Kinetics of the Acid Hydrolysis of Diazepam, Bromazepam, and Flunitrazepam in Aqueous and Micellar Systems

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Received March 20, 1989, from the *Departamento de Química física, Facultad de Farmacia, Universidad de Salamanca, Apartado 449, 37080 Salamanca, Spain.* Accepted for publication June 13, 1990.

Abstract □ A kinetic study of the acid hydrolysis of aqueous diazepam, bromazepam, and flunitrazepam was carried out at 25 °C using a spectrophotometric method. For diazepam and flunitrazepam, the experimental pseudo first-order rate constant decreased as the acid concentration was increased. The contrary behavior was found in the case of bromazepam. A kinetic scheme that includes the hydrolysis reaction of both protonated and nonprotonated species of the drug can account for these results. Also, the kinetics of the acid hydrolysis of the same drugs in the presence of micellar aggregates [nonionic polyoxyethylene-23-dodecanol (Brij 35); cationic cetyl trymethyl ammonium bromide (CTAB); and anionic sodium decyl (SdeS), dodecyl (SDS), and tetradecyl (STS) sulfate] was studied at 25 °C. Negligible effects were observed in the cases of nonionic and cationic micelles. Anionic micelles produced an inhibitory effect in the reaction velocity. This effect increased as the hydrophobic nature of the surfactant increased. All these facts are interpreted quantitatively by means of a pseudophase model.

1.4-Benzodiazepines are compounds used as psychotropic drugs and may undergo acid-base hydrolysis in aqueous solution. Study of this reaction, within the physiological pH range, is of great importance because the absorption of these drugs in the gastrointestinal tract is affected by the nature of the chemical species involved.

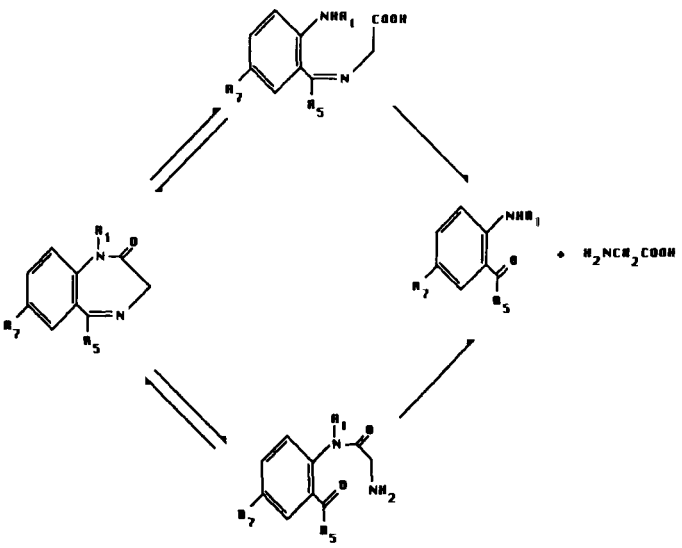
Owing to the importance of the mechanism of this reaction, the degradation kinetics of such drugs has been studied by many authors. The results show that the reaction is rather complex and is affected by diverse factors such as acid concentration, the value of the protonation constant of the drug, the nature of the functional groups, the presence of organic solvents used in dissolving the reagents, etc.[1] As a general rule, the acid hydrolysis of these compounds can be thought of as occurring in two stages. In the first stage, breakage of the ring is produced at the 1.2-amidic or at the 4.5-azomethine bonds, depending on the structure of the drug.[2] In compounds such as diazepam, nitrazepam, flunitrazepam, or clonazepam, the reaction occurs exclusively by breakage of the cycle in the 4.5-azomethine position,[3,4] while in the case of bromazepam, the possibility of breakage caused by the 1.2-amidic bond has been proposed.[2,5] Another example of breakage of the bond at the 4.5-azomethine position is the hydrolysis of triazolbenzodiazepines, in which the presence of the triazol group prevents the rupture of the 1.2-amidic group.[6] In any case, this first stage of the reaction seems to be either reversible or irreversible, depending on the pH of the medium.[7-9] The second stage consists of the rupture of the remaining bond, either amidic or azomethine, giving rise to derivatives of glycine and aminobenzophenone. This stage only takes place in drastic conditions of temperature and acidity.[1]

In previous works carried out by other authors[10-12] the effect of the addition of surfactants on both the acid and alkaline hydrolysis rates of some benzodiazepines has been studied. In these works it was observed that anionic micelles of sodium dodecylsulfate (SDS) produce a strong inhibition of the acid hydrolysis reaction of diazepam,[10] whereas they catalyze the reaction of nitrazepam derivatives at high acid concentrations.[11]

In an attempt to devise a kinetic scheme that would quantitatively account for the reaction of acid hydrolysis of these drugs in aqueous and in micellar systems, the present studies on the effect of the acid concentration and of the addition of different surfactants on the hydrolysis rate of diazepam, bromazepam, and flunitrazepam were undertaken (Scheme I). The aims of this work are threefold: (*a*) to gain knowledge on the mechanism of aqueous hydrolysis; (*b*) to propose a kinetic scheme that is capable of accounting for the inhibitory behavior of the anionic micelles of SDS; and (*c*) to study the effect produced by a change in the type of surfactant.

