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Acid–Base and Spectroscopic Properties of 1,4-Benzodiazepines in Sodium Dodecyl Sulfate Micellar Solutions

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Acid-base equilibria and spectroscopic properties of diazepam and chlorodiazepoxide were investigated in sodium dodecyl sulfate (SDS) micellar solutions as functions of pH. The results were compared with the behaviors in homogeneous aqueous media. The presence of SDS increased the pK_a of chlorodiazepoxide to 6.3 from 4.7, while it has little effect on the pK_a of diazepam. The acidic protonated form of diazepam was moderately fluorescent when the solution was excited at 350 nm, and emission intensity of the species was enhanced about 5 fold by the presence of SDS. On the other hand, the acidic solution of chlorodiazepoxide was non-fluorescent, but the neutral solution of the compound was fluorescent upon excitation at 350 nm. The emission peak of the neutral chlorodiazepoxide shifted to shorter wavelength region without significant change in the emission intensity upon the addition of SDS. Procedures for assay of the individual drugs from their mixture by the use of SDS micelle were discussed.

Introduction

Surfactants are amphiphilic molecules composed of a hydrophobic portion and a hydrophilic portion. At certain concentration (cmc), surfactat molecules aggregate to form a self-assembled aggregates, micelles. Micelle solubilizes and/or binds many organic substances and ionic species. The physico-chemical properties of chemical species are usually significantly modified upon binding on micelles. Hence, the micellar systems have been used as novel media for chemical and photochemical reactions.[1] Recently, the micellar chemical and photochemical reactions.[1] Recently, the micellar systems have also been employed to modify and improve many analytical schemes.[2]

1,4-Benzodiazepines are a class of physiologically active drugs and widely prescribed as anti-anxiety agents. Thus assay of the drugs in formulation and biological fluids has been the subject of intensive studies. Much emphasis has been placed on the electro-chemical methods for the analysis of 1,4-benzodiazepines.[3-5] Absorption[6] and fluorescence[7-10] spectrophotometry were also utilized to determine some of 1,4-benzodiazepines. Recently, the interaction of 1,4-benzodiazepines with surfactants and resultant enhancement of fluorescence emission were reported.[7-10] Also studies on the kinetics of hydrolysis[11] and solubilization[12] of the drugs in micellar solutions were described.

1,4-Benzodiazepines are protonated in acidic media. The protonated and deprotonated species of organic molecules usually show different chemical stability and physico-chemical properties. Since the acid-base equilibria of organic molecules are greatly influenced by the presence of ionic micelles,[2b] the studies on the acid-base behavior and pH-dependent spectroscopic properties of 1,4-benzodiazepines in micellar solutions would provide valuable information on the chemistry of the compounds. The results can be utilized to develop a micelle-improved spectroscopic method for the analysis of the drugs.

In this report, we present the studies of acid-base and

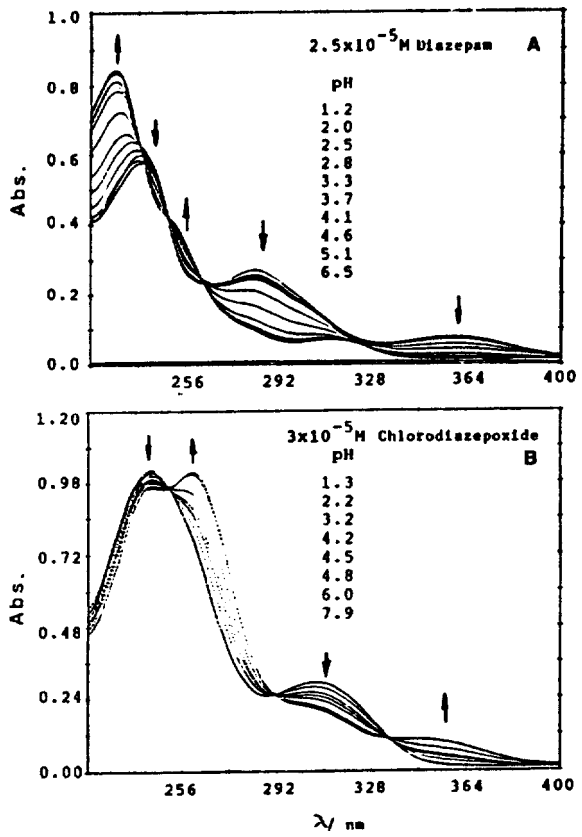
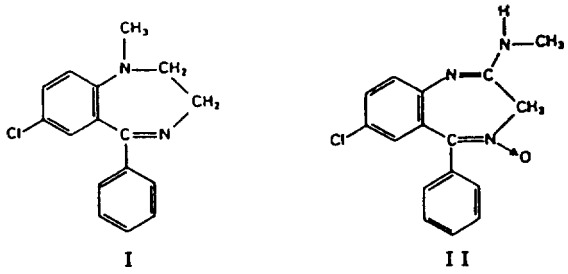


