

(2) W. Berg, K. Korsan-Bengsten, and J. Ygge, *Thromb. Diath. Haemorrh.*, **19**, 169 (1968).
 (3) R. J. Merrills and J. T. B. Shaw, *Biochem. J.*, **106**, 101 (1968).
 (4) W. Liniger and P. Rueggsegger, *Math. Biosci.*, **1**, 263 (1967).
 (5) W. Liniger and P. Rueggsegger, *Thromb. Diath. Haemorrh.*, **17**, 412 (1967).
 (6) J. Daver, E. Edmond, E. Panak, and M. Ricoeur, *Rev. Eur. Etudes Clin. Biol.*, **15**, 333 (1970).
 (7) J. Daver, P. Desnoyers, and M. Ricoeur, in "Synthetic Fibrinolytic Thrombolytic Agents," K. N. Von Kaulla and J. F. Davidson, Eds., Charles C Thomas, Springfield, Ill., 1975, pp. 245–264.
 (8) P. Desnoyers, J. Daver, J. Labaume, and J. Sebastien, in *ibid.*, pp. 265–275.
 (9) C. A. Bouvier and J. Gründlinger, *Schweiz. Med. Wochenschr.*, **93** (41), 1451 (1963).
 (10) J. Gründlinger and C. A. Bouvier, *Thromb. Diath. Haemorrh.*, **12**, 425 (1964).

(11) C. A. Bouvier, M. Beretta-Piccoli, and M. Giacometti, in "Progress in Chemical Fibrinolysis and Thrombolysis," J. F. Davidson, M. Samama, and P. Desnoyers, Eds., Raven, New York, N.Y., 1975, pp. 281–288.
 (12) L. A. Nelder and R. Mead, *Computer J.*, **7**, 308 (1965).
 (13) R. Bierme, J. Daver, B. Boneu, M. Ricoeur, and M. Verry, "Third Congress of International Society on Thrombosis and Haemostasis," Washington, D.C., Aug. 22–26, 1972, Abstr. p. 276.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Kwang Eun Kim and Mlle. Françoise Chamboine for assistance in evaluating the data and to Dr. Richard Süverkrup for the programming of the Simplex algorithm on the computer. Appreciation is also expressed to Dr. Jean Claude Arnáud, Director of Research of the Servier Research Laboratories, whose support and encouragement made this collaborative endeavor possible.

Physicochemical Properties of Glycyrrhizic Acid in Aqueous Media II: Effect on Flocculation–Deflocculation Behavior of Suspensions of Sulfathiazole and Graphite

AKINOBU OTSUKA *, YORINOBU YONEZAWA, and YASUHIKO NAKAMURA

Received January 19, 1977, from the Faculty of Pharmacy, Meijo University, Tempaku-ku, Nagoya, Japan. Accepted for publication April 22, 1977.

Abstract □ The flocculation–deflocculation behavior of sulfathiazole and graphite in aqueous solutions of glycyrrhizic acid was studied by measuring the sedimentation volume and turbidity of supernates. The dispersing effect of glycyrrhizic acid on suspensions of sulfathiazole showed a maximum in the pH 3–4 region, the same pH region where the ζ -potential of sulfathiazole particles showed a negative maximum. The results were explained by the variation of degrees of ionization of glycyrrhizic acid and sulfathiazole with pH. With graphite suspensions, the pH region where the dispersing effect of glycyrrhizic acid showed a maximum shifted to a higher pH compared with sulfathiazole. This result can be attributed to the fact that graphite is a nonpolar substance so the surface properties are not affected by a pH change. Hence, the adsorption of glycyrrhizic acid occurs even in a fairly high pH range.