Experimental Section

Materials—Drugs were kindly donated by different laboratories. Diazepam (mp 128 °C) was obtained from Prodes, and bromazepam (mp 273 °C) and flunitrazepam (mp 165 °C) were obtained from Roche. All drugs were sufficiently well characterized by the manufacturers to be used without further purification. The anionic surfactants sodium decyl sulfate (SdeS), sodium dodecyl sulfate (SDS), and sodium tetradecyl sulfate (STS) together with cetyl trimethyl ammonium bromide (CTAB) and HCl were from Merck. Polyoxyethylene 23-dodecanol (Brij 35) was from Sigma Chemical.



	R_1	R_5	R_7
BROMAZEPAM	-H	2-Pyridyl-	-Br
DIAZEPAM	$-CH_3$	Phenyl-	-Cl
FLUNITRAZEPAM	$-CH_3$	2-Fluorophenyl-	$-NO_2$

Scheme I

0022-3549/91/0500-0459$01.00/0

Kinetic Studies—Kinetic measurements were made using a spectrophotometric method. The hydrolysis reaction was followed by monitoring the absorbance at 235 nm for bromazepam and diazepam and at 280 nm for flunitrazepam. These wavelengths were found to be the most appropriate in preliminary spectrophotometric studies. All kinetic runs were carried out at 25 °C using a Hitachi 150-20 spectrophotometer. The pH of the reaction medium did not change during the course of the reaction. In all cases, absorbance measurements were carried out until a constant value with time was attained. Rate measurements showed good first-order behavior. The experimental rate constants were determined from plots of log $(A_t - A_\infty)$ versus time.

An HPLC study of the hydrolysis reaction was carried out in an acid medium in the case of aqueous diazepam. A Varian 5000 chromatograph with a variable wavelength UV detector set at 235 nm was used. The column, composed of a stationary phase of RP-18, was 10 μm × 30 cm in length and had a 0.4-cm i.d. The mobile phase consisted of a methanol:water mixture (65:35) with a flow rate of 1 mL/min. The retention time of diazepam was 10.6 min.

Results and Discussion

Spectrophotometric Study of the Reaction—Previous to the establishment of the best spectral wavelength for monitoring the course of the reaction, spectra for each one of the three drugs studied were recorded between 200 and 400 nm at $t = 0$ and $t = \infty$, in acid solution and in the absence and presence of surfactant (SDS). Deconvolution analysis of the absorbance values revealed the evolution of the innermost spectral details during the reaction. These results are presented in Figures 1 and 2. The deconvolution procedure used[13] considers the absorption spectral envelope as composed of a number (N) of Gaussian bands, allowing expression of the absorbance $[A(\nu)]$ as a function of frequency (ν) in the following way:

$$A(\nu) = \sum^{N} A_i(\nu_i) \exp\{-\ln 2[(\nu - \nu_i)/\delta_i]^2\} \qquad (1)$$

where A_i is the maximum absorbance at each band central frequency (ν_i) and δ_i is the half-width, which refers to the frequency width of the band at a point where absorbance is half the maximum.

Tables I–III present the best UV deconvolution parameters for bromazepan, diazepam, and flunitrazepam and their hydrolysis products in aqueous acid medium with and without SDS added. From these results a number of important features are evident. In all cases, the initial and final spectra maintain the same characteristic bands, with only minor changes in some central frequencies and half-widths. Also, as the course of the reaction proceeds, it can be seen that most of the component bands decrease in their intensities, but no one disappears completely in the corresponding final spectrum. This fact could be a point in favor of the reversibility of the first step of reaction, as stated in Scheme I and previously proposed in earlier mechanistic schemes for this kind of reaction.[3] Finally, the presence of surfactant in the reaction medium, though modifying the intensity of some bands, does not seem to make any significant change in the spectral behavior of the reaction.

In order to confirm in a more definite way the reversibility of the degradation reaction, an HPLC assay of the hydrolysis reaction was carried out. Aqueous diazepam in 0.0109 M HCl was subjected to HPLC at $t = 0$ and at different stages of the course of the reaction. The results show unequivocally that after >10 half-lives, diazepam is present in the reaction medium in the proportion $[\text{diazepam}]_\infty/[\text{diazepam}]_0 = 0.88$, which may prove in an alternate way the aforementioned reaction reversibility. This value agrees acceptably well with the ratio of the central absorbances at $t = \infty$ and $t = 0$ for the deconvolution bands of the UV spectra of this drug in the same medium, as shown in Table II.