Figure 1. pH-dependent absorption spectra of 2.5×10^{-5} M diazepam (A) and 3.0×10^{-5} M chlorodiazepoxide (B) in water. Arrows indicate the direction of increasing pH.

spectroscopic properties of two 1,4-benzodiazepines, diazepam (I) and chlorodiazepoxide (II), in anionic sodium dodecyl sulfate (SDS) micellar solutions.



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Experimental

Sodium dodecyl sulfate (SDS) was obtained from Fluka and recrystallized three times from ethyl alcohol after washing with ether. Diazepam and chlorodiazepoxide were kindly provided by NIH. Solutions were prepared with deionized glass-distilled water. pH of solutions was adjusted with sodium cacodylate (final concentration was 0.04 M) and HCl. Ionic strength of the solution was held constant at 0.1 M with NaCl.

Absorption spectra were recorded with a Gilford 2600 X-Y UV-VIS spectrophotometer. A Hitachi 650-10S fluorescence spectrophotometer, equipped with a thermostatic cell holder, was used to take fluorescence spectra at 25 °C. The slit widths of excitation and emission beams were 5 and 10 nm, respectively. Since chlorodiazepoxide is photoisomerizable,[13] fresh solution which was kept in dark was used to take the spectra of the compound.

Table 1. The UV-VIS Absorption Spectral Features of Diazepam and Chlorodiazepoxide in Water

Compounds	Form	Peaks	Isosbestic points
		λ/nm (ε× M cm)	λ/nm (ε× M cm)
Diazepam	acidic	240 (23,000)	250 (17,000)
		285 (11,000)	262 (9,600)
		360 (3,100)	
	neutral	230 (34,000)	
		255 (15,000)^{sh}	
		318 (3,100)	
Chlorodiazepoxide	acidic	244 (33,400)	252 (31,500)
		307 (9,900)	291 (8,400)
	neutral	260 (33,000)	335 (3,300)
		355 (3,300)^{sh}	

sh: shoulder

Results and Discussion

Absorption Spectra and Acid-Base Equilibria. The UV-Vis absorption spectra of diazepam and chlorodiazepoxide exhibited a series of bands. Both the blue-shift and change in shape of the absorption spectra occurred with decreasing pH of the solutions. The isosbestic points were observed. The pH-dependent absorption spectra of diazepam and chlorodiazepoxide were shown in Figure 1. The spectral features of the spectra were summarized in Table 1. Observation of isosbestic points with varying pH of solutions is a clear indication that the drugs behave as monoacidic bases in the pH range studied, pH 1.5-8.

The presence of an anionic surfactant SDS in solutions did not cause significant change in spectral shape. However, the pH dependence of absorbance (A) was modified by SDS. In Figure 2, we presented variation of absorbance of diazepam at 283 nm and chlorodiazepoxide at 307 nm with pH of the solutions in the absence and presence of 50 mM SDS. The absorbance values in plateau regions in Figure 2 can be regarded as those of the protonated (DH^+) form (at low pH) and the neutral (D) form (at high pH) of the respective compounds. We denote these as A_{DH} and A_D, respectively.