Keyphrases □ Glycyrrhizic acid—effect on flocculation–deflocculation behavior of sulfathiazole and graphite, aqueous solutions, various pH values □ Sulfathiazole—flocculation–deflocculation behavior, effect of glycyrrhizic acid, aqueous solutions, various pH values □ Graphite—flocculation–deflocculation behavior, effect of glycyrrhizic acid, aqueous solutions, various pH values □ Flocculation–deflocculation behavior—sulfathiazole and graphite, effect of glycyrrhizic acid, aqueous solutions, various pH values □ Antibacterials—sulfathiazole, flocculation–deflocculation behavior, effect of glycyrrhizic acid, aqueous solutions, various pH values

A previous report from this laboratory (1) dealt with the surface-active properties and the formation of molecular aggregates of glycyrrhizic acid, a triterpenoid acidic glucoside extracted from *Glycyrrhiza glabra* L. This paper describes the dispersing effect of this substance on aqueous suspensions of sulfathiazole and graphite. While the use of natural products for suspending agents or emulsifying agents has attracted special interest recently, studies of this kind on glycyrrhizic acid have not been published.

EXPERIMENTAL

Materials—Monopotassium glycyrrhizinate¹ was used as received. Graphite² and sulfathiazole³ were obtained as commercial samples. The specific surface area of the graphite determined by the air permeation method⁴ was 0.25 m²/g. The sulfathiazole was finely ground, and the sample with a specific surface area of 0.62 m²/g was used. All other reagents were analytical grade.

Sedimentation Measurements—To 2.50 g of sulfathiazole or 2.00 g of graphite in a 50-ml graduated test tube was added 50 ml of an aqueous solution of monopotassium glycyrrhizinate whose pH had been adjusted with hydrochloric acid or potassium hydroxide solutions. The mixture was shaken in a constant-temperature bath at 25° for 24 hr and subsequently allowed to stand for 24 hr at the same temperature; the sedimentation volume was then measured.

Turbidity Tests—After the measurements of sedimentation volume, the turbidity of the cloudy liquid phase above the sediment was measured at 500 nm according to:

$$\tau = -\ln T/l \quad (\text{Eq. 1})$$

where τ is the turbidity, T is the transmittance, and l is the cell path length.

Adsorption Measurements—After the sedimentation measurements and/or the turbidity tests, the suspensions were centrifuged quickly. The glycyrrhizic acid concentration in the supernate was determined as follows. For the sulfathiazole–glycyrrhizic acid system, 10 ml of supernate was transferred into a test tube, and then 1 ml of concentrated hydrochloric acid and 5 ml of water were added. The mixture was heated to 95° and then allowed to cool. The formed precipitate was dissolved in 5 ml of chloroform, and the absorbance was measured at 258 nm.

For the graphite–glycyrrhizic acid system, the absorbance of the su-

¹ Maruzen Seiyaku Co., Tokyo, Japan.

² Yoneyama Chemical Industries, Osaka, Japan.

³ Sankyo Co., Tokyo, Japan.

⁴ Model SS-100, Shimadzu Seisakusho, Kyoto, Japan.

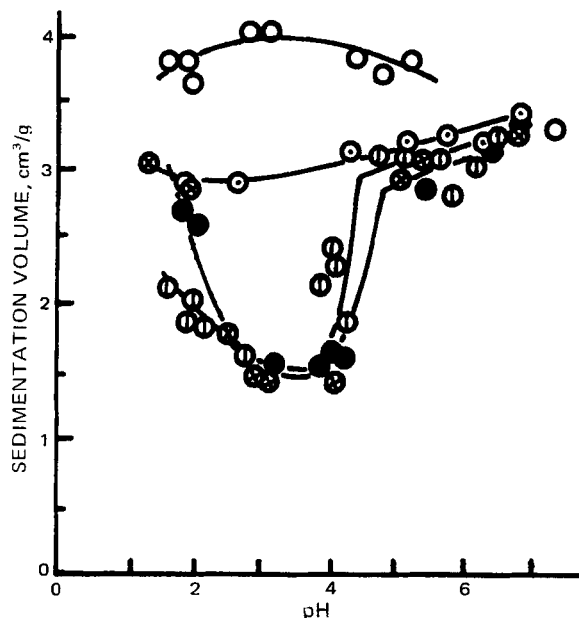


Figure 1—Relationship between sedimentation volume and pH for sulfathiazole suspensions in the absence and presence of glycyrrhizic acid. Key [concentration of glycyrrhizic acid (mM)]: ○, 0; ○, 0.10; ⊕, 0.30; ●, 0.50; and ⊗, 0.75.

pernate at 258 nm was measured directly. The amount of glycyrrhizic acid adsorbed was calculated from the difference in the concentrations before and after adsorption.

