Interactions of Surface Active Agents with Congo Red

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(Received April 11, 1960)

Interactions between various surface active agents and many substances such as protein, dyes and polymers have been studied for contribution to the elucidation of fundamental properties of surface active agents to or extend their applications[1-4]. It has been reported that some dyes such as azo, thiazine or triphenylmethane dyes were changed in their spectra with the addition of various substances such as agar, chondroitin sulfate, nucleic acid or albumin[5-9]. The similar effects of surface active agents, though the greater part of these are ionic ones, on the spectral changes of some dyes have also been studied in recent years[10-15]. In the case of non-ionic detergents, a complex formation has been predicted between dyes and detergents[12,15], but details are not known.

In this paper, the metachromatic effects of a non-ionic surface active agent, Tween 80 and a cationic one, cetyl pyridinium chloride on the absorption spectrum of Congo Red solution are reported.

Experimental

Reagents.—The Congo Red used in this experiment was purified by salting out with sodium acetate and recrystallizing several times from water. Tween 80 (Atlas Powder Co.) was dissolved in water to about 5%, ionic impurities were removed by means of ion-exchange resins (Amberlite IR-120, IRA-400), then evaporated and dried in vacuo at about 50°C. Other reagents were recrystallized from water before use.

Absorption Spectra.—The absorption spectra of the solutions were measured with a Beckman type spectrophotometer using a 1 cm. glass absorption cell.

Procedures.—The procedure used in the preparation of the dye solutions to be measured was as follows: 1 ml. of the Congo Red stock solution (2.34×10^{-3} mol./l.) was measured in a 100 ml. volumetric flask. Then 1 ml. of the concentrated buffer solution was pipetted in and an appropriate amount of 4.9% Tween 80 solution was added to the flask to obtain the desired pH and detergent concentrations. The entire contents of the flask were then diluted to 100 ml. and stirred sufficiently. It was left to stand for about 30 min. after the preparation in order to secure an equilibrium (it had been confirmed that 30 min. was sufficient for the equilibrium), then the spectrum was measured using an aliquot part of the solution. Another part of solution was used to determine the pH of the solution. In the experiments of cetyl pyridinium chloride, the sample solutions prepared were centrifuged and then the supernatant solutions were used to measure the spectra.

Results

Fig. 1 represents the absorption spectra of Congo Red solution of 2.34×10^{-5} mol./l. in the presence of various amounts of Tween 80 at the fixed pH of 3.4. The spectrum varies gradually with the addition of Tween 80 and the new absorption peak appears at about 510 mμ. This peak increases in height with increasing concentration of Tween 80 and it becomes constant above the Tween 80 concentration of 0.309%. The isosbestic point is clearly observed among these spectra, which may suggest that there is a definite molecular constitution of the product. An isosbestic point of this kind was also observed in other reports[3,13,15] which studied the interaction between some dyes such as Methyl Orange, Benzopurpurine 4B and Erythrosine and non-ionic or cationic detergents.

Fig. 2 shows the results measured at pH 4.3. As a whole the spectra are similar to those in Fig. 1, but the spectral change occurs with the addition of a smaller amount of Tween 80 than in the case of that at pH 3.4. The wavelength of the absorption peak of the product is the same in both cases.

Fig. 3 shows the change of the absorbance at the absorption peak with the concentration

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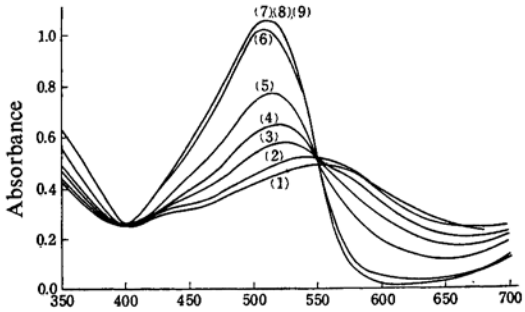

Fig. 1. Absorption spectra of Congo Red solutions containing various amounts of Tween 80 at fixed pH of 3.4; concentration of Congo Red, 2.34×10^{-5} mol./l.; concentration of Tween 80 (%): (1), 0.00; (2), 0.00483; (3), 0.0193; (4), 0.0386; (5), 0.078; (6), 0.155; (7), 0.309; (8), 0.525; (9), 1.05.

