve and are compared with the values thus obtained, the deviations are generally found to be great and especially so in the neighbourhood of the discontinuous points, which represent boundary points between two distinct steps; they are two to nine times greater than the experimental errors, which have adopted the values three times as much as the probable errors, and several neighbouring points lie only on one side of the assumed single curve. In consequence, it is possible to demonstrate from these facts that all the points do not belong to a single smooth curve, but that they exist in a step-wise manner. In the same way, it can be demonstrated that two neighbouring steps are separated from each other. Since the experimental point 13 is deviated from the third step by a value twice as much as an experimental error, it is evident that the point does not belong to the third step. It may be considered that in this region adsorption of two different types occur and that in the two flasks, which correspond to two neighbouring experimental points, occurred adsorptions of different type each in its own way. This phenomenon will be seen more clearly in the case of hemoglobin.

The experiments were repeated four times, and the discontinuous isotherms were obtained every time. Hence, it can be concluded that in adsorption of lead ions by active charcoal the isotherm is discontinuous and that it conforms to Freundlich's equation sectionally.

2. Adsorption of lead ions by ox hemoglobin from lead nitrate solution.

The experiments were carried out in the way as that described in the section on experimental method. When a lead nitrate aqueous solution is added to a hemoglobin aqueous solution, it causes gradual aggregation and precipitation, and gross aggregated particles settle down soon after ceasing to stir the

mixture, and the supernatant liquid becomes clear. 10 ml of the supernatant liquid is then withdrawn and KNO_3 added to the extent of 0.1 N. Analysis is carried out by the polarographic method. As the isotherms obtained by experiments repeated five times under the same conditions are in every case the same, and displacement is not seen, the data from five experiments were averaged. They are shown in table 3, figs. 3 and 4. The number of experimental points are 23 at concentrations lying between 0.078 and 10.39×10^{-4} M/L.

As shown in fig. 3, the isotherm is distinctly step-wise in shape, or discontinuous, composed of four steps. The ascending portion of each step is parabolic, but becomes a straight line parallel to the abscissa, or horizontal, at the height of the ascending portion, namely, between two parabolic curves there exists a horizontal straight line. Consequently, from this fact alone, it may be said evident that the curved portion in each step is separated from each other, or discontinuous. It becomes more obvious when the logarithms of c and x/m are plotted (fig. 4). When we examine the conformability of a curved portion

Table 3. Adsorption of lead ions by hemoglobin at 20° C.
Hemoglobin 0.221 g/L

Experimental point No.	Initial concentration 10^{-4} M/L	Equilibrium concentration 10^{-4} M/L	x/L 10^{-4} g-atom/L	x/m 10^{-4} g-atom/1 g-hemoglobin	Ratio of adsorption %	Constants of $\frac{x}{m} = \alpha c^{1/n}$	x/m calculated
1	0.500	0.078	0.422	1.91	84.4	$1/n = 0.2717$ $\log \alpha = 0.5833$ $r = 0.016$	1.92
2	0.750	0.199	0.551	2.49	73.5		2.49
3	1.000	0.361	0.639	2.89	63.9		2.90
4	1.250	0.594	0.656	2.97	52.5	$1/n = 0.2783$ $\log \alpha = 0.5385$ $r = 0.022$	2.99
5	1.500	0.778	0.722	3.27	48.1		3.22
6	1.750	0.993	0.757	3.43	43.3		3.45
7	2.000	1.203	0.797	3.61	39.9		3.64
8	2.250	1.405	0.845	3.82	37.6		3.80
9	2.500	1.626	0.874	3.95	35.0		3.95
10	2.750	1.878	0.872	3.95	31.7	$1/n = 0.7820$ $\log \alpha = 0.3574$ $r = 0.074$	4.06 4.37 4.62
11	3.000	2.093	0.907	4.10	30.2		
12	3.250	2.304	0.946	4.28	29.1		
13	3.500	2.469	1.031	4.67	29.5		
14	3.750	2.70	1.05	4.75	28.0	$1/n = 0.6088$ $\log \alpha = 0.3373$ $r = 0.165$	5.00 5.59 6.21 6.81
15	4.000	2.96	1.04	4.71	26.0		
16	4.500	3.46	1.04	4.71	23.1		
17	5.000	3.93	1.07	4.84	21.4		
18	6.00	4.71	1.29	5.84	21.5		
19	7.00	5.61	1.39	6.29	19.9		
20	8.00	6.53	1.47	6.65	18.4		
21	9.00	7.53	1.47	6.65	16.3		
22	10.00	8.56	1.44	6.52	14.4		
23	12.00	10.39	1.61	7.29	13.4

Fig. 3. Adsorption of lead ions by hemoglobin.

