[Chem. Pharm. Bull.
16(11)2248—2256(1968)]

UDC 615.033/.034 : 547.466.09 : 615.451

Physico-chemical Approach to Biopharmaceutical Phenomena. I.[1) Adsorption of Tryptophan from Aqueous Solution[2)]

Hisashi Nogami, Tsuneji Nagai,[3)] Eihei Fukuoka,[3a)]
and Hiroshi Uchida[3b)]

Faculty of Pharmaceutical Sciences, University of Tokyo[3)]

(Received June 13, 1968)

The adsorption of tryptophan by carbon black from aqueous solution was studied for an approach to an understanding of biopharmaceutical phenomena, comparing with the adsorptions by keratin, silica gel and graphite.

The stereoselective adsorption, which took place on keratin, was not observed in the case of carbon black. The interaction of tryptophan with the carbon black surface might be originated by the orientation of hydrophobic moiety on the surface, being free from steric hindrance of the other moiety.

While the amount of tryptophan adsorbed on silica gel increased with the acidity, the adsorption by carbon black did not depend distinctively on pH in the region of pH 3 to 9, though some part of the carbon black surface was considered to have a polarity. Tryptophan might be adsorbed preferentially on the nonpolar part of the surface where the indole group adopted the parallel orientation.

The adsorption of tryptophan by carbon black increased with temperature. In order to discuss this unusual phenomenon, the thermodynamic functions were calculated, showing the entropy effect was predominant in the course of adsorption. It was suggested that the adsorption in this system might be forced with the entropy increase through the change of water structure caused by break-down of the iceberg enclosing the tryptophan molecule in solution.

Studies of adsorption from solution in the pharmaceutical field have been done with two views to searching an adsorbent useful to sustained-release preparations[4)] and to investigating the adsorption of drug on container wall during the storage.[5)] The adsorption studies in this series were carried out for an approach to an understanding of biopharmaceutical phenomena.

An interaction between drug and protein or lipid plays an important role in biopharmaceutical phenomena. For example, the gastrointestinal absorption of drug proceeds initially upon the uptake of drug to gut membrane composed of protein and lipid. Both protein and lipid generally are of hydrophobic character in the aspect of surface chemistry. Therefore, the gut membrane also is considered to be of the same character. Approaching biopharmaceutical phenomena, it is significant to investigate the adsorption of drug on some hydrophobic surface, which seems to be related to the uptake process of drug on gut wall.

---

1) This paper forms Part XXI of "Studies on Powdered Preparations," Preceding paper, Part XX: *Chem. Pharm. Bull.* (Tokyo), **15**, 279 (1967).

2) This work was presented at the 87th Annual Meeting of the Pharmaceutical Society of Japan, Kyoto, April 1967, being taken in part from the theses of Eihei Fukuoka and Hiroshi Uchida for the degree of Doctor of Pharmaceutical Sciences, University of Tokyo, 1968.

3) Location: *Hongo, Tokyo*; *a*) Present address: *Faculty of Pharmaceutical Sciences, Chiba University, Chiba*; *b*) Fellow from *Chemical Laboratory, Kyorin Pharmaceutical Co., Ltd., Ukima-machi, Kita-ku, Tokyo.*

4) *a*) A. Grollman, W.H. Frior, and E. Grollman, *J. Biol. Chem.*, **109**, 189 (1935); *b*) J. Sklow, *Endocrinology*, **32**, 109 (1943); *c*) "Remington's Pharmaceutical Sciences," 13th ed., Mack Publishing Co., p. 620.

5) W. Saski, *J. Pharm. Sci.*, **52**, 264 (1963).

NII-Electronic Library Service

Based on the above consideration, carbon black, which might have a similarity to the gut membrane in the sense of hydrophobic character, was chosen in this study as a model substance. Tryptophan, one of essential amino acids, was chosen as the adsorbate, because 1) a stereoselective adsorption and 2) electrostatic interaction with adsorbent could be investigated from its stereoisomerism and amphoteric property, respectively, 3) the good adsorbability was expected from its hydrophobic character, thus making quantitative measurements possible, and 4) the analytical method by UV absorption was easily established.

Keratin and silica gel also were used as the adsorbents to investigate the selective adsorption of optical antipodes and the effect of surface polarity on adsorption, respectively.

The surface of carbon black is inhomogeneous, as is well known. Accordingly the adsorption on graphite, which is of more homogeneous surface and is composed from the same atom, was examined for comparison with carbon black.