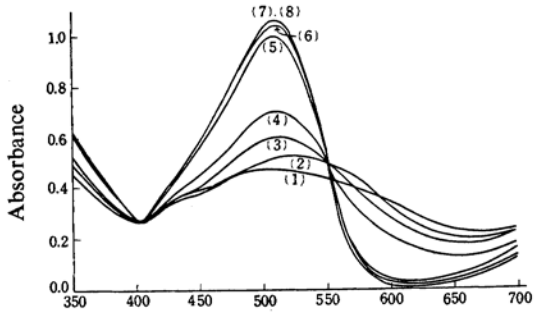

Fig. 2. Absorption spectra of Congo Red solutions containing various amouts of Tween 80 at pH 4.3; concentration of Congo Red, 2.34×10^{-5} mol./l.; concentration of Tween 80 (%): (1), 0.00; (2), 0.005; (3), 0.02; (4), 0.04; (5), 0.08; (6), 0.15; (7), 0.29; (8), 0.58.

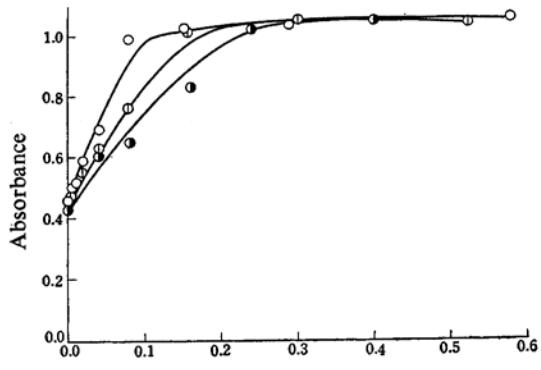

Fig. 3. The absorbances at 510 mμ of Congo Red solutions plotted against increasing concentrations of Tween 80 at three values of pH; ◑ pH 3.0; ◫ pH 3.4; ○ pH 4.3.

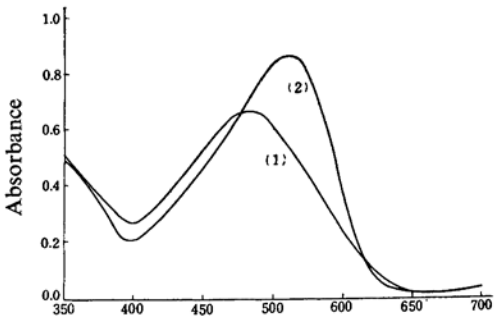

Fig. 4. Effect of the addition of Tween 80 on the absorption spectrum of Congo Red solution at pH 7.0; concentration of Congo Red, 1.87×10^{-5} mol./l.; conccentration of Tween 80 (%): (1), 0.00; (2), 0.35.

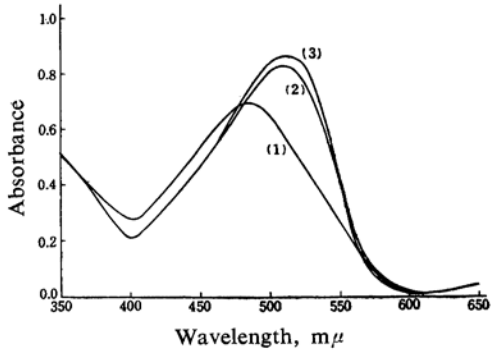

Fig. 5. Effect of the addition Tween 80 on the absorption spectrum of Congo Red solution at pH 9.2; concentration of Congo Red, 1.87×10^{-5} mol./l.; concentration of Tween 80 (%): (1), 0.00; (2), 0.016; (3), 0.16.

of Tween 80 at three values of pH of the dye solution.