Aqueous Hydrolysis—In all cases, experimental rate constants for the hydrolysis reaction were determined at different acid concentrations (from 0.001 M to 1.75 M for bromazepam, to 0.545 M for diazepam, and to 0.305 M for flunitrazepam). It was evident that an excess of inert electrolyte added (0.20 M NaCl) left the value of the experimental rate constant unchanged (within experimental error) with respect to the expected value in the absence of added electrolyte. This fact made it unnecessary to carry out the kinetic study under conditions of controlled ionic strength. These results are presented on a semi-logarithmic plot in Figures 3–5. As can be seen in these figures, except for the acid hydrolysis of bromazepam, the experimental rate constant decreases with an increase in the acid concentration. Concerning diazepam, note that the trend observed at 25 °C is contrary to the one previously reported at 80 °C in the same pH range.[8]

This kind of behavior can be accounted for in terms of a general hydrolysis scheme (Scheme II) in which the hydrolysis reaction of both the protonated and nonprotonated species is produced by breakage of the ring either at the 4,5-azomethine bond (diazepam and flunitrazepam)[1] or at the 1,2-amidic bond (bromazepam).[2] According to this, the kinetics of the hydrolysis reaction can be written as shown in Scheme II, where P represents either the 4,5-open ring derivative (diazepam and flunitrazepam) or the 1,2-open ring derivative (bromazepam). According to this kinetic network and considering the rate equation of each step, the mass balance for the drug, and the protonation balances, and also considering that all the species, products, and reagents contribute to the absorbance at the working wavelengths, the following equation is obtained for the variation of absorbance with time:

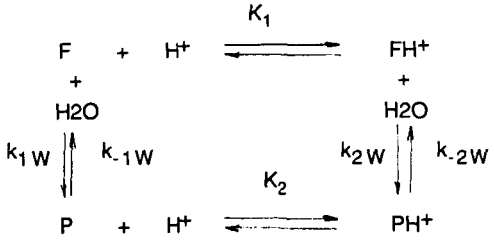
$$(A - A_\infty) = (A_0 - A_\infty) \exp(-k_{exp}\, t) \qquad (2)$$

where:

$$k_{exp} = \frac{k_{1W} + k_{2W}K_1(H^+)}{1 + K_1(H^+)} + \frac{k_{-1W} + k_{-2W}K_2(H^+)}{1 + K_2(H^+)} \qquad (3)$$

and A_0 and A_∞ are the absorbances at $t = 0$ and $t = \infty$, respectively, that depend on the molar absorptivities and on the initial and final concentrations of the species F, FH^+, P, and PH^+.

Numerical fitting of the obtained data for the experimental rate constant (k_{exp}) to a six-parameter expression, as shown in eq 3, will yield, to a high degree of probability, an ambiguous result because the values that these parameters can take in order to make both terms numerically separable