Experimental

Materials——Carbon black marketed as "Seisei Shirasagi" by Takeda Chemical Ind., Ltd. was used after the activation by heating. Keratin marketed by Tokyo Kasei Kogyo Co., Ltd. was washed with distilled water several times, dried in a desiccator, and the part passing through 250 mesh (40μ) sieve was used as the sample. Silica gel marketed as "Wakogel B-O" by Wako Pure Chemical Ind., Ltd. was used without further treatment. Graphite[6] was purified through washing with HCl solution to remove iron checking with $K_3[Fe(CN)_6]$ reagent, washing with distilled water to remove chloric ion checking with $AgNO_3$ reagent, finally pH of the washing being in a neutral region, and then the powder was dried at 100°, the part passing through 250 mesh (40μ) sieve being used as the sample. Surface properties of these powder samples are shown in Table I.

TABLE I. Surface Properties of Adsorbent Samples

	Carbon black	Graphite	Silica gel
Specific surface area[a] (m^2/g)	1250	353	282
X-ray analysis[b]	amorphous	crystal	amorphous
Zero point of charge[c] (pH)	6.9	3.5	7.3
Maximum peak of pore size distribution[d] (Å)	22.5	32.5	—
Pore volume[d] (ml/g)	1.41	0.386	—

a) Determined by BET method using nitrogen.
b) Observed by Cu-radiation with a Toshiba X-Ray Diffractometer Model ADX-102.
c) Determined from pH profile of the apparent amount of hydrogen ion and hydroxyl ion adsorbed at the equilibrium at 40° after shaking the adsorbent for 24 hr in a mixture of 1/10 N HCl and 1/10 N KOH of a given initial pH.[10] See Fig. 3 and 4.
d) Calculated from adsorption–desorption isotherm of nitorogen according to Inkley's method (R.W. Grauston and F.A. Inkley, "Advances in Catalysis," **9**, 143 (1957)).

D-Tryptophan, L-tryptophan, and DL-tryptophan of the purest reagent grade were obtained commercially.

Procedure for Determination of the Amount Adsorbed and Equilibrium pH by Batch Method——a) In the Case of Carbon Black: 20 mg of the adsorbent was added in 10 ml of a completely clear solution of tryptophan of a given concentration, being shaken in a constant temperature incubator (Taiyo M-1) for 24 hr[7] and filtrated very rapidly with a glass–filter.[8] The concentration of tryptophan in the filtrate was determined according to ultra violet (UV) absorption method after diluting with distilled water, using a Hitachi Perkin–Elmer UV–VS spectrophotometer. The optical density of D-tryptophan was determined at 280 mμ, L-tryptophan at 279 mμ, and DL-tryptophan at 280 mμ, being independent of pH of the solution. The amount of tryptophan adsorbed[9] was calculated as $V \cdot \Delta C/M$, where V was the volume of solution, ΔC the difference between initial and equilibrium concentrations, and M the amount of carbon black.

---

6) The authors are most grateful to Dr. Mitsuo Tsunoda of Central Laboratory, Hitachi Ltd. for the generous supply of graphite.
7) This was satisfactorily long because the adsorption attained to equilibrium in 4—6 hr and it attained also to another equilibrium in 4—6 hr in the perturbed experiment regarding the temperature.
8) The adsorption of solute by the filter was preliminarily examined to be negligible.
9) The amount of solvent adsorbed was neglected because tryptophan was considered to be adsorbed preferentially.

For the investigation of pH dependence of adsorption, tryptophan was dissolved in a mixture of 1/10 N HCl and 1/10 N KOH[10] at a given pH, and then the adsorption procedure followed in the same way as above, pH of the filtrate being determined with a Hitachi–Horiba Model P pH meter.

b) In the Case of Keratin: 700 mg of the adsorbent was added in 10 ml of aqueous solution of tryptophan. The concentration of tryptophan in the filtrate was respectively corrected for blank determinations containing no tryptophan, because it had been found that some substance dissolved out of the keratin sample. The others were the same procedures as in the case of carbon black.

c) In the Case of Silica Gel: This experiment was to investigate the pH dependence of adsorption. Thus, tryptophan was dissolved in a mixture of 1/10 N HCl and 1/10 N KOH[10] at a given pH. 1 g of the adsorbent was added in 9 ml of the tryptophan solution, because of rather low adsorbability. The suspension was centrifuged after shaking for 24 hr, and pH of the supernatant and the concentration of tryptophan in it were determined. The others were the same procedures as in the case of carbon black.

d) In the Case of Graphite: 40 mg of the adsorbent was added in 10 ml of aqueous solution of tryptophan and the suspension was filtrated with a cotton–filter[8] after shaking because, if a glass–filter was used, it was difficult to remove the graphite packed in the meshes. The others were the same procedures as in the case of carbon balck.