Fig. 4. Adsorption of lead ions by hemoglobin at 20° C.

in each step to Freundlich's equation, all of the curved portions conforms well, as shown in table 3 (7 and 8 columns). Consequently, it may be concluded that at these four ascending curved portions the adsorption which occurs is in general of the same type as in the case of adsorption by solids in solutions and that the horizontal portion following the curve indicates the phenomenon of saturation of adsorption. Further, it can be demonstrated in the same way as in the case of active charcoal that the discontinuity in this isotherm is not due to an experimental error. As to the relation between the degree of aggregation

and the steps in the isotherm, the degree of aggregation varies with each step, but there is found no great difference in one and the same step. At the discontinuous, or horizontal, portion the degree of aggregation corresponds, in some cases, to that of the next step.

Reappearance and dimorphism of discontinuous adsorption isotherm: In five experiments under the same conditions, the isotherms obtained are always the same in type and the concentration at which the steps occur are also generally constant. Most steps are very stable and show always the same shape, while some show slight instability and show dimorphism. It is marked at the third step, and, only in one case was it found at the fourth step. Steps showing dimorphism are shown in fig. 5. There were three cases: the first in which the first half of the third step appeared early as an independent step (III') between the second and third steps (experiments 1 and 3), the second in which no steps of this kind appeared (experiments 2 and 4), and the third case in which both III and III' appeared together in one and the same experiment (experiment 5).

Fig. 5. Dimorphism in adsorption of lead ions by hemoglobin at 20° C.

Adsorption isotherm at low temperature: In this experiment the temperature was $1.2^{\circ}\text{C.} \pm 0.5^{\circ}$, while other conditions were exactly the same as in the former (fig. 3), the adsorbed amount of lead ions by hemoglobin decreases considerably at low temperature. However, the discontinuity of the isotherm never varies and is well seen.

3. Adsorption of lead ions by egg albumin from lead nitrate solution.

The experiments were carried out in the same way as in the case of hemoglobin, taking 25 experimental points at concentrations lying between 0.068 and $15.55 \times 10^{-4}\text{ M/L}$. Temperature was $20.0^{\circ}\text{C.} \pm 0.1^{\circ}$. In order to observe a discontinuous portion of an isotherm in detail, the experimental points were taken as closely as possible, as long as the interval of points do not lie within experimental error. The results are shown in table 4, fig. 6. The isotherms obtained show a marked steplike shape, or are discontinuous, composed of four

Table 4. Adsorption of lead ions by egg albumin at 20. C.
Egg albumin 0.9029 g/L

Experimental point No.	Initial concentration 10^{-4} M/L	Equilibrium concentration 10^{-4} M/L	x/L 10^{-4} g-atom/L	x/m 10^{-4} g-atom/1 g-albumin	Ratio of adsorption %	Constants of $\frac{x}{m} = \alpha c^{1/n}$	x/m calculated
1	0.250	0.068	0.182	0.202	72.8	$1/n = 0.9657$ $\log \alpha = 0.4690$ $r = 0.0317$	0.220
2	0.500	0.116	0.384	0.425	76.8		0.368
3	0.750	0.201	0.549	0.608	73.2		0.625
4	1.000	0.271	0.729	0.807	72.9		0.834
5	1.250	0.417	0.833	0.922	66.6		
6	1.375	0.563	0.812	0.899	59.1		
7	1.500	0.632	0.868	0.961	57.9		
8	1.625	0.779	0.846	0.937	52.1		
9	1.750	0.877	0.873	0.967	49.9		
10	1.875	0.895	0.980	1.08	50.5		
11	2.000	1.054	0.946	1.05	47.3	$1/n = 0.98366$ $\log \alpha = -0.15057$ $r = 0.0499$	1.294
12	2.125	1.159	0.966	1.07	45.5		1.508
13	2.250	1.215	1.035	1.146	46.0		1.686
14	2.375	1.308	1.067	1.182	44.9		1.897
15	2.500	1.433	1.067	1.182	42.7		2.165
16	3.000	1.849	1.151	1.274	38.4		
17	3.500	2.160	1.340	1.484	38.3		
18	4.000	2.419	1.581	1.751	39.5		
19	4.500	2.728	1.772	1.963	39.4		
20	5.000	3.12	1.88	2.08	37.6		
21	7.50	5.22	2.28	2.52	30.4		
22	10.00	6.08	3.92	4.34	39.2		
23	15.00	10.04	4.96	5.49	33.1		
24	20.00	12.22	7.78	8.62	38.9		
25	25.00	15.55	9.45	10.4	37.8