The identical effects of Tween 80 are also observed in either the neutral or basic medium, the results being shown in Figs. 4 and 5. In these cases the absorbance at 510 mμ became constant with the addition of a very small amount of the detergent and their spectral changes were not remarkable as compared with that in the acidic medium.

When cetyl pyridinium chloride (c. p. c.) is added to the Congo Red solution at pH 4.2, a spectral shift occurs and indicates the presence of an interaction product between c. p. c. and Congo Red, as shown in Fig. 6. In this case, Congo Red is dispersed by a small quantity of c. p. c., and has an absorption peak at about 660 mμ. When the concentration of the detergent increases, a new peak appears at 460 mμ which may be assigned to the interaction product between Congo Red and c. p. c. This peak increases in height with increasing

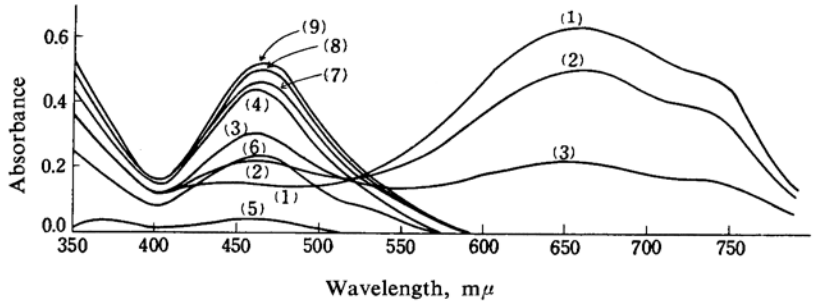

Fig. 6. Absorption spectra of Congo Red solutions containing various amounts of cetyl pyridinium chloride at fixed pH of 4.2; concentration of Congo Red, 2.17×10^{-5} mol./l.; concentration of cetyl pyridinium chloride (mol./l.$\times10^{5}$): (1), 0.725; (2), 1.45; (3), 2.90; (4), 3.63; (5), 4.35; (6), 10.1; (7), 11.6; (8), 14.5; (9), 160.

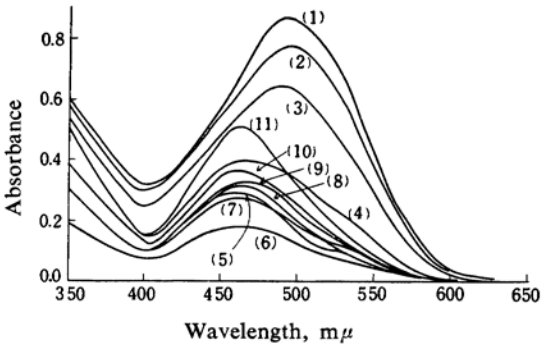

Fig. 7. Absorption spectra of Congo Red solutions containing various amounts of cetyl pyridinium chloride at fixed pH of 7.0; concentration of Congo Red, 2.17×10^{-5} mol./l.; concentration of cetyl pyridinium chloride (mol./l.$\times10^{5}$): (1), 0.00; (2), 0.685; (3), 1.37; (4), 2.74; (5), 3.43; (6), 8.22; (7), 9.59; (8), 11.0; (9), 16.4; (10), 27.4; (11), 68.5.

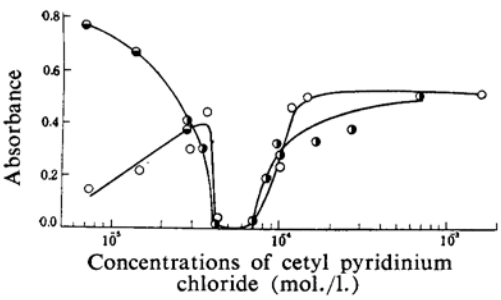

Fig. 8. The absorbances at 464 mμ and 490 mμ (pH 7.0) of Congo Red solutions (from which the precipitates are removed) plotted against the concentrations of cetyl pyridinium chloride at two values of pH: ○, 464 mμ, pH 4.2; ◑, 464 mμ, pH 7.0; ◒, 490 mμ, pH 7.0; concentration of Congo Red, 2.17×10^{-5}mol./l.