Results and Discussion

Selective Adsorption of Optical Antipodes of Tryptophan from Aqueous Solution

Selective adsorption of optical antipodes has been observed on many naturally occuring substances as wool, casein and starch.[11–13] In physiological phenomena also, there have been found various stereoselective behaviors.[14–16] McMenamy and Oncley reported that the binding amount of L-tryptophan to serum albumin was about 100 times more than that of D-tryptophan.[17] Therefore, the adsorption of D- and L-tryptophan was interesting enough to be investigated.

1) Adsorption of D- and L-Tryptophan by Carbon Black——Adsorption isotherms obtained are shown in Fig. 1 and the stereoselectivity was not found. Selective adsorption of optical antipodes is expected to take place when there is a difference between the optical antipodes in the structural property in solution and/or in the interaction with adsorbent surface. As

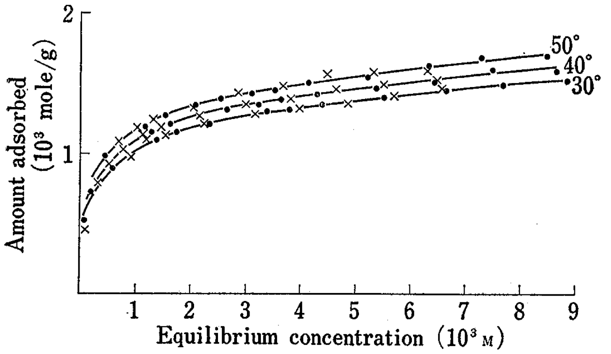

Fig. 1. Adsorption Isotherms of L- and D-Tryptophan by Carbon Balck from Aqueous Solution

—·— L-tryptophan —×— D-tryptophan

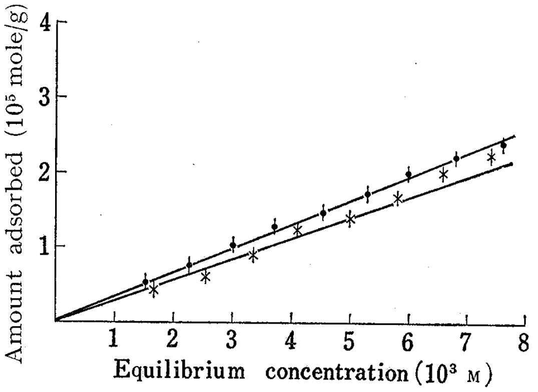

Fig. 2. Adsorption Isotherms of L- and D-Tryptophan by Keratin from Aqueous Solution at 30°

—┼— : L-tryptophan —✳— : D-tryptophan

---

[10] This was to keep the ionic strength 0.1.

[11] W. Bradley and D. Easty, *J. Chem. Soc.*, **1951**, 499.

[12] M. Ohara, K. Ohta, and T. Kwan, *Bull. Chem. Soc. Japan*, 37, 76 (1964).

[13] J. Koga, N. Morita, K. Konishi, and Y. Kuroki, Presentation No. 4R3 26 at the 20th Annual Meeting of Chemical Society of Japan, Tokyo, March 1967.

[14] W.T. Agar, F.J.R. Hird, and G.S. Sidhu, *J. Physiol.*, 121, 255 (1953).

[15] L.R. Finch and F.J.R. Hird, *Biochim. Biophys. Acta*, 43, 278 (1960).

[16] O. Gibson and G. Wiseman, *Biochem. J.*, **48**, 426 (1951).

[17] R.H. McMenamy and J.L. Oncley, *J. Biol. Chem.*, 233, 1436 (1958).

NII-Electronic Library Service

will be discussed in the following paper,[18)] an iceberg of the same size and structure may be formed enclosing D- and L-tryptophan molecule in aqueous solution, respectively. The interaction of tryptophan with carbon black surface might be originated by the orientation of hydrophobic moiety on the surface, that is, the orientation of indole group might be an important factor and also might be free from steric hindrance of the other group. Accordingly it seemed reasonable that D- and L-tryptophan gave the same adsorption isotherms, contrary to the case of keratin which will be described next. It is well known that many organic medicinals are optically inactive and gastrointestinal absorptions of such exogenous substances are controlled by passive transport (or simple diffusion),[19)] being independent of stereoselectivity. Moreover, it is considered that carbon black has a similarity to the gut membrane components in the surface–chemical aspect. Therefore, some correlation between the adsorption by carbon black and the gastrointestinal absorption of drug might be obtainable.