Fig. 6. Adsorption of lead ions by egg albumin at 20° C.

steps. The ascending curved portion of the first and second steps conform well to Freundlich's equation. It can be demonstrated in the same way as in the case of hemoglobin that this discontinuity depends neither upon experimental error nor due to accident.

In this case such dimorphism as seen in the case of hemoglobin is not seen and the degrees of aggregation are almost the same over the whole range with no differences according to steps.

4. Adsorption of lead ions by gelatin from lead nitrate solution.

In this case, adsorption was carried out at $25.0^{\circ}\text{C} \pm 0.1^{\circ}$ and analysis at $23.0^{\circ}\text{C} \pm 1.0^{\circ}$. The number of experimental points are 28 at concentrations lying between 0.152 and $21.1 \times 10^{-4}\text{ M/L}$. The results are shown in table 5, fig. 7.

Table 5. Adsorption of lead ions by gelatin at 25°C .
Gelatin 1.000 g/L

Experimental point No. .	Initial concentration 10^{-4} M/L	Equilibrium concentration 10^{-4} M/L	x/L 10^{-4} g-atom/L	Ratio of adsorption %	Constants of $\frac{x}{m} = \alpha c^{1/n}$	x/m calculated
1	0.250	0.152	0.098	39.2	$\left. \begin{array}{l} 1/n = 0.5389 \\ \log \alpha = -0.2934 \\ r = 0.0134 \end{array} \right\}$	0.247
2	0.500	0.263	0.237	47.4		0.315
3	0.750	0.412	0.338	45.1		0.388
4	1.000	0.607	0.393	39.3		0.454
5	1.250	0.811	0.439	35.1		
6	1.500	1.057	0.443	29.5	$\left. \begin{array}{l} 1/n = 1.189 \\ \log \alpha = -0.4237 \\ r = 0.0175 \end{array} \right\}$	0.496
7	1.750	1.260	0.490	28.0		0.575
8	2.000	1.427	0.573	28.7		0.646
9	2.250	1.574	0.676	30.1		0.745
10	2.500	1.775	0.725	29.0		
11	3.000	1.995	1.005	33.5	$\left. \begin{array}{l} 1/n = 0.2721 \\ \log \alpha = -0.0643 \\ r = 0.0330 \end{array} \right\}$	1.041
12	3.500	2.438	1.062	30.3		1.099
13	4.000	2.826	1.174	29.4		1.137
14	4.500	3.28	1.22	27.1		1.191
15	5.000	3.87	1.13	22.6		
16	6.00	4.77	1.23	20.5	$\left. \begin{array}{l} 1/n = 1.607 \\ \log \alpha = -1.015 \\ r = 0.162 \end{array} \right\}$	1.19
17	8.00	6.12	1.88	23.5		1.77
18	10.00	7.67	2.33	23.3		2.33
19	12.00	9.79	2.21	18.4		
20	14.00	11.6	2.4	17.1		
21	15.00	11.6	3.4	22.7		
22	16.00	11.5	4.5	28.1		
23	18.00	13.6	4.4	24.4		
24	20.00	15.4	4.6	23.0		
25	22.00	15.2	6.8	30.9		
26	24.00	17.4	6.6	27.5		
27	26.00	19.3	6.7	25.8		
28	28.00	21.1	6.9	24.6		

The value of x/m ($10^{-4}\text{ g-atom/l g-gelatin}$) is equal to that of x/L .