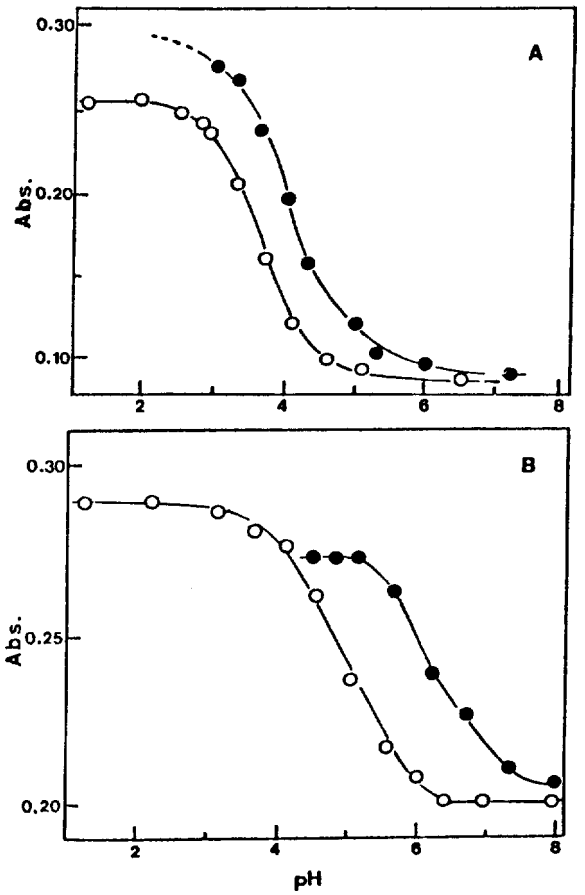
The acid dissociation constants of the conjugated acids are defined as following.

$$DH^+ \rightleftharpoons H^+ + D \quad K_a = \frac{[H^+][D]}{[DH^+]} \tag{1}$$

The apparent pK_a of the acid is related to pH of the solution and the fraction (α) of 1,4-benzodiazepines present as a neutral species at the pH by;

$$pH = pK_a + \log\left(\frac{\alpha}{1-\alpha}\right) \tag{2}$$

The α values are calculated from the absorbance at the pH, A_{DH} and A_D values of the compound by $\alpha = (A_{DH}-A)/(A_{DH}-A_D)$. The pK_a values of the compounds were determined from the intercepts of the pH *vs.* $\log\left(\frac{\alpha}{1-\alpha}\right)$ plots. For dia-



Fiure 2. Effects of SDS on the absorbance of 2.5×10^{-5} M diazepam at 283 nm (A) and 3.0×10^{-5} M chlorodiazepoxide at 307 nm (B) at various pH. Open circles are data taken from the surfactant-free solutions and filled circles are those from 50 mM SDS solutions.

zepam in SDS solution, we could not obtain A_{DH} unambiguously, presumably due to the second protonation in highly acidic SDS solution. In this case, an adjustable parameter was used for A_{DH}, and α_{pH} values were expressed as the function of the parameter. The pK_a of diazepam in SDS solution was estimated from best-fitted pH *vs.* log $(\frac{\alpha}{1-\alpha})$ plot. The pK_a values were summarized in Table 2.

The pK_a of diazepam in water obtained in this study (3.7 ± 0.15) is slightly higher than the reported values (3.3-3.5) by others.[10,11] The difference can be attributed to the high ionic strength (0.1 M) of the medium employed in this study.[14]

Table 2 shows that the pK_a values of both diazepam and chlorodiazepoxide are higher in the SDS solutions than in SDS-free water. The change in pK_a is much greater in the conjugated acid of chlorodiazepoxide than diazepam. Cirugeda and Soriano[10] reported that pK_a of diazepam in surfactant free solution (3.4 ± 0.1) was coincident with that in SDS solution (3.5 ± 0.1) in experimental error range. From this, they deduced a conclusion that SDS micelle has *no* influence on the dissociation of 1,4-benzodiazepines. Obviously, our results in Table 2 indicate that their conclusion was overgeneralized and show that the extent of the effect of SDS micelle on pK_a of 1,4-benzodiazepines depends on the nature of the drugs.