Scheme II

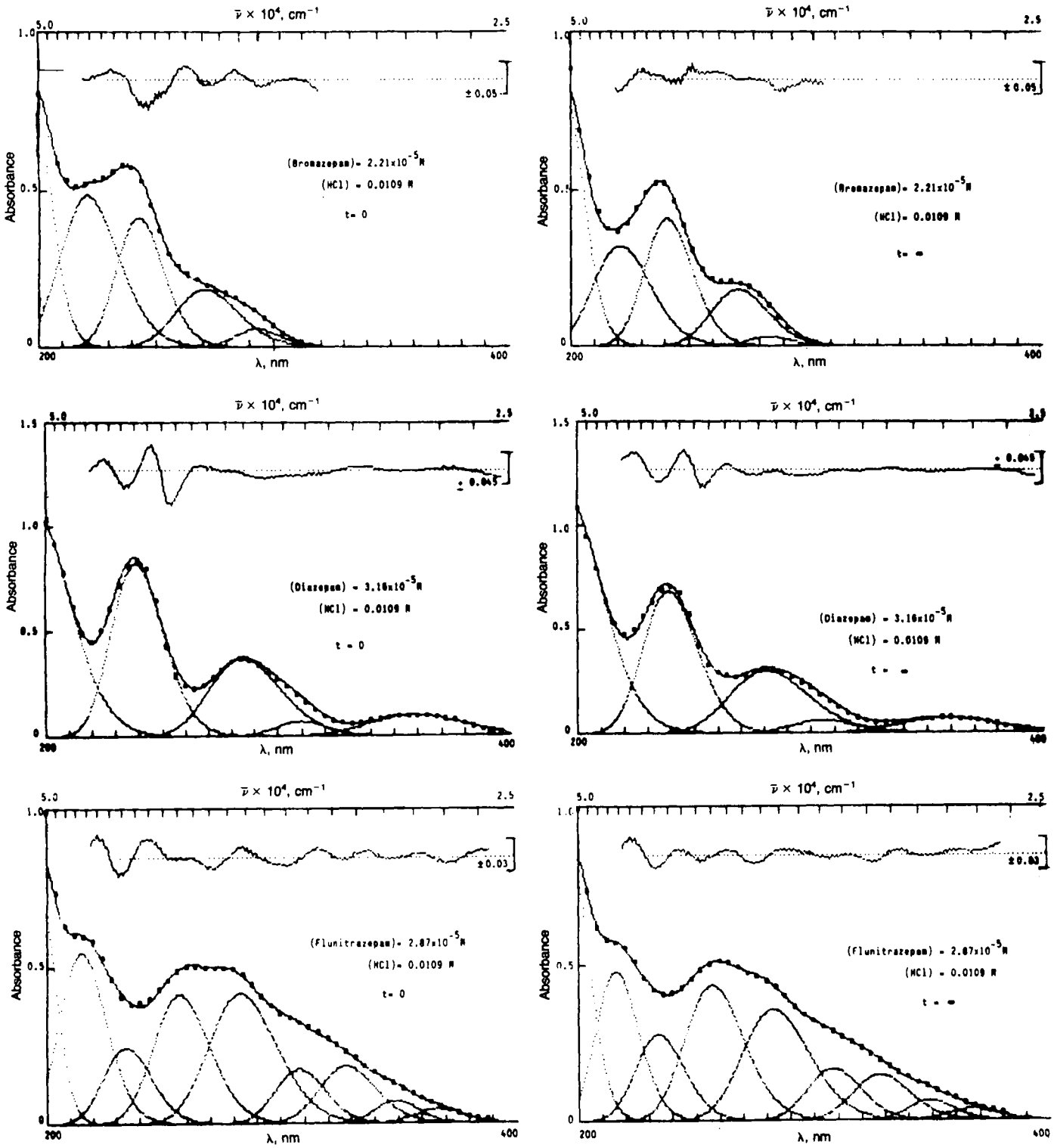


Figure 1—Deconvolution of UV absorption spectra of 1,4-benzodiazepines in aqueous acidic solution and of their hydrolysis products. Experimental absorbances are plotted at 4-nm intervals for clarity. Dotted lines are the Gaussian component bands and solid lines denote calculated spectra contours. The upper line is the absolute deviation from experimental values.

seem to lie in very restrictive ranges.[14] In fact, the best unambiguous fit of the experimental data is obtained by the following reduced type of equation:

$$k_{exp} = \frac{k_{0W} + k_\infty K(H^+)}{1 + K(H^+)} \quad (4)$$

Compared with eq 3, eq 4 permits one to identify k_{0W} and k_∞ as follows:

$$k_{0W} = k_{1W} + k_{-1W}$$
$$k_\infty = k_{2W} + k_{-2W} \quad (5)$$

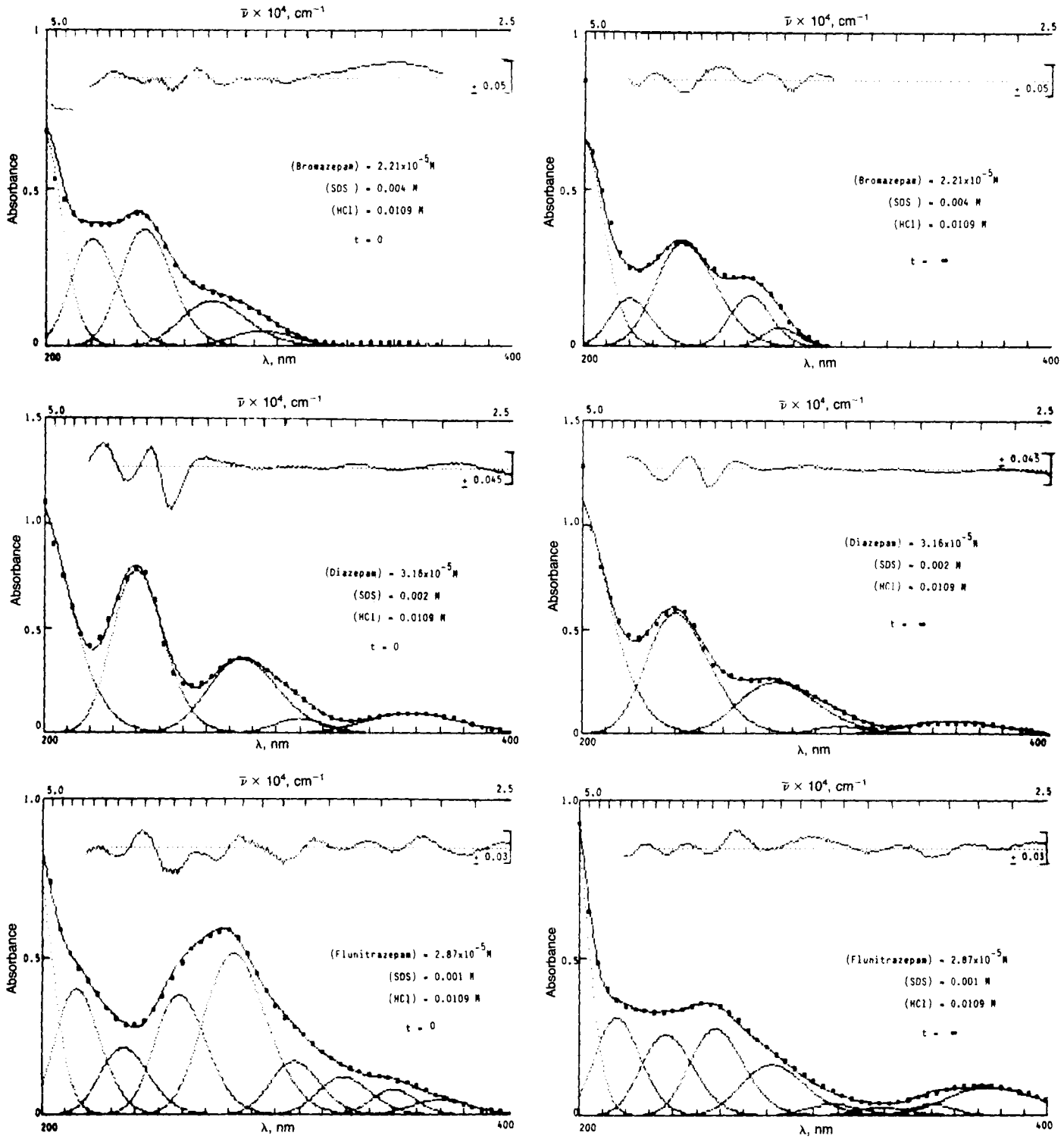


Figure 2—Deconvolution of UV absorption spectra of 1,4-benzodiazepines in aqueous acidic solution and of their hydrolysis products with SDS added. Experimental absorbances are plotted at 4-nm intervals for clarity. Dotted lines are the Gaussian component bands and solid lines denote calculated spectra contours. The upper line is the absolute deviation from experimental values.

where K is a fitting parameter averaging in some intricate way the equilibrium as well as the kinetic constants contained in the scheme. This makes inappropriate any eventual comparison of its value to the protonation constant of the respective drug.

Numerical fits were carried out by means of a nonlinear least-squares method (an iterative grid method).[15] The best values obtained for the parameters k_{0W}, k_{∞}, and K are shown in Table IV.

In the light of these values for diazepam and flunitrazepam it is evident that the nonprotonated species is hydrolyzed to equilibrium more readily than the protonated species. It seems that in the protonated drug the hydrogen atom bound to the azomethine group is able to stabilize this structure with respect to the hydrolytic attack. However, in the hydrolysis of bromazepam, since the hydrolytic attack is carried out at the

Table I—Deconvolution Parameters for Ultraviolet Absorption Spectra in Acidic and Micellar Media

ν/kK (λ/nm)	Bromazepam (2.21×10^{-5} M) plus HCl (0.0109 M)				Bromazepam (2.21×10^{-5} M) plus SDS (0.004 M) and HCl (0.0109 M)			
	$t = 0$		$t = \infty$		$t = 0$		$t = \infty$	
	A	δ/kK	A	δ/kK	A	δ/kK	A	δ/kK
52.63 (198)	0.765	2.20	0.790	2.27	—	—	—	—
50.25 (199)	—	—	—	—	0.670	2.30	—	—
50.00 (200)	—	—	—	—	—	—	0.650	2.45
45.25 (221)	0.484	2.98	0.321	2.90	0.338	2.40	0.151	2.10
41.49 (241)	—	—	0.406	2.05	—	—	—	—
41.15 (243)	0.414	2.00	—	—	0.370	2.15	0.330	2.50
36.90 (271)	0.182	2.10	0.180	1.90	0.143	2.20	0.161	1.40
35.21 (284)	—	—	0.030	1.22	—	—	0.060	1.10
34.13 (293)	0.057	1.25	—	—	0.050	1.70	—	—

Table II—Deconvolution Parameters for Ultraviolet Absorption Spectra in Acidic and Micellar Media

ν/kK (λ/nm)	Diazepam (3.16×10^{-5} M) plus HCl (0.0109 M)				Diazepam (3.16×10^{-5} M) plus SDS (0.002 M) and HCl (0.0109 M)			
	$t = 0$		$t = \infty$		$t = 0$		$t = \infty$	
	A	δ/kK	A	δ/kK	A	δ/kK	A	δ/kK
50.76 (197)	1.05	3.95	1.12	3.95	1.10	3.85	1.15	3.85
41.84 (239)	0.82	2.30	0.682	2.40	—	—	—	—
41.67 (240)	—	—	—	—	0.78	2.25	0.58	2.70
35.59 (281)	—	—	0.30	2.50	—	—	—	—
35.40 (282.5)	—	—	—	—	—	—	0.247	2.45
35.09 (285)	0.37	2.20	—	—	0.355	2.15	—	—
32.79 (305)	—	—	0.06	1.70	—	—	—	—
32.26 (310)	0.07	1.30	—	—	0.07	1.30	0.035	1.30
28.01 (357)	—	—	—	—	0.098	1.80	—	—
27.93 (358)	0.10	1.80	0.075	1.80	—	—	0.060	1.80

Table III—Deconvolution Parameters for Ultraviolet Absorption Spectra in Acidic and Micellar Media