The temperature dependence, as is observed in Fig. 1, will be discussed later.

2) Adsorption of D- and L-Tryptophan by Keratin——Considering the results reported by McMenamy and Oncley on the stereoselective binding of tryptophan to serum albumin,[17)] the selective adsorption of tryptophan was expected to take place by keratin, which is insoluble in water and is composed of L-amino acids only as serum albumin is. The isotherms obtained are shown in Fig. 2.

The difference was significant between D- and L-isomer, though it was not so remarkable compared with the result by McMenamy and Oncley.[17)] Comparing with the adsorption by such a non-selective adsorbent as carbon black, the result seemed due to the structure of keratin surface and showed the stereoselectivity that the L-isomer had a greater affinity to keratin than D-isomer, corresponding to general stereospecific phenomena in biological system.

pH Dependence of the Adsorption of Tryptophan[20)] from Aqueous Solution

It is known from the heat of immersion,[21)] wettability,[22)] and others that carbon black surface is nonpolar. However, as shown in Fig. 3, some polar group might exist on the surface, having a possibility to cause an electrostatic interaction with tryptophan. On the other hand,

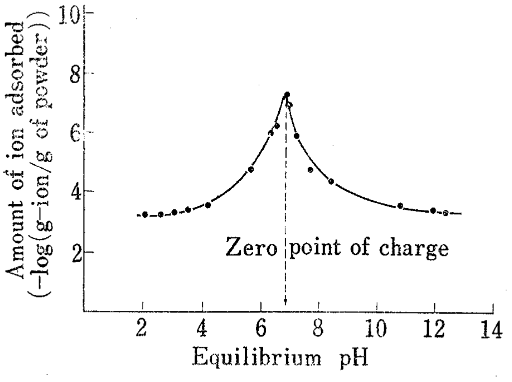

Fig. 3. Apparent Adsorption of Hydrogen Ion and Hydroxyl Ion by Carbon Black at 40°

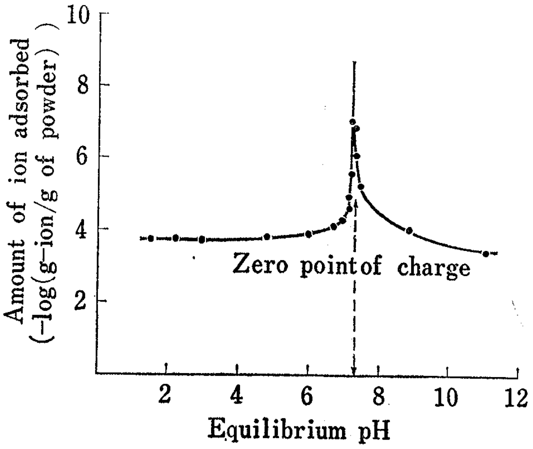

Fig. 4. Apparent Adsorption of Hydrogen Ion and Hydroxyl Ion by Silica Gel at 40°

---

18) Part II: H. Nogami, T. Nagai, and H. Uchida, *Chem. Pharm. Bull.* (Tokyo), **16**, 2265 (1968).
19) S. Mayer, R.P. Maickel, and B.B. Brodie, *J. Pharmacol. Exptl. Therap.*, **127**, 205 (1959).
20) DL-Tryptophan was used.
21) B.J. Young, J.J. Chessick, A.C. Zettlemoyer, and F.H. Healey, *J. Phys. Chem.*, **58**, 313 (1954).
22) F.M. Fowkes and W.D. Harkins, *J. Am. Chem. Soc.*, **62**, 3377 (1940).

NII-Electronic Library Service

silica gel is known to have polar surface.[21,23,24] Fig. 4 expresses such a surface polarity of silica gel used in this study.

1) Adsorption of Tryptophan by Carbon Black from Aqueous Solution of Various pH——Adsorption isotherms obtained were in accordance with Langmuir equation expressed as

$$M=\frac{abC}{1+bC} \tag{1}$$

where M is the amount adsorbed at concentration C in solution, a the amount adsobed when the entire surface is covered by a monolayer, and b the equilibrium constant of adsorption process. Equation (1) is rearranged as