The shift in the dissociation constant of organic compounds in the presence of micelles was observed in a variety of systems.[14,15] Several quantitative models including the pseudophase ion-exchange model (PIE) were developed to interpret the phenomena.[14] According to the PIE model, the organic molecules bind to micelles by hydrophobic interaction. As OH^- ions do not bind to the anionic micelles such as SDS due to electrostatic repulsion, the deprotonation of the micelle-bound acid is disfavored and thus the pK_a of a micelle-bound cationic species increases. Our results accord well with this interpretation.

Table 2. The pK_a Values of Protonated Diazepam and Chlorodiazepoxide in Water and 50 mM SDS Solutions[a]

Compounds	water	50 mM SDS
Diazepam	3.7	4.1
Chlorodiazepoxide	4.8	6.3

[a]Estimated error range of the pK_a values was ±0.15.

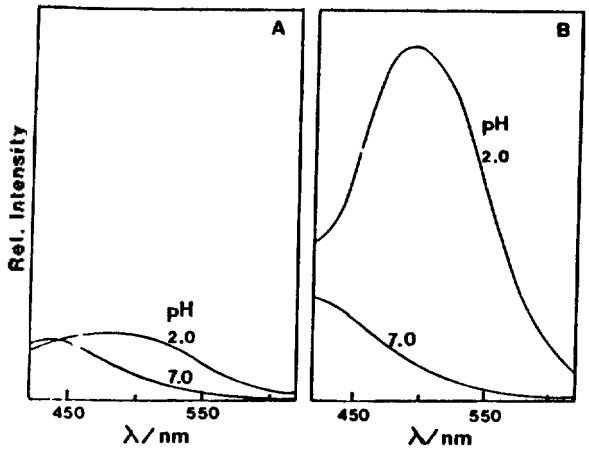


Figure 3. Fluorescence spectra of diazepam (pH 7) and its protonated form (pH 2) in the absence of SDS (A) and in the presence of 50 mM SDS (B). The excitation wavelength was 350 nm.

Effects of SDS on the Fluorescence Spectra. When diazepam solution was excited at 350 nm, the solution was scarcely fouorescent at $pH > pK_a$. Lowering pH of the solutions resulted in moderately strong emission at 490 nm. This indicates that the protonated form of diazepam is fluorescent. The presence of SDS enhanced the fluorescence emission from the acidic diazepam solutions. This agrees with the previous reports[9,10] which showed the enhancement of the fluorescence emission of diazepam upon the addition of SDS to the acidic solutions of diazepam above cmc of the surfactant. The pH-dependent fluorescence spectra of diazepam and the effects of SDS on the spectra were illustrated in Figure 3.

In Figure 4, we presented the variation of the emission intensity of diazepam with pH. The emission intensity *vs.* pH profiles resemble the patterns of absorbance *vs.* pH plots shown in Figure 2A. The slight decrease in the emission intensity in SDS solution at pH 1.3 seems to reflect the second protonation of the compound. The similarity of the pH dependency of the absorption (Figure 2A) and emission (Figure 4) indicates that the enhanced emission of diazepam at lower pH arises from the increased concentration of the protonated form which absorbs the exciting light more strongly. We used the pH-dependent emission data to eval-

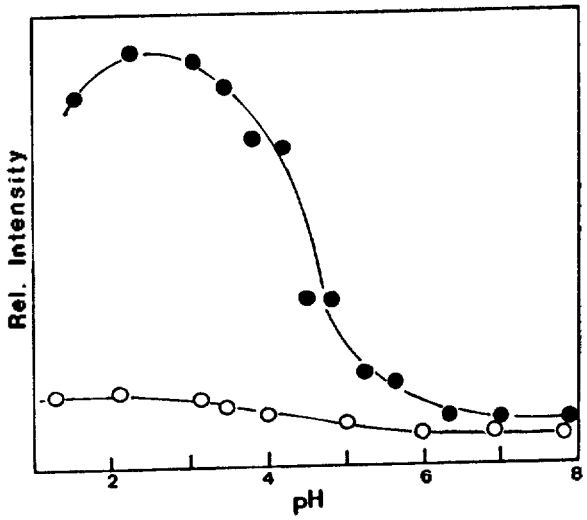


Figure 4. Variation of emission intensity of diazepam at 490 nm with pH of media in the surfactant-free solutions (○) and 50 mM SDS solutions (●). λ_{ex} was 350 nm.