Fig. 7. Adsorption of lead ions by gelatin at 25° C.

The isotherms show a marked step-wise shape, but are somewhat different from the preceding three in detail. At a concentration lower than 11.0×10^{-4} M/L they are almost similar in type to the preceding three, but at higher concentrations than this they assume completely rectangular steps. It can be demonstrated in the same way as in the case of hemoglobin that this discontinuity is not due to experimental error. On examining the conformability of the curved portion of the first half to Freundlich's equation, we find that the values of x/m calculated are in agreement with that observed, but on the second and fourth curves they differ somewhat from Freundlich's equation for the value of $1/n$ becomes greater than 1.0. Nevertheless, it may be considered that at the first half adsorption similar in type to the preceding three occurs, determined by the shape and position of steps. On the contrary, the second half of the isotherm at concentrations between 11.00 and 21.00×10^{-4} M/L is entirely different from the preceding type. When the concentration reaches a certain value, combination begins suddenly and, with no increase in concentration, the adsorbed amount reaches a certain extent, at which the combination ceases suddenly and thereafter no increase of the adsorbed amount occurs in spite of increase in concentration. Thus, two completely rectangular steps appear. Regarding the nature of this combination, it is not discussed here, but it is evident that a combination occurs, entirely different from that at the first half.

In the case of gelatin, different from the preceding two cases, no aggregation upon addition of a lead solution occurs; the mixture remains quite clear with no change visible to the naked eye. Such dimorphism as in the case of hemoglobin does not appear.

MECHANISM BY WHICH THE DISCONTINUITY OCCURS IN ADSORPTION ISOTHERMS

In the present work, the adsorbate is in each case lead ions from a lead

nitrate aqueous solution, but the adsorbents are active charcoal, ox hemoglobin, egg albumin and gelatin, which are entirely different from each other in property. In results, however, the adsorption isotherm is in all cases discontinuous without exception, and, in spite of slight differences in detail they essentially agree in the following points. Namely, with continuous increase of concentration, the amount adsorbed also increases continuously. Within this period the adsorption isotherm conforms to Freundlich's equation. However, soon after it reaches a state of saturation of adsorption, thereafter the amount adsorbed is constant without any increase or decrease, in spite of an increase of concentration. After continuation of such a state for some time, an increase in the amount adsorbed occurs suddenly at a certain concentration and the same course of events is repeated as in the previous one. Thus, several steps are formed.

Let us now call a spot on adsorbent where lead ions are adsorbed an adsorbing centre or active centre provisionally. If all adsorbing centres of an adsorbent were the same and there were no differences in property, then the amount adsorbed should increase continuously with a continuous increase of concentration. It is true that eventually a state of saturation of adsorption is reached, but it cannot be considered that strong adsorption and saturation occur reciprocally within such a narrow range as in the case of the present experiments. Consequently there must necessarily be several kinds of adsorbing centres, which are different from each other in adsorbing capacity. And the number of these must be not less than that of the steps. Now, in order to explain this simply, let us suppose that there are as many kinds of centre as there are steps, and call them first class centre, second class centre, etc. respectively. We assume then the first class centre as having a capacity of adsorbing in the lowest concentration, so that it starts adsorbing in the lowest concentration. As all the first class centres have the same property concerning adsorption, adsorption occurs gradually at each centre of the first class in succession with a gradual increase in concentration, and with this appears a continuous isotherm. When no more centre remains, then the increase in the amount adsorbed will not be seen in spite of the increase in concentration. This corresponds to a state of saturation and the isotherm lies parallel to the abscissa, or horizontal, and we can call this the discontinuous portion. The isotherm thus formed by the first class centres corresponds to the first step. When concentration increases further and reaches the sphere of the second class, then adsorption occurs at the second class centres and the second step is formed in the same way as the first. Thus in the interval between adsorptions occurring at two kinds of centres, there is a period where no adsorption occurs. Namely these several types of adsorption occur discontinuously and in consequence a discontinuous isotherm is obtained as a whole.