ν/kK (λ/nm)	Flunitrazepam (2.87×10^{-5} M) plus HCl (0.0109 M)				Flunitrazepam (2.87×10^{-5} M) plus SDS (0.001 M) and HCl (0.0109 M)			
	$t = 0$		$t = \infty$		$t = 0$		$t = \infty$	
	A	δ/kK	A	δ/kK	A	δ/kK	A	δ/kK
50.76 (197)	0.760	1.95	0.850	2.20	0.800	2.20	0.950	2.00
46.51 (215)	0.545	2.51	—	—	0.400	2.40	—	—
46.30 (216)	—	—	0.476	2.26	—	—	0.310	2.40
42.73 (234)	0.240	2.20	0.274	2.20	—	—	—	—
42.55 (235)	—	—	—	—	0.213	2.20	—	—
42.19 (237)	—	—	—	—	—	—	0.255	2.30
38.91 (257)	0.408	2.12	0.430	2.20	—	—	—	—
38.76 (258)	—	—	—	—	—	—	0.275	2.00
38.61 (259)	—	—	—	—	0.382	1.90	—	—
35.46 (282)	—	—	—	—	0.515	1.95	0.160	2.00
35.33 (283)	0.411	2.10	0.353	2.05	—	—	—	—
32.47 (308)	0.170	1.30	0.163	1.38	0.170	1.30	0.035	1.40
30.49 (328)	0.180	1.30	0.143	1.30	—	—	—	—
30.39 (329)	—	—	—	—	0.120	1.20	0.025	1.20
28.65 (349)	0.070	0.85	0.060	0.90	—	—	—	—
28.57 (350)	—	—	—	—	0.080	0.89	0.035	0.89
27.25 (367)	0.042	0.88	0.033	0.90	—	—	—	—
27.03 (370)	—	—	—	—	0.049	1.05	—	—
26.67 (375)	—	—	—	—	—	—	0.090	1.65

1,2-amidic bond, protonation of the drug does not produce this protective effect.

On the other hand, the nature of the −R5 radical strongly affects the rate of hydrolysis of these drugs when the reaction is carried out through breakage of the azomethine bond. Thus, the presence of a fluoride atom in flunitrazepam facilitates the hydrolysis reaction of this drug. This has already been reported in the hydrolysis of other fluorobenzodiazepines.[16] The electronegative character of fluoride can produce an electrodeficiency in the carbon corresponding to the imine group, facilitating the hydrolytic attack of this group.

Micellar Hydrolysis—Nonionic Brij 35 and cationic CTAB aggregates do not have appreciable effects on the hydrolysis rate of these drugs. These findings are in agreement with

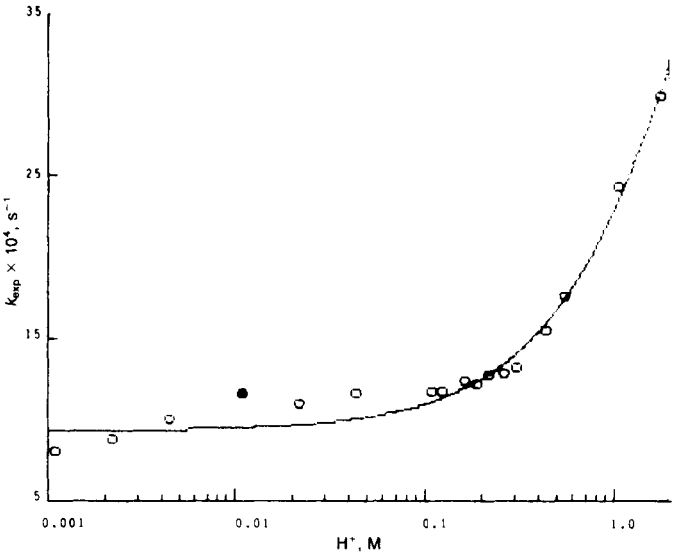


Figure 3—Pseudo first-order experimental rate constant versus HCl concentration for the hydrolysis of bromazepam. The full point was obtained with 0.20 M NaCl added. The curve has been calculated according eq 4 and Table IV.

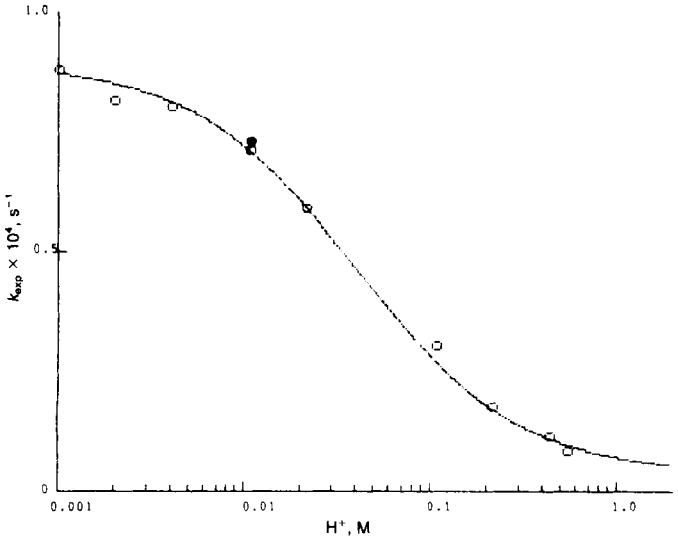


Figure 4—Pseudo first-order experimental rate constant versus HCl concentration for the hydrolysis of diazepam. The full point was obtained with 0.20 M NaCl added. The curve has been calculated according to eq 4 and Table IV.

electrostatic considerations already reported by Hartley.[17] The presence of anionic micelles of SDS leads to a considerable decrease in the rate constant when the surfactant concentration is increased. This inhibitory effect is observed at all HCl concentrations employed (0.0109–0.218 M) for the three drugs studied. The values of the experimental rate constant (k_{exp}) as a function of the concentration of SDS are shown in Table V. For illustrative purposes some examples of this kind of behavior are shown in Figures 6 to 8.