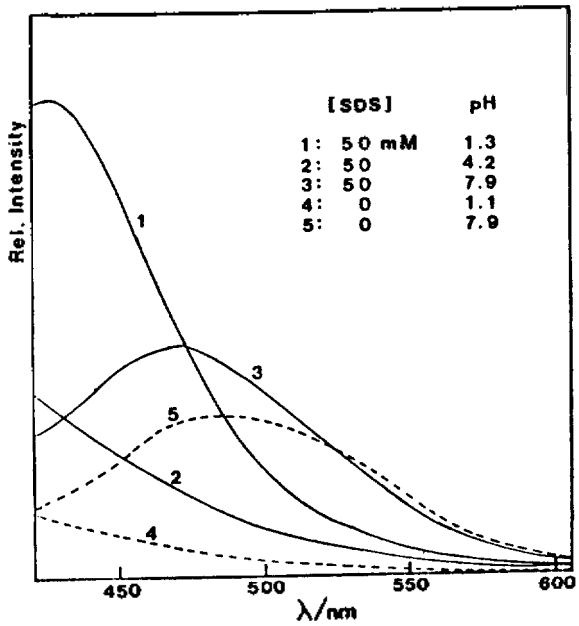


Figure 5. Fluorescence spectra of chlorodiazepoxide in the presence (1–3) and absence of SDS (4, 5) at different pH shown. λ_{ex} was 350 nm.

uate the pK_a values of diazepam by the same manner discussed in the previous section by replacing the absorbance value (A) with emission intensity. The results agreed well with those from absorbance data (Table 2).

Since the abdsorbance values of diazepam were not significantly modified by the presence of SDS, the large enhancement of emission intensity of diazepam in SDS micellar solutions can be attributed to the increase in emission quantum yield of the protonated form. The protection of excited state of organic molecules from the non-fluorescent deactivation processes was observed in many other systems in micellar solutions.[1b]

Figure 5 shows the fluorescence spectra of chlorodiazepoxide excited at 350 nm. The spectra show the fluorescence emission at 490 nm from solutions at $pH > pK_a$. This is the opposite behavior to the one observed with diazepam, but agrees well with the absorption data. This shows that the absorbance at the excitation wavelength is mainly due to the deprotonated neutral species of chlorodiazepoxide. Again, the pK_a value calculated from emission data agreed well with that from absorbance data given in Table 2.

The presence of SDS shifted the emission maximum of chlorodiazepoxide to *ca.* 460 nm (see, Figure 5). Unlike diazepam, the emission intensity of chlorodiazepoxide increased only about 30% by the addition of SDS. Interestingly, the emission from chlorodiazepoxide in SDS solutions displayed large blue-shift with enhanced intensity in strongly acidic condition. This behavior was not observed in diazepam solutions, which excludes the possibility of artifact. One possible explanation for this is the second protonation of chlorodiazepoxide.

Implications to the Micelle-Improved Analysis of 1,4-Benzodiazepines. The use of an anionic micelle SDS provides several advantages to the analysis of 1,4-benzodiazepines. One is obviously the increased solubility of the drugs in aqueous media.[12] 1,4-Benzodiazepines are hydrophobic and sparingly soluble in water unless they are protonated in acidic condition: the solubility of diazepam in surfactant-free water was reported as 2.5×10^{-4} M. SDS micelle increases the solubility of the drugs. The other types of micelles (non-ionic or cationic) can also be used for this purpose.