ζ-Potential Measurements—The ζ-potential was measured using a streaming potential measuring apparatus, which was an improvement on the Hazel type (2). The ζ-potential was calculated from the Helmholtz-Smoluchowski equation:

$$\zeta = 4\pi\eta\kappa H/PD \quad (\text{Eq. 2})$$

where H is the streaming potential, P is the pressure that drives the liquid through a powder bed, η is the viscosity, κ is the specific conductance, and D is the dielectric constant of the liquid.

RESULTS

The relationship between the sedimentation volume and the pH for sulfathiazole is given in Fig. 1. Figures 2 and 3 show the turbidity of the liquid phase against the pH for sulfathiazole and graphite, respectively. In each case, the parameter is the glycyrrhizic acid concentration present.

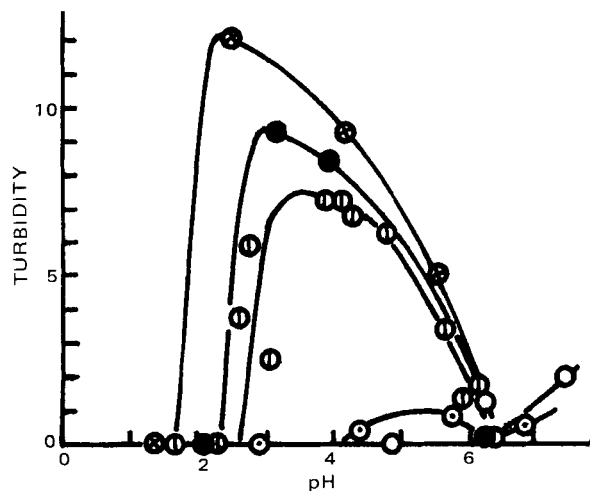


Figure 2—Relationship between turbidity of the liquid phase above the sediment and pH for sulfathiazole suspensions in the absence and presence of glycyrrhizic acid. Key [concentration of glycyrrhizic acid (mM)]: ○, 0; ○, 0.10; ⊕, 0.30; ●, 0.50; and ⊗, 0.75.

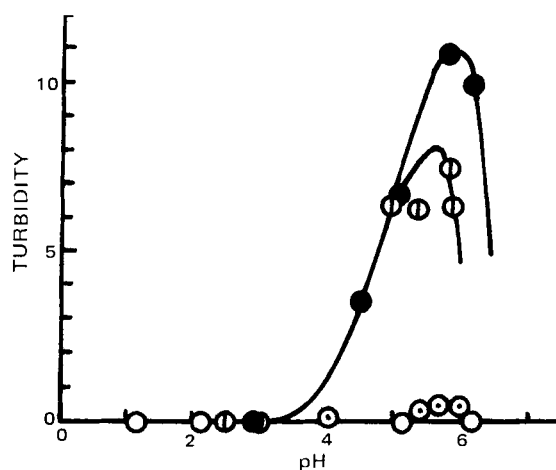


Figure 3—Relationship between turbidity of the liquid phase above the sediment and pH for graphite suspensions in the absence and presence of glycyrrhizic acid. Key [concentration of glycyrrhizic acid (mM)]: ○, 0; ○, 0.10; ⊕, 0.30; and ●, 0.50.

In the absence of glycyrrhizic acid, the sedimentation volume of sulfathiazole was extremely large, leaving a clear layer of liquid above a sediment. This result means that sulfathiazole particles were in a flocculated state. In the presence of sufficient glycyrrhizic acid, the sedimentation volume decreased as the pH rose to 3–4, but it increased with a further rise in pH. The turbidity of the liquid phase showed a maximum in the same pH range. These results indicate that the dispersibility of sulfathiazole in aqueous solution of glycyrrhizic acid was optimal in this pH range.