On the contrary, if there were a great many varieties of adsorbing centres of different adsorbing capacity, a number of minute steps corresponding to each centre should appear intercrossed and the isotherm would be smooth, or continuous, as a whole. Therefore, it seems logical to assume that even if the types of centres are more than the number of steps, they are relatively few in kind,

and some of them which are alike in the power to adsorb form one group and that this group of different kinds of centres form one step in the isotherm. Dimorphism shown by hemoglobin seems to correspond to this special type.

In the case of hemoglobin, aggregation occurs when lead ions are adsorbed and the state and degree correspond in general to the occurrence of steps. This must account for that differences in state of aggregation of hemoglobin molecules not giving rise to discontinuous isotherms, but that differences in type of adsorption corresponding to each step produce various states of aggregation. This is clear from the following reasons. Namely, the isotherms are equally discontinuous in the case of gelatin where no aggregation occurs, and in the case of egg albumin where, though aggregation occurs, its degree has no relation with an occurrence of steps, and also in the case of active charcoal.

MOLECULAR-WEIGHT DETERMINATION OF PROTEINS BY THE APPLICATION
OF THE DISCONTINUITY IN ADSORPTION ISOTHERM

In the previous section where the mechanism by which the discontinuities in adsorption isotherms occur, it has been explained that a step in an isotherm occurs when one or more kinds of adsorbing centres adsorb as a group. According to this hypothesis, the height of a step, or the amount adsorbed in the corresponding concentration range, corresponds to the amount of lead ions adsorbed by one group of adsorbing centres. Here, by the height of a step we do not mean the height from the abscissa, but the height from the highest portion of a horizontal portion of a previous step. As the unit of the amount adsorbed, x/m is not and x/L is used. An explanation of the unit x/L has been given in the section on experimental methods. The height of each step in the case of hemoglobin is shown in table 6. As molecules of hemoglobin in the aqueous solution employed in the experiments are uniform in property, all

Table 6. Adsorption of lead ions by ox hemoglobin at 20° C.
Amount of lead ions adsorbed per step (10^{-4} g-atom/L)

Step No.	Experiment 1	Experiment 2	Experiment 3	Experiment 4	Experiment 5
I	0.671 = 0.033 × 20	0.758 = 0.033 × 23	0.633 = 0.030 × 21	0.714 = 0.033 × 22	0.663 = 0.033 × 20
II	0.164 = 0.033 × 5	0.134 = 0.033 × 4	0.150 = 0.030 × 5	0.168 = 0.033 × 5	0.135 = 0.033 × 4
III'	0.067 = 0.033 × 2		0.122 = 0.030 × 4		0.098 = 0.033 × 3
III	0.098 = 0.033 × 3	0.198 = 0.033 × 6	0.125 = 0.030 × 4	0.198 = 0.033 × 6	0.18 = 0.033 × 5
IV	0.34 = 0.033 × 10	0.47 = 0.033 × 14	0.42 = 0.030 × 14	0.31 = 0.033 × 9	0.36 = 0.033 × 11
IV'	0.17 = 0.033 × 5				
Total number of adsorbed ions per mole- cule	45	47	48	42	43

of the molecules should equally have all kinds of and the same number of adsorbing centres. If m represents the number of molecules of hemoglobin existing in one litre of solution employed in the experiment, i_I the number of lead ions adsorbed by all the first class centres of one molecule, i_{II} that by the second class, i_{III} that by the third class and i_{IV} that by the fourth class, and N Avogadro's Number, then the gram-atom of lead ions adsorbed in each step is expressed as follows,

$$\frac{i_I \times m}{N}, \frac{i_{II} \times m}{N}, \frac{i_{III} \times m}{N}, \frac{i_{IV} \times m}{N} \dots \dots \dots (1)$$