$$\frac{C}{M}=\frac{1}{ab}+\frac{1}{a}C \tag{2}$$

Table II shows the values of a and b at various pH obtained from Langmuir plot according to equation (2). Table III shows the apparent amounts of tryptophan and hydrogen ion or hydroxyl ion adsorbed at various pH, both measured at the same time. It can be said from these tables that the adsorption of tryptophan by carbon black was not so dependent on pH, and that the amount of tryptophan adsorbed was more than that of hydrogen or hydroxyl ion adsorbed, especially in pH 3 to 9 region. Tamamushi and Tamaki reported that pH effect was very minor on the adsorption of long-chain electrolytes by carbon black.[25] Sorby examined the adsorption of phenothiazine derivatives by kaolin, talc, and activated charcoal, and reported that the adsorption by activated charcoal was least influenced by pH.[26] The result obtained in this study may correspond to these reports, showing that carbon black is intrinsically of nonpolar and is completely different from such a polar material as silica gel described later.

As shown in Table III, the amount of tryptophan adsorbed decreased in strong acidic and strong alkaline pH regions, but the amount of hydrogen or hydroxyl ion adsorbed increased.

TABLE II. Langmuir Constants of Adsorption of Tryptophan by Carbon Balck from Aqueous Solution at Various pH

pH_e	Temp. (°C)	a-Value $\times 10^3$ (mole/g)	b-Value $\times 10^{-3}$ (liter/mole)
1.98	30	1.29	1.08
1.93	40	1.45	1.15
2.45	30	1.67	1.22
2.45	40	1.82	1.77
2.74	30	1.63	1.34
2.74	40	1.71	1.95
3.49	30	1.65	1.29
3.80	30	1.77	—
6.07	30	1.71	1.46
6.23	40	1.82	1.39
6.73	30	1.78	1.56
6.79	40	1.85	2.00
9.45	30	1.81	1.01
9.33	40	1.85	1.35
10.0	30	1.55	0.89
9.8	40	1.72	1.02

---

23) A.M. Gaudin and D.W. Fuerstenau, *Trans. Am. Inst. Mining, Met. Engrs.*, **202**, 66 (1955).
24) T.W. Healy and D.W. Fuerstenau, *J. Colloid Sci.*, **20**, 376 (1965).
25) *a)* T. Tamamushi and K. Tamaki, *J. Chem. Soc.*, **1962**, 855; *b)* *Idem*, *ibid.*, **1963**, 3382.
26) D.L. Sorby, E.M. Plein, and J.D. Bemmaman, *J. Pharm. Sci.*, **55**, 785 (1966).

Table III. Adsorption of Tryptophan and Hydrogen Ion or Hydroxyl Ion by Carbon Black

Temp. (°C)	pH_i	pH_e	$M_a\times10^3$ (mole/g)	$M_b\times10^3$ (mole/g)	$M_h\times10^3$ (g-ion/g)	$(M_a+M_h)\times10^3$ (mole/g)	$(M_b+M_h)\times10^3$ (mole/g)
30°	1.90	1.95	1.16	1.20	0.60	1.76	1.80
	2.30	2.35	1.48	1.50	0.26	1.74	1.76
	2.63	2.74	1.45	1.50	0.26	1.71	1.76
	3.11	3.49	1.48	1.52a)	0.23	1.71	1.75
	3.25	3.80	1.45	1.52	0.20	1.65	1.72
	3.64	6.07	1.58	1.62a)	0.12	1.70	1.74
	4.20	6.73	1.64	1.72	0.03	1.97	1.75
	10.35	9.38	1.60	1.66a)	0.10	1.70	1.76
	10.94	10.00	1.34	1.37	0.38	1.72	1.75
40°	1.90	1.93	1.30	1.34	0.45	1.75	1.79
	2.30	2.36	1.65	1.70	0.25	1 90	1.95
	2.63	2.74	1.60	1.65	0.26	1.86	1.91
	3.64	6.27	1.68	1.72a)	0.12	1.80	1.84
	10.35	9.31	1.68	1.75a)	0.10	1.78	1.85
	10.94	9.79	1.52	1.54	0.30	1.82	1.84

pH_i : pH of solution before adsorption
pH_e : pH of solution after adsorption
M_a : amount of tryptophan adsorbed at the equilibrium concentration 0.8×10^{-2} M at the respective equilibrium pH, obtained from adsorption isotherm.
M_b : amount of tryptophan adsorbed at the equilibrium concentration 1.0×10^{-2} M at the respective equilibrium pH, obtained from adsorption isotherm.
M_h : apparent adsorption of hydrogen ion or hydroxyl ion
a) Value estimated by extrapolation.