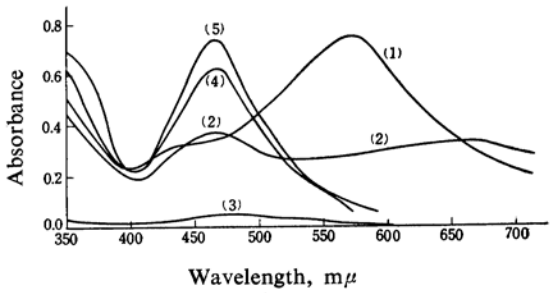

Fig. 9. Effect of the addition of cetyl pyridinium chloride at pH 3.8; concentration of Congo Red, 2.88×10^{-5} mol./l.; concentration of cetyl pyridinium chloride (mol./l.$\times10^{5}$): (1), 0.00; (2), 1.5; (3), 4.5; (4), 30.0; (5), 300.

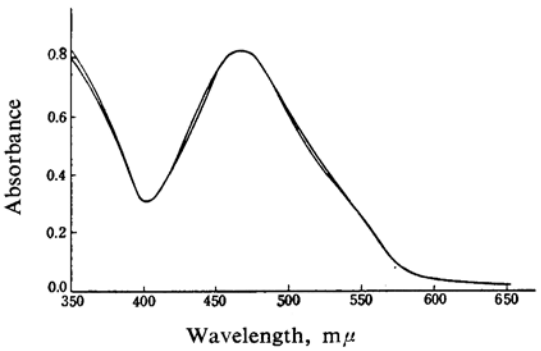

Fig. 10. Effect of the addition of cetyl pyridinium chloride at pH 1.45; concentration of Congo Red, 2.88×10^{-5} mol./l; concentration of cetyl pyridinium chloride, 7.3 and 14.5×10^{-5} mol./l.

concentration of c. p. c. The interaction products are precipitated in the presence of a certain amount of the detergent and then dispersed again with further increase of the concentration of c. p. c. The same effect of c. p. c. is observed in the neutral medium as shown in Fig. 7. In either case the interaction products are completely precipitated in the concentration range where the mole ratio of Congo Red to c. p. c. is about 1/2 and begins to be dispersed with further increase of c. p. c. concentration as shown in Fig. 8. Figs. 9 and 10 show that the same interaction product is also formed in the more acidic medium.

Discussion

The absorption peak at 510 mμ, as seen from Figs. 1, 2, 4 and 5, is considered to indicate that Congo Red and Tween 80 make a definite molecular compound irrespective of the pH of the solution. The results of Fig. 3 show that the hydrogen ion in the medium plays an important role in the equilibrium of the interaction between Congo Red and Tween 80. Either the acid or the base form of Congo Red is similarly varied in its spectrum and well dispersed by the addition of a sufficient amount of Tween 80. However, the amount of detergent required to show just sufficient change increases with lowering the pH of the medium. Although in acidic Congo Red solutions containing insufficient Tween 80 (below 0.309% of Tween 80 concentration at pH 3.4), precipitates appear by aging (about 24 hr. after preparation), in the systems containing sufficient detergent, no precipitation occurs. This is probably due to a kind of solubilization, but the precise mechanism is not known at the present.