The results for graphite suspensions were qualitatively similar to those for sulfathiazole except that the pH range in which there was a marked increase in dispersibility of suspensions shifted to 4–6. The dispersibility of sulfathiazole or graphite in aqueous solutions of glycyrrhizic acid decreased as the ionic strength increased. Figure 4 illustrates that the sedimentation volume increased when potassium chloride was added to suspensions. Moreover, the addition of the salt caused the complete disappearance of turbidity in the liquid phase above the sediment layer of graphite.

Figure 5 shows the effect of pH on the amount of glycyrrhizic acid adsorbed by sulfathiazole. The parameter here is the initial concentration of glycyrrhizic acid. Figure 6 presents the adsorption isotherms drawn on the basis of the data in Fig. 5. The amount adsorbed was large in a low pH range and decreased with rising pH. At any pH above 4.5, virtually

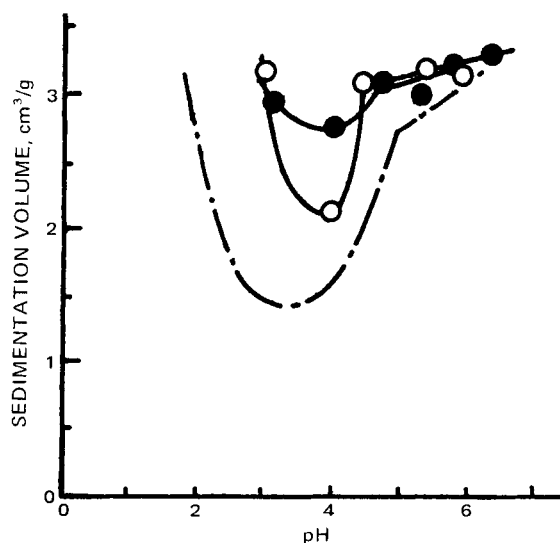


Figure 4—Effect of potassium chloride addition on the sedimentation volume of sulfathiazole in the solution of glycyrrhizic acid. Key [concentration of potassium chloride (M)]: ---, 0; ○, 0.10; and ●, 0.50. Concentration of glycyrrhizic acid is 0.50 mM.

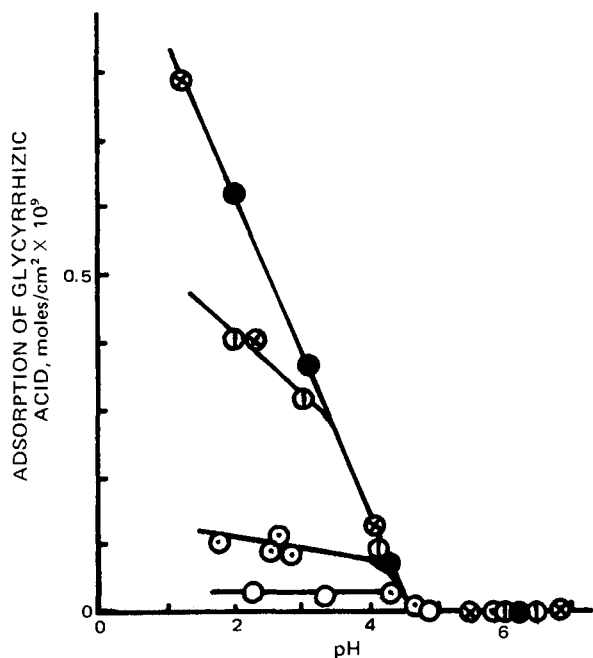


Figure 5—Amount of adsorption of glycyrrhizic acid on sulfathiazole versus pH. Key [initial concentration of glycyrrhizic acid (mM)]: ○, 0.05; ◐, 0.10; ◑, 0.30; ●, 0.50; and ⊗, 0.75.

no adsorption occurred. With graphite (Figs. 7 and 8), the amount of glycyrrhizic acid adsorbed fell to practically zero above pH 6.5.