This means that no ion exchange adsorption took place. Considering that the sum of tryptophan and hydrogen or hydroxyl ion adsorbed was constant as shown in Table III, the adsorption was similar to a competitive one. Hydrogen or hydroxyl ion may be adsorbed on the polar part of surface and tryptophan on the nonpolar part. Because of its hydrophobic property, tryptophan might be preferentially adsorbed on the nonpolar part where the indole group adopted the parallel orientation, the other group hindering hydrogen or hydroxyl ion from attaching to the polar part, and thus the amount of such ion adsorbed was considered to decrease when the amount of tryptophan adsorbed increased, giving a similar result to a competitive adsorption.

The parallel orientation of indole group on the surface mentioned above was expected from the following calculation. The area occupied by one molecule of tryptophan was calculated as 110Å^2 by using the value of *a* in Table II and specific surface area of carbon black shown in Table I. Considering that the surface area obtained by gas adsorption method may be different from the one available for the adsorption from solution, the value calculated above seemed reasonable compared with the cross-sectional area of 70Å^2 obtained by a graphical projection of the molecular structure model, meaning that the largest plane of tryptophan molecule might adopt the parallel orientation on the carbon black surface, as corresponded to such orientation of indole group.

Conclusively, carbon black might be of nonpolar surface and pH had no distinct effect on the adsorption of tryptophan by this adsorbent from aqueous solution of pH 3 to 9.

2) Adsorption of Tryptophan by Silica Gel from Aqueous Solution of Various pH——Adsorption isotherms[27] obtained are shown in Fig. 5. Depending on the amount of trypto-

---

[27] The amount adsorbed by silica gel should be an apparent one because some amount of the solvent (water) was considered to be adsorbed on such polar surface.

NII-Electronic Library Service

phan adsorbed, *i.e.*, on the initial concentration of tryptophan, the equilibrium pH varied from the initial pH more remarkably than that in the case of carbon black. Since the equilibrium pH should be taken in consideration for discussing the pH effect on the adsorption, the data obtained under various conditions were arranged in the five classes according to equilibrium pH as shown in Fig. 5, and the amount of tryptophan adsorbed was plotted against the relative concentration, *i.e.*, the ratio of equilibrium concentration to solubility at the pH.[18]

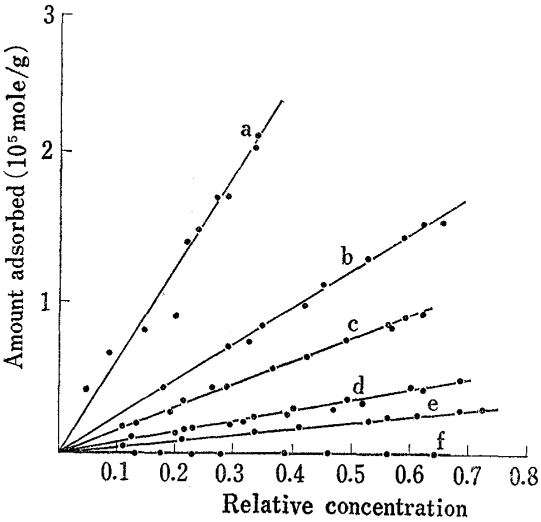

Fig. 5. Apparent Adsorption Isotherms of Tryptophan by Silica Gel from Aqueous Solution at 30°

equilibrium pH
a : 1.5
b : 2.3—3.0
c : 3.2—5.5
d : 6.0—7.5
e : 8.0—8.5
f : 9.3—9.6

The result showed that the adsorption depended remarkably on pH, increasing with acidity. The affinity of tryptophan to silica gel seemed so small that the isotherm was on a straight line in Fig. 5, as is explained by Langmuir equation. The amount of tryptophan adsorbed by silica gel was much smaller than that by carbon black described above, and was also much smaller than the amount of hydrogen or hydroxyl ion adsorbed. Considering that silica gel is hydrophilic and carbon black is hydrophobic, the above results may indicate that tryptophan is of hydrophobic property.

In more acidic region than the zero point of charge, pH always got close to the zero point of charge after tryptophan was adsorbed. It is, therefore, understood that no ion exchange adsorption took place, as was the same in the case of carbon black, while Otsuka reported on the ion exchange adsorption of amino acids by synthetic aluminum silicate,[28] a substance similar to silica gel in the polarity of surface. The amount of both hydrogen ion and tryptophan adsorbed increased with the acidity of solution at equilibrium, while tryptophan was hardly adsorbed in more alkaline pH region than zero point of charge. This kind of pH dependence has been reported on the adsorption of glycine by montomorillonite,[29] while the discussion in detail has never been given. The following explanation might be possible for the result obtained in this study. In more acidic pH region than zero point of charge, hydrogen ion is considered to be adsorbed preferentially on silica gel, SiOH and $SiOH_2^+$ being formed.[24] SiOH formed in this way seems to have an ability to adsorb both hydrogen ion and tryptophan. Thus, the amount of tryptophan adsorbed may increase with acidity, though it is much smaller than that of hydrogen ion adsorbed. In more alkaline pH region than zero point of charge, hydrogen ion is desorbed and SiO^- may be formed, repulsing the tryptophan anion, as seems to be a negative adsorption of tryptophan.