In view of these results, it may be considered as follows: in an acidic medium, an acid form of Congo Red is equilibrated to a base form as in the following formula,

$$R\begin{matrix}NH_2\\NH_2\end{matrix} + 2H^+ \rightleftharpoons R\begin{matrix}NH_3^+\\NH_3^+\end{matrix} \qquad (1)$$

It is possible to assume the following equilibrium process for the spectral change,

$$R\begin{matrix}NH_2\\NH_2\end{matrix} + 2\ \text{Tween 80} \rightleftharpoons R\begin{matrix}NH_2-\text{Tween 80}\\NH_2-\text{Tween 80}\end{matrix} \qquad (2)$$

In this expression $R\begin{matrix}NH_2\\NH_2\end{matrix}$, $R\begin{matrix}NH_3^+\\NH_3^+\end{matrix}$ and $R\begin{matrix}NH_2-\text{Tween 80}\\NH_2-\text{Tween 80}\end{matrix}$ represent the base form of Congo Red, the acid one and the interaction product respectively.

The effect of pH (Fig. 3) may be interpreted qualitatively by these assumptions as follows. When the pH of the medium is lowered, the concentration of the base form decreases according to the formulation 1, which results in an increase of the amount of Tween 80 required to give a definite absorbance. In either neutral or basic medium, the same processes are probably considered to occur from the results of Figs. 4 and 5. In these cases, however, the concentrations of the base form become so great that the process is mainly represented by the formulation 2 and the same effect is produced with a smaller amount of Tween 80 than that in the acidic medium.

Thus, the amino groups of Congo Red molecule are considered to be blocked with Tween 80 and the products are well dispersed, since the aggregation of Congo Red molecules is prevented by the attachement of Tween 80. It has been observed that the adsorption of Congo Red for filter paper or cotton (which is considered to have a negative surface), was reduced remarkably by the addition of Tween 80, which may also be interpreted by a similar process.

In the case of cetyl pyridinium chloride, the wavelength of the new peak is 460 mμ, the position of which is not affected by the medium pH as shown in Figs. 6 and 7.

When the concentration of the detergent is 4.2×10^{-5} mol./l., at which the mole ratio of Congo Red to detergent is 1/2, the products are most hydrophobic and precipitated. They have a trend to be dispersed both sides of this ratio. In the lower concentrations of the detergent, in which the mole ratio is below about 1/1, neither precipitation nor metachromatic color is oberved. In the higher concentrations above the mole ratio of about 1/4, the products are well dispersed. The dispersion in the latter case is not considered to be due always to solubilization[11], since the system being dispersed with sufficient c. p. c. produces no precipitation even by means of dilution.

From these facts, it is considered that Congo Red is bound at the anionic loci, sulfonates, with c. p. c. by ionic force. It is probable that the products whose sulfonates are blocked become hydrophobic, but with further c. p. c. they will become hydrophilic again owing to the adsorption of the excess of c. p. c. with the hydrophilic groups towards water.

Summary

The spectral changes of Congo Red solution with the addition of Tween 80 and cetyl pyridinium chloride were measured at various pH.

The wavelength of the absorption peak of

the interaction product between Congo Red and Tween 80 is 510 mμ in the acidic medium as well as those in the neutral and the basic ones, from which it is considered that they produce a molecular compound of a definite constitution irrespective of the medium pH. However, since the amount of Tween 80 required to show the same effect in spectra increases with lowering pH, it is considered that only a base form of Congo Red is probably active and binds with Tween 80 at the amino groups.

The solutions of Congo Red containing sufficient cetyl pyridinium chloride have an absorption peak at 460 mμ. The product is completely precipitated as the ratio of Congo Red to cetyl pyridinium chloride is 1/2 and dispersed in both sides of this ratio, which depends little on the medium pH. This is considered to show that cetyl pyridinium chloride binds with Congo Red at the sulfonate groups, the product becomes most hydrophobic as two sulfonates in a molecule are blocked and hydrophilic with an excess of the detergent.

In conclusion, the author wishes to express his sincere thanks to Professor R. Matsuura of Kyushu University for his kind guidance and encouragement during this experiment, and also to Dr. H. Kimizuka of Kyushu University for his supply of the cationic detergent and discussion concerning this work.

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