Since the difference in the pK_a values of diazepam and chlorodiazepoxide becomes greater by the presence of SDS, the use of SDS enables one to assay individual drugs from their mixture spectroscopically. As evident from Figure 2, more than 95% of chlorodiazepoxide exists as acidic form in SDS solution at pH 5.3, while only less than 5% of diazepam is present as acidic form at the same condition. Thus the absorbance change of a solution containing both diazepam and chlorodiazepoxide brought by raising or lowering pH of the solution from pH 5.3 is mainly due to the chlorodiazepoxide (when pH is raised) or diazepam (when pH is decreased). It would be possible to separate each component from their mixture with less than 10% uncertainty. A similar scheme can be used without SDS by choosing the measuring wavelengthes at isosbestic points of individual components. However, the sensitivity of the analysis would be much less as the absorbance change of a drug with pH at the isosbestic point of the other drug is small.

The sensitivity of fluorometric determination of diazepam in acidic solution is greatly enhanced by the presence of SDS. Since the protonated and thus positively charged diazepam does not bind to cationic or non-ionic micelle, the enhancement of the emission intensity of diazepam is not expected in the cationic or non-ionic micellar solutions. Again, the assay of diazepam and chlorodiazepoxide from their mixture is possible by following the change in emission intensity upon raising (for chlorodiazepoxide) or decreasing (for diazepam) pH of the SDS solutions from pH 5.3.

In conclusion, it has been shown that the pK_a of chlorodiazepoxide increases in the presence of anionic SDS micelles to much greater extent than diazepam. When diazepam and chlorodiazepoxide solutions are excited at 350 nm, the former solution shows fluorescence emission in acidic media and the emission intensity is enhanced about five-fold by the

presence of SDS, while the latter solution fluoresces at high pH with little dependence of the emission intensity on the presence of SDS. These different properties of diazepam and chlorodiazepoxide on pH can be used to facilitate the design of micelle-improved chemical analysis of the drugs.

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The Synthesis of Selectively Substituted *p*-Acetylcalix[4]arene

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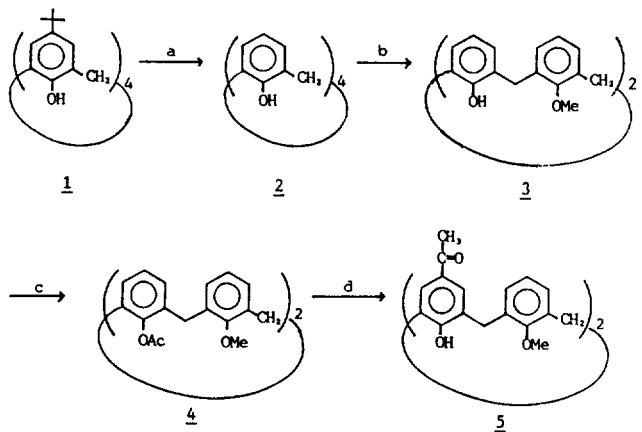
A method is described for the selective functionalization of calix[4]arene at the para positions of the phenyl rings. The diametrically substituted calix[4]arene dimethyl ether **3**, obtained from the treatment of calix[4]arene **2** with methyl iodide in the presence of K_2CO_3, is converted to the diacetyloxy calix[4]arene dimethyl ether **4**. This compound undergoes Fries rearrangement to yield the diametrically *p*-diacetylcalix[4]arene dimethyl ether **5** in 68% yield.

Introduction

The functionalization of calixarenes on the phenyl rings has attracted our attention[1] and that of several research groups[2,3] because of the possibility of easily obtaining new host molecules for the complexation of ions and neutral molecules or new type of enzyme mimics. Although several routes have been developed to introduce functional groups at the para positions of the phenyl rings, they all lead to tetra-substituted calix[4]arenes, having the same substituent at all the para positions. The stepwise route to give access to differently substituted calix[4]arenes were developed by Gutsche and No[4] and Böhmer *et al.*[5], but the methods are relatively long, tedious and low with respect to yield. The purpose of the present work is to exploit the possibility of adapting the short synthesis to the preparation of selectively functionalized calixarenes which can be used for the synthesis of calixarenes containing more than two different functional groups. Here we report the synthesis of diametrically substituted *p*-diacetylcalix[4]arene dimethyl ether **5** as shown on scheme.



Reagents: a. $AlCl_3$/Benzene, b. MeI/K_2CO_3/Acetone, c. Ac_2O/H^+, d. $AlCl_3$

Scheme