A quantitative interpretation of such phenomena can be gained from a pseudophase model[18] which considers that the reaction takes place in the aqueous and micellar phases simultaneously, but at different rates. Scheme III would take into account this situation. Taking into account the rate equations for the reactions expressed in Scheme III, the corresponding mass balances, and the condition that all the species in which the drug is found (together with the reaction

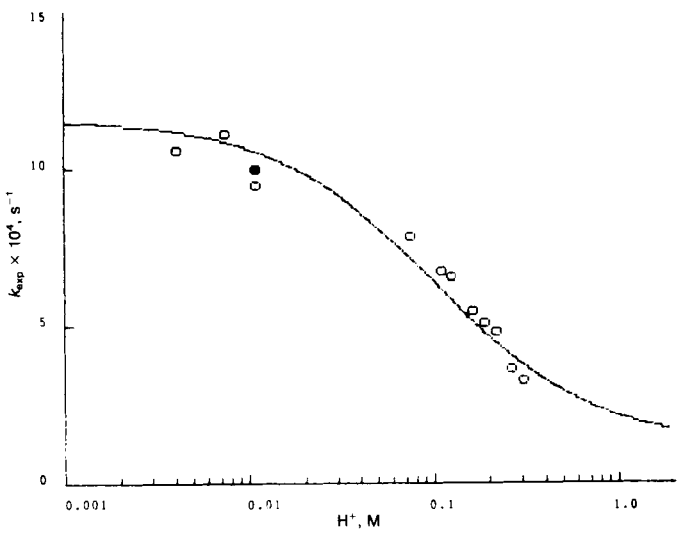


Figure 5—Pseudo first-order experimental rate constant versus HCl concentration for the hydrolysis of flunitrazepam. The full point was obtained with 0.20 M NaCl added. The curve has been calculated according to eq 4 and Table IV.

Table IV—Kinetic and Binding Parameters for the Acid Hydrolysis of Some Aqueous 1,4-Benzodiazepines

1,4-Benzodiazepine	$10^5 k_{ow}$, s^{-1}	$10^5 k_{\infty}$, s^{-1}	K, M^{-1}
Bromazepam	93.2 ± 0.8	838 ± 75	0.223 ± 0.017
Diazepam	8.92 ± 0.09	0.36 ± 0.04	24.7 ± 0.4
Flunitrazepam	116 ± 1	11.6 ± 0.2	10.1 ± 0.2

products) contribute to the measured absorbance, one obtains for A versus t a dependence that is characteristic of a first-order reaction (similar to that shown in eq 2). In this case the experimental rate constant (k_{exp}) is given by the following expression:

$$k_{exp} = \frac{k_{FW} + k_{FM}K_{FM}(D_n)}{1 + K_{FM}(D_n)} + \frac{k_{PW} + k_{PM}K_{PM}(D_n)}{1 + K_{PM}(D_n)} \quad (6)$$

where:

$$k_{FW} = \frac{k_{1W} + k_{2W}K_1(H^+)}{1 + K_1(H^+)} \quad (7)$$

$$k_{PW} = \frac{k_{-1W} + k_{-2W}K_2(H^+)}{1 + K_2(H^+)} \quad (8)$$

$$k_{FM} = \frac{k_{1M} + k_{2M}(K_1K_{FH}/K_F)(H^+)}{1 + (K_1K_{FH}/K_F)(H^+)} \quad (9)$$

$$k_{PM} = \frac{k_{-1M} + k_{-2M}(K_2K_{PH}/K_P)(H^+)}{1 + (K_2K_{PH}/K_P)(H^+)} \quad (10)$$

Table V—Experimental Rate Constants for the Acid Hydrolysis of 1,4-Benzodiazepines in Aqueous and Micellar Media