The addition of potassium chloride caused an increase in the amount of glycyrrhizic acid adsorbed on sulfathiazole whereas the adsorption on graphite was hardly affected (Fig. 9).

The curves of ζ -potential against pH for sulfathiazole are given in Fig. 10. In the absence of glycyrrhizic acid, a gradual reduction in the ζ -potential with rising pH was observed. In the presence of glycyrrhizic acid, the ζ -potential dropped rapidly with an increase in pH and reached negative maxima in the pH 3–4 region.

DISCUSSION

When discussing the effects of pH on the adsorption of glycyrrhizic acid by sulfathiazole or graphite and on the flocculation–deflocculation behavior of these suspensions, the variation of the charge on both the powder surface and the glycyrrhizic acid molecule with pH should be considered. Although glycyrrhizic acid is a tribasic acid, it is impossible to detect the first and second end-points in the titration curve of its

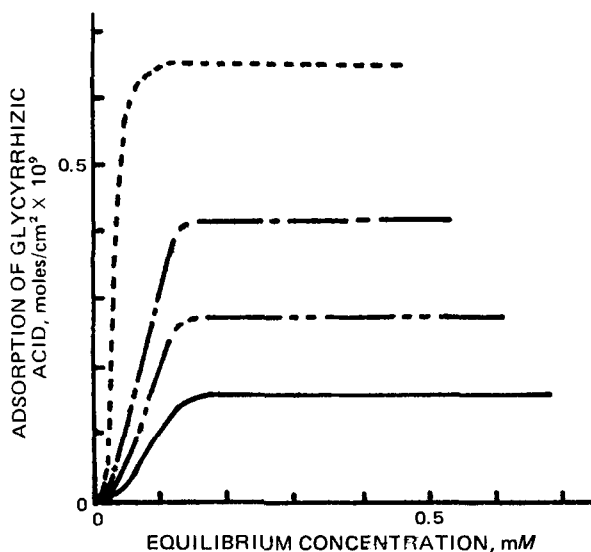


Figure 6—Adsorption isotherms for sulfathiazole at various pH values. Key: ---, pH 2.0; - - -, pH 3.0; - · - · -, pH 3.5; and —, pH 4.0.

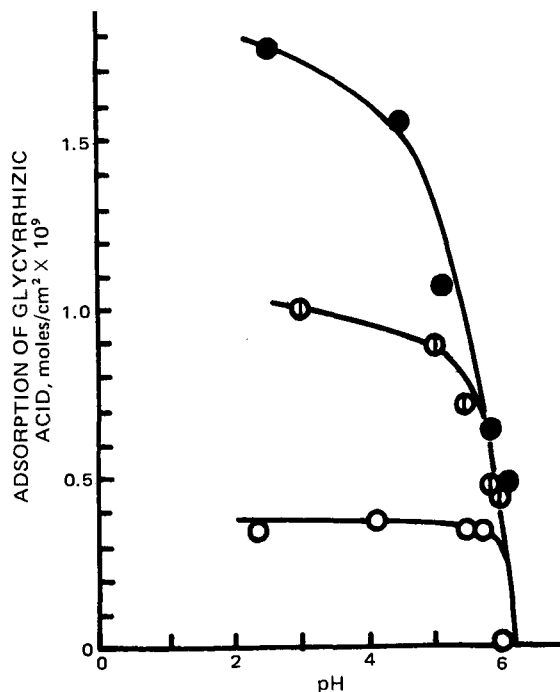


Figure 7—Amount of adsorption of glycyrrhizic acid on graphite versus pH. Key [initial concentration of glycyrrhizic acid (mM)]: ○, 0.10; ◐, 0.30; and ●, 0.50.

aqueous solution by a strong base. The titration curve, however, yields the apparent average dissociation constant.

The variation of the degree of ionization of carboxyl groups with pH is shown in Fig. 11a. The distribution of the various forms of sulfathiazole as a function of pH was calculated from literature values of its dissociation constants (3, 4) and is given in Fig. 11b, which explains the variation of the ζ -potential with pH in the absence of glycyrrhizic acid.