Each of them should be equal to the height of each step obtained from the experimental data. Then, the height of each step ought to have a common measure, m/N . As shown in table 6, it agrees well with the results of the experiments, namely, in all of the five experiments each of the steps has 0.033 or 0.030×10^{-4} as the greatest common measure. In other words, there is a simple ratio in all of the steps. Let us call the greatest common measure an adsorption unit amount. Then, m/N equals $1/n \times 0.033 \times 10^{-4}$ or $1/n \times 0.030 \times 10^{-4}$. As m/N corresponds to the gram-molecule of hemoglobin in the solution employed in the experiments, the molarity of hemoglobin solution can be obtained by calculating the common measure of all of the steps. Further, as the concentration in grams per litre of a hemoglobin solution is known in the experiments, the molecular weight of hemoglobin could be calculated from it. Namely,

$$\text{Mol. wt. of Hb.} = \frac{\text{grams/litre of Hb.}}{\text{Molarity of Hb.}} \dots \dots \dots (2)$$

The molarity of a hemoglobin solution is $1/n \times (\text{adsorption unit amount})$. Hence,

$$\text{Mol. wt. of Hb.} = \frac{\text{grams/litre of Hb.}}{\text{Adsorption unit amount}} \times n \dots \dots \dots (2')$$

As is evident from this, when the value of n is unknown, the value obtained means the minimal molecular weight.

Next an attempt can be made to calculate the values of $i_I \dots i_{IV}$ in table 6. As the adsorption unit amount is n multiples of m/N , the number of lead ions adsorbed at one centre of one molecule, $i_I \dots i_{IV}$, is respectively n multiples of the values shown in the table. Namely, they become $20 \times n$, $5 \times n$, $2 \times n$, $3 \times n$, $10 \times n$, $5 \times n$, in the first experiment. This means that the number of lead ions adsorbed at each centre corresponding to each step becomes also multiples of n . It seems improbable to surmise as above, for each type of centre is markedly different from each other in property. Presumably it may be considered to be $n = 1$. The molecular weight of ox hemoglobin calculated by this method is shown in table 7. The average value, 6.8×10^4 is then obtained. In both experiments at 20°C. and at 1.2°C. , the values obtained of molecular weight are exactly the same. This value corresponds to G. S. Adair's value of 67,000 or 68,000 determined by osmotic pressure measurements (7), and with T. Svedberg's value of 68,000 on horse hemoglobin determined by means of the ultracentrifuge (8).

Table 7. Molecular weight of ox hemoglobin

Experiment NO.	Hemoglobin g/L	Hemoglobin M/L	Molecular weight
At 20° C.	1 0.221	0.033×10^{-4}	6.7×10^4
	2 0.228	0.033×10^{-4}	6.9×10^4
	3 0.200	0.030×10^{-4}	6.7×10^4
	4 0.230	0.033×10^{-4}	7.0×10^4
	5 0.225	0.033×10^{-4}	6.8×10^4
Average			6.82×10^4
At 1.2° C. Average	0.263	0.038×10^{-4}	6.9×10^4

In the case of egg albumin, the molecular weight can be determined in the same way (table 8). The adsorption unit amount is 0.267×10^{-4} . Since n may be considered to be equal to 1 for the same reason as in the former, the concentration of an egg albumin solution employed in the experiment becomes 0.267×10^{-4} M/L. The concentration of the albumin solution is known to be 0.9029 g/L. Hence, the molecular weight is

$$\frac{0.9029}{0.267 \times 10^{-4}} = 3.38 \times 10^4$$

This value agrees with the minimal molecular weight of 33,800 calculated by E. J. Cohn (9) from the equivalent combining weights, and with T. Svedberg's value of 34,500 by means of the ultracentrifuge (8).

Table 8. Adsorption of lead ions by egg albumin at 20° C.
Amount of lead ions adsorbed per step (10^{-4} g-atom/L)

Step, No.	Amount adsorbed per step
I	$1.067 = 0.267 \times 4$
II	$0.813 = 0.271 \times 3$
III	$2.08 = 0.260 \times 8$
IV	$4.49 = 0.264 \times 17$

In the case of gelatin it differs somewhat from the previous two. Gelatin is considered to be a polydispersed sol and a mixture of particles of various sizes. Now, when an examination is made as to what relation exists between the heights of steps (table 9), the first, the fourth, the fifth and the sixth steps have a simple integral ratio and 0.22×10^{-4} as the greatest common measure.