Conclusively, tryptophan is considered to have a very small affinity to silica gel and to behave as a nonpolar molecule.

Temperature Dependence of the Adsorption of Tryptophan[20] from Aqueous Solution

In order to discuss the mechanism of adsorption process of tryptophan by carbon black, adsorption isotherms were investigated at different temperatures. It was interesting that the amount adsorbed increased with temperature. Few examples have been reported for the adsorption increasing with temperature, *e.g.*, polyvinyl acetate by metal powder,[30] glycin

---

28) A. Otsuka and T. Otani, Presentation No. 5 at the 17th Koroido Kakagu Toronkai (Symposium on Colloid Chemistry), Japan, Tokyo, 1965.
29) O. Sieskind and R. Wey, *Compt. Rend.*, **1953**, 1652.
30) J. Koral, R. Ullman, and F.R. Eirich, *J. Phys. Chem.*, **62**, 541 (1958).

NII-Electronic Library Service

and its peptides by montomorillonite,[31] and Janus Red B by silica and Lissamine Green NB by alumina.[32]

As shown in Fig. 6, each isotherm regarding carbon black and graphite was in accordance with Langmuir equation, *i.e.*, equation (2), except in the low equilibrium concentration in the case of carbon black. Such a deviation in the low equilibrium concentration was observed upon the adsorption of barbiturates by carbon black from aqueous solution,[33] seeming due to surface heterogeneity of the adsorbent. Since the adsorption is considered to take place preferentially on the site of the stronger affinity, it may be reasonable that the Langmuir plot in the case of carbon black deviated to the lower part in the low equilibrium concentration as shown in Fig. 6a. For reference, the free energy change calculated at the deviating part was larger than that at the straight line part in both cases of tryptophan and barbiturates.[33] However, the straight line, as shown in Fig. 6a, extends in wide range to represent the carbon black surface to be largely homogeneous.

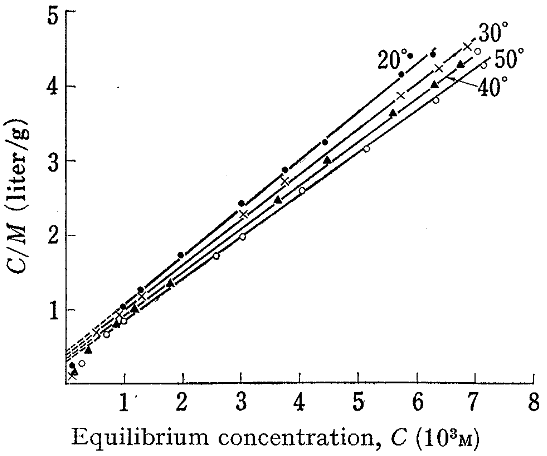

Fig. 6a. Langmuir Plots of Adsorption of Tryptophan by Carbon Black from Aqueous Solution

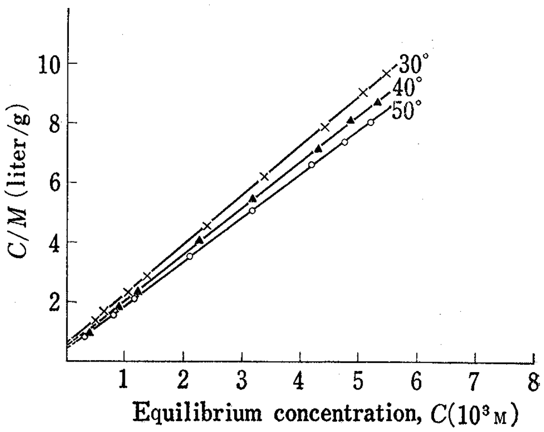

Fig. 6b. Langmuir Plots of Adsorption of Tryptophan by Graphite from Aqueous Solution

Since b in equations (1) and (2) is the equilibrium constant of adsoprtion process, the following equations are derived.

$$\Delta F = -RT \ln b \tag{3}$$

$$\Delta H = d(\Delta F/T)/d(1/T) \tag{4}$$

$$\Delta S = (\Delta H - \Delta F)/T \tag{5}$$

where ΔF, ΔH, and ΔS are the free energy change, the enthalpy change and the entropy change of the adsorption process, respectively, at temperature T. From these equations the thermodynamic functions are obtained.