When glycyrrhizic acid is added to the suspensions, glycyrrhizate anions are adsorbed on the surface of sulfathiazole; the negative charge of particles then becomes higher than in the absence of glycyrrhizic acid.

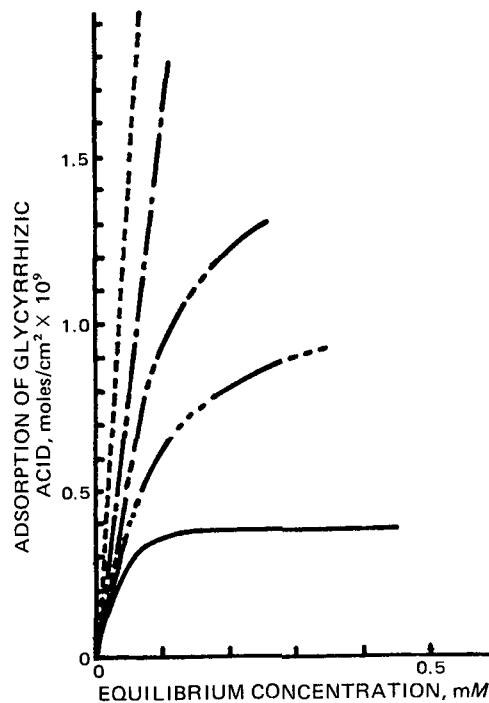


Figure 8—Adsorption isotherms for graphite at various pH values. Key: ---, pH 3.0; - - -, pH 4.0; - · - · -, pH 5.0; - · - · - · -, pH 5.5; and —, pH 6.0.

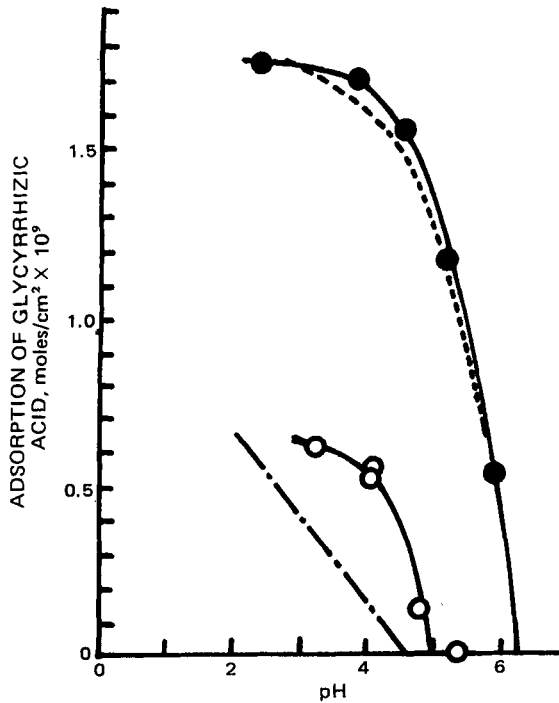


Figure 9—Effect of the addition of potassium chloride on the amount of adsorption of glycyrrhizic acid. Key: - - -, in the absence of potassium chloride for sulfathiazole; O, in 0.5 M KCl solution for sulfathiazole; - · - ·, in the absence of potassium chloride for graphite; and ●, in 0.5 M KCl solution for graphite. Initial concentration of glycyrrhizic acid was 0.50 mM.