Table 9. Adsorption of lead ions by gelatin at 25° C.
Amount of lead ions adsorbed per step (10^{-4} g-atom/L)

Step, No.	Amount adsorbed per step
I	$0.443 = 0.221 \times 2$
II	$0.282 = 0.282 \times 1$
III	$0.465 = 0.233 \times 2$
IV	$1.12 = 0.224 \times 5$
V	$2.2 = 0.22 \times 10$
VI	$2.2 = 0.22 \times 10$

} or $0.747 = 0.249 \times 3$

or the adsorption unit amount, while the second and the third steps have no common measure with the other steps. Even if the sum of the second and the third steps are taken as one step with an unclear boundary, no common measure is found. This means that all gelatin molecules are uniform concerning the possession of the first, the fourth, the fifth and the sixth class centres, but not uniform regarding the second and the third class centres.

It is not possible to deduce what effects this fact has upon the size of a particle from the data obtained in the present experiments, but it might be possible to estimate the number of gelatin molecules by comparing the amounts adsorbed at the first, the fourth, the fifth and the sixth steps with one another. It can be assumed with the same accuracy as in the preceding two that in this case too $n = 1$. Then the value determined in the same way as in the previous two is the mean molecular weight of gelatin:

$$\frac{1.000}{0.22 \times 10^{-4}} = 4.5 \times 10^4$$

When this value is compared with the values obtained by other methods, it lies in the range of T. Svedberg's value (8), of 10,000 to 70,000, obtained by means of the ultracentrifuge.

In molecular-weight determination of proteins, the calculated heights of steps in the discontinuous isotherm in the case of active charcoal is shown in table 10. In this case, again, all of the steps have simple integral ratio and 0.128×10^{-4} as the adsorption unit amount. It is deduced from this that active charcoal which consists of particles of various sizes is uniform concerning the possession of various kinds of adsorbing centres. Then, the unit particle size of active charcoal which has a set of different kinds of centres as in the case of a molecule of proteins, becomes

$$\frac{3.500}{0.128 \times 10^{-4}} = 27.3 \times 10^4$$

It may be considered that actually these unit particles conglomerate and form larger particles of various sizes.

Table 10. Adsorption of lead ions by active charcoal at 20° C.
Amount of lead ions adsorbed per step (10^{-4} g-atom/L)

Step, No.	Amount adsorbed per step
I	0.634 = 0.127×5
II	0.619 = 0.124×5
III	0.260 = 0.130×2
	0.396 = 0.132×3

DISCUSSION

In the investigation of the discontinuity in adsorption isotherms, especially of ions from solutions, the most important problem is the magnitude of experimental error. Of various sources of errors numerated, the greatest one is the quantitative-determination error. It has a decisive influence not only upon

the examination of conformability of values observed, to experimental formula, but also upon the decision of intervals of experimental points. If intervals of experimental points are too wide, the detection and demonstration of the discontinuity is not only difficult, but it also becomes impossible to examine the exact form, and in consequence we cannot obtain the basis for studying the mechanism. As shown in the results of the experiments, one step of discontinuous isotherms is so minute that in many cases it is included within experimental error in other common methods of analysis employed in the investigation of adsorption from solutions. This may be the greatest cause of failure to demonstrate the discontinuity in adsorption isotherms in previous investigations. I have, however, succeeded in solving this difficulty by employing the polarographic method of analysis, namely, in the present work, this method was satisfactorily employed in showing the sensitiveness and accuracy of the quantitative determination.

As a second factor which has an influence on the accuracy of results, it is necessary to complete the quantitative determination of all samples within as short a time as possible, while the various conditions are constant, once the determination has to be done after an equilibrium is reached. It is more important in a case like the present where samples are numerous in proportion to experimental points. In this respect the method employed is also suitable.