Table IV shows the result. Both ΔH and ΔS were positive and the entropy term was predominant in the free energy change. Graphite showed the same tendency. Bartel, *et al.*[34] reported that the absolute value of ΔH was not higher than that of ΔF on the adsorption of *n*-butyl alcohol by graphite or blood char from aqueous solution, ΔS being positive, but no explanation was given for the adsorption mechanism.

As will be discussed in the following paper,[18] the hydrophobic hydration may take place in aqueous solution of tryptophan, forming an iceberg enclosing the tryptophan molecule.

---

[31] D.J. Greenland, R.H. Laby, and J.P. Quirk, *Trans. Faraday Soc.*, **58**, 829 (1962).

[32] C.H. Giles, J.J. Greczek, and S.N. Nakhwa, *J. Chem. Soc.*, **1961**, 93.

[33] H. Nogami, T. Nagai, and H. Uchida, *Chem. Pharm. Bull.* (Tokyo), in press.

[34] F.E. Bartell, T.L. Thomas, and Y. Fu, *J. Phys. Chem.*, **55**, 1456 (1951).

Table IV. Langmuir Constants and Thermodynamic Functions of Adsorption of Tryptophan by Carbon Black or Graphite from Aqueous Solution

Temp. (°C)	a-Value × 10^3 (mole/g)	b-Value × 10^{-3} (liter/mole)	ΔF (kcal/mole)	ΔH (kcal/mole)	ΔS (e.u.)
Carbon balck					
20	1.51	1.63	-4.32	0.22	15.5
30	1.61	1.70	-4.45	0.22	15.4
40	1.67	1.87	-4.67	0.22	15.6
50	1.76	2.03	-4.88	0.22	15.8
Graphite					
30	0.590	3.55	-4.90	0.18	16.7
40	0.633	3.60	-5.06	0.18	16.7
50	0.690	3.63	-5.25	0.18	16.7

It was discussed before that, on the adsorption by carbon black, the largest plane of tryptophan molecule might adopt the parallel orientation to the carbon black surface. Therefore, the iceberg was considered to be broken down through the adsorption process. Considering that the endothermal effect and the entropy increase caused by the break-down of iceberg might overcome the exothermal effect and the entropy decrease, respectively, the positive values of ΔH and ΔS, as shown in Table IV, could be expected.

Since the term $T\Delta S$ was larger than ΔH, the adsorption in this system was considered due mainly to the entropy effect. Although it has usually been considered that an adsorption takes place predominantly through energy change, the adsorption in the tryptophan/carbon black system might be forced with entropy increase through the destruction of the iceberg, as could be an explanation for the fact that the adsorption increased with temperature. In other words, hydrophobic bonding might participate in this adsorption phenomenon. Scheraga, *et al.*35) reported that a carboxylic acid of larger molecular weight was adsorbed more on polystyrene accompanying the increase of entropy, giving an explanation based on the hydrophobic bonding.

Various phenomena depending on hydrophobic bonding have been reported, *e.g.*, depressions of surface tension and micelle formation by surfactants,36) adsorption of dyes by fiber,37) and binding of low molecules to polymers.38) In biochemical field also, the concept of hydrophobic bonding have been introduced for an explanation for such phenomena as the denaturation of protein in aqueous solution, 39) and binding of organic substances of low molecular weight to protein.40)

The adsorption by carbon black, which might depend on hydrophobic bonding as described in this study, could be a model for biopharmaceutical phenomena in which hydrophobic bonding was considered to participate.

---

35) H. Schneider, G.C. Kresheck, and H.A. Scheraga, *J. Phys. Chem.*, **69**, 1311 (1966).

36) P. Mukerjee and A. Ray, *J. Phys. Chem.*, **67**, 190 (1963).

37) A. Katayama, Y. Kuroki, and K. Konishi, *Senigaku-Kaishi*, **19**, 828 (1963).

38) P. Molyneux and H.P. Frank, *J. Am. Chem. Soc.*, **83**, 3169 (1961).

39) W. Kauzmann, *Advan. Protein Chem.*, **14**, 1 (1959).

40) *a*) W. Scholtan, *Arzneimittel-Forsch.*, **14**, 469 (1964); *b*) M. Sahyun, *Nature*, **209**, 613 (1966).