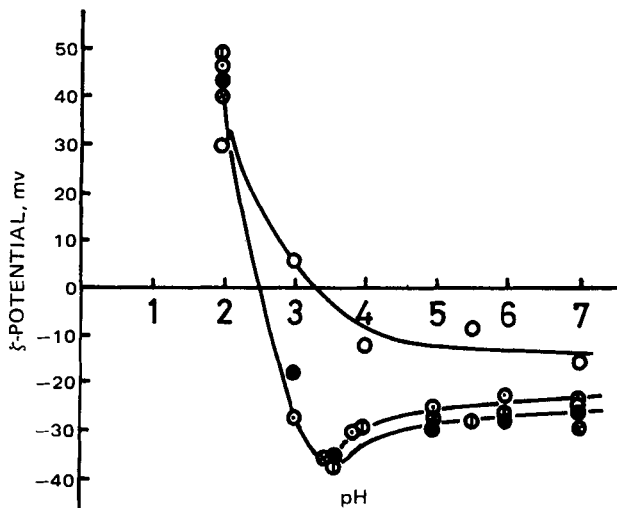


Figure 10— ζ -Potential of sulfathiazole in the absence and presence of glycyrrhizic acid versus pH. Key [concentration of glycyrrhizic acid (mM)]: O, 0; ○, 0.10; ⊖, 0.30; ●, 0.50; and ⊗, 0.75.

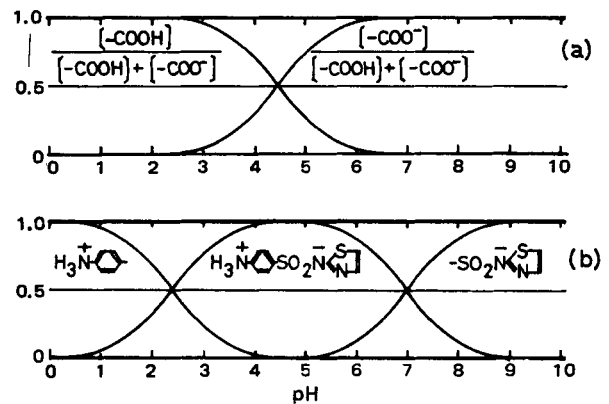


Figure 11—Distribution of various forms of glycyrrhizic acid (a) and sulfathiazole (b) as functions of pH.

As a result, negative maxima appear in the ζ -potential-pH relationship. This result may be due to two effects which counteract each other:

1. With rising pH, the degree of ionization of adsorbed glycyrrhizic acid increases, which causes an increase in the negative charge of particles.
2. An increase in the negative charge of particles and glycyrrhizic acid molecules increases the escaping tendency of the molecules into the liquid phase and, hence, the amount of adsorption decreases.

The dispersibility of sulfathiazole in the presence of glycyrrhizic acid increases in the pH region over which an increase in the negative charge on particles occurs, and the optimum pH for dispersibility agrees with the pH where the ζ -potential shows negative maxima.

The existence of an optimum pH region is also known for the dispersibility of graphite in the solutions of glycyrrhizic acid. There is no essential difference between sulfathiazole and graphite regarding the stabilization mechanism of suspensions. However, graphite is a nonpolar substance; therefore, the surface properties of graphite are not affected by a pH change, and glycyrrhizic acid adsorption occurs even at a fairly high pH. As a result, the pH region where the dispersibility of graphite shows maxima shifts to a higher pH compared with sulfathiazole.

With the addition of electrolytes to the sulfathiazole-glycyrrhizic acid system, the electrostatic repulsive forces between negatively charged sulfathiazole surfaces and glycyrrhizinate anions are diminished. Consequently, glycyrrhizic acid adsorption increases. However, as the salt concentration increases, the repulsive force between particles decreases, resulting in the flocculation of suspensions.

With graphite, the electrostatic repulsion between particles and anions may be ignored; electrolyte addition does not produce such a marked increase in adsorption as in the case of sulfathiazole. The lowering of dispersibility of graphite by electrolytes can be explained in the same manner as for sulfathiazole.

REFERENCES

- (1) A. Otsuka, Y. Yonezawa, K. Iba, T. Tatsumi, and H. Sunada, *Yakugaku Zasshi*, **96**, 203 (1976).
- (2) M. Nakagaki and Y. Nakamura, *ibid.*, **92**, 1000 (1972).
- (3) M. Nakagaki, N. Koga, and H. Terada, *ibid.*, **83**, 586 (1963).
- (4) T. Koizumi, T. Arita, and K. Kakemi, *Chem. Pharm. Bull.*, **12**, 413 (1964).