Further, in the case of adsorption in solutions, there lies the factor that the adsorbents are not only solids, but are colloidal particles in many cases. In the experiments of adsorption of ions by protein molecules it is absolutely necessary to determine precisely free ions only in the presence of proteins without injuring the protein molecules. It has been demonstrated in the present work that the polarographic method is most suitable for this purpose. It was not possible to solve completely whether lead ions adsorbed on protein molecules cannot be liberated during the time required by one drop of mercury to fall at the cathode, 3.5 seconds, but, even if the adsorption in these cases is completely reversible, the amount of lead ions liberated during so short a time as 3.5 seconds may be negligible, considering that it requires a long time to attain the equilibrium in adsorption from solutions. It is evident that this point cannot be a problem against the real existence of the discontinuous adsorption isotherms, because in the case of active charcoal where the pure supernatant liquid containing no charcoal is analysed the same type of discontinuity was demonstrated.

Experiments were carried out for adsorption of lead ions from lead nitrate solution by active charcoal, hemoglobin, egg albumin and gelatin, and the discontinuity in adsorption isotherms were found in all these cases, as shown in the section of results of experiments. For the reasons that equilibrium was attained completely, that experimental errors were well examined, that discontinuity reappears always in all the experiments, and that discontinuities of a similar type were obtained with various kinds of adsorbents markedly different from each other in property, there seems no doubt that these discontinuous adsorption isotherms really exist.

Regarding the mechanism by which this phenomenon occurs, this can be explained when we assume that in the adsorbents there are several kinds of adsorbing centres different from each other in their adsorbing capacity. The essential qualities of the adsorbing centre and of the adsorbing power, however, cannot be concluded from the present work. Whatever they may be, the ascending curved portion of a step in discontinuous isotherms conforms to Freundlich's equation in many cases, and it is evident that different kinds of adsorption occur discontinuously, which can be explained by comparing the constants of the equation and from the differences in the concentration at which adsorption begins or ends. And this accords, without any contradictions, to the results of the experiments—in the heights of all steps in the cases of hemoglobin and egg albumin, which are monodispersed sols, there is found a simple integral ratio, but not in the case of gelatin which is polydispersed sol.

As to the method of determination of the molecular weight of proteins by the application of the discontinuity in adsorption isotherms, this phenomenon was employed to determine the molarity of protein solutions used in the experiments.

In this work it was assumed that the value of n is equal to 1 in order to estimate the number of adsorbed ions per centre, besides the assumption for the explanation of the mechanism of the phenomenon. As there was good basis for this assumption (as explained in the previous section), the molecular weight determined by this method may be more properly called a true molecular weight rather than a minimal molecular weight. In order to estimate the value of n , another way would be to employ other types of ions as adsorbents besides lead ions, and compare the values with each other.

The molecular weights, determined by this method, of ox hemoglobin, egg albumin and gelatin are in agreement with values obtained by other methods as indicated in the previous section. By this method, no special apparatus except a polarograph is necessary, 0.5 g of a sample is sufficient for one experiment and the time required for one experiment is only two days. Moreover, its accuracy seems not inferior to other methods. So this may be considered to be one of the most excellent. Regarding the solvent of proteins, it is possible to determine the molecular weight under any condition and in the presence of any solvent, so long as the solvent is indifferent to the ions as adsorbents and the solvent does not disturb the adsorption of the ions. No great determination errors seem to occur in most cases, even when impurities are present in a protein solution. In this respect, too, this method is superior.

Though by this method we can find whether the various adsorbing centres possessed by molecules or particles are uniform or not, we cannot determine if the lack of uniformity of molecular or particle weight is accompanied by variations in adsorbing capacity. This is a drawback to this method. In such a case however we always can determine only the mean molecular or particle weight.

SUMMARY

In the present investigation it was demonstrated that the polarographic

method is excellent for the analysis of experiments on adsorption from solutions. By this method the adsorption of lead ions from a lead nitrate solution of low concentration by active charcoal, ox hemoglobin, egg albumin and gelatin was investigated and it was found and shown that the adsorption isotherms in all of these cases are discontinuous. A hypothesis is presented regarding the mechanism by which the discontinuity in adsorption isotherms occurs. It was found that molecular weight of proteins can be determined simply and accurately by applying the discontinuous isotherms. The molecular weights obtained by this method are for ox hemoglobin 6.8×10^4 , for egg albumin 3.38×10^4 and for gelatin 4.5×10^4 .

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