Drug	Micellar Media[a]	$k_{exp} \times 10^5$, s^{-1}		
Diazepam	SDS	HCl[b]	HCl[c]	HCl[d]
	0.6	—	—	1.69
	0.8	—	—	1.25
	1.0	—	1.86	0.831
	1.5	—	0.721	0.467
	2.3	3.12	—	—
	2.4	2.24	—	—
	2.5	—	0.336	0.285
	2.7	1.67	—	—
	2.8	1.64	—	—
	3.0	1.31	0.268	0.253
	4.0	0.58	0.241	0.213
	5.0	0.33	0.195	0.188
	10.0	0.18	0.160	0.121
Flunitrazepam	SDS	HCl[b]	HCl[c]	HCl[d]
	0.5	—	65.2	42.1
	1.0	—	—	33.2
	1.5	—	38.1	20.8
	2.5	—	18.4	14.4
	3.0	78.1	—	—
	4.0	36.3	—	—
	5.0	19.6	9.18	8.11
	6.0	11.0	—	—
	8.0	6.55	6.50	6.39
	10.0	5.23	5.07	5.18
	20.0	4.70	3.81	3.85
	30.0	5.03	3.81	3.48
	40.0	4.81	3.44	3.38
	60.0	5.00	—	2.94
	80.0	4.36	—	2.81
	100.0	4.16	3.33	2.73
Bromazepam	SDS	HCl[b]	HCl[c]	HCl[d]
	1.0	—	113	127
	5.0	105	106	119
	10.0	97.5	103	111
	20.0	93.9	100	104
	30.0	91.3	96.4	101
	40.0	90.1	94.4	99.5
	60.0	89.3	93.9	98.3
	80.0	88.1	93.6	96.1
	100.0	88.4	92.3	95.4
	SdeS	HCl[b]	HCl[c]	HCl[d]
	2.3	—	115	128
	3.8	—	114	125
	3.9	104	—	—
	7.7	102	110	—
	19.0	99.1	107	115
	38.0	—	103	—
	46.0	96.4	102	107
	51.0	94.8	—	—
	61.0	—	—	105
	77.0	—	102	—
	92.0	93.8	—	102
	107.0	93.3	100	102
	123.0	—	100	101
	153.0	92.1	—	101
	STS	HCl[e]	HCl[b]	HCl[f]
	0.20	102	—	106
	0.27	101	104	104
	0.45	99.7	103	103
	0.67	—	—	103
	0.75	98.8	102	—
	0.90	97.6	102	101
	1.1	97.3	101	100
	1.6	95.6	—	98.0
	2.2	91.8	98.9	95.3
	3.5	87.7	97.0	92.6

[a] All media concentrations are 10^{-3} M. [b] 0.0109 M. [c] 0.109 M. [d] 0.218 M. [e] 0.0085 M. [f] 0.0218 M.

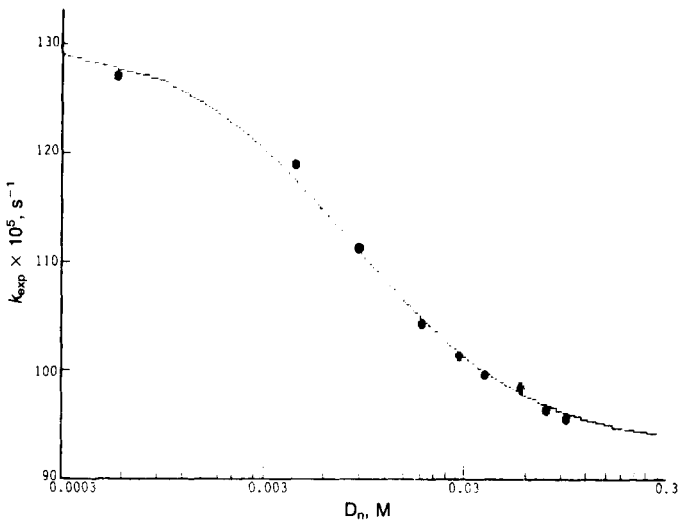


Figure 6—Pseudo first-order experimental rate constant versus SDS concentration for the hydrolysis of bromazepam at an HCl concentration of 0.218 M. The curve has been calculated according to eq 13 and Table VI.

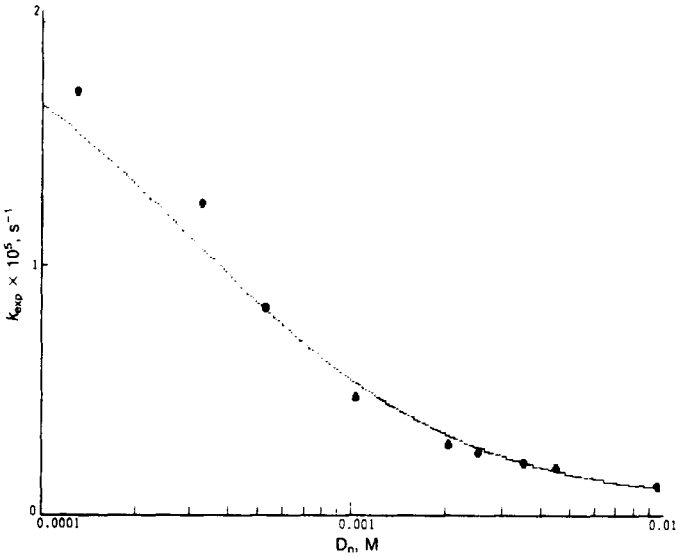


Figure 7—Pseudo first-order experimental rate constant versus SDS concentration for the hydrolysis of diazepam at an HCl concentration of 0.218 M. The curve has been calculated according to eq 13 and Table VI.

$$K_{FM} = \frac{K_F + K_{FH} K_1 (H^+)}{1 + K_1 (H^+)} \quad (11)$$

$$K_{PM} = \frac{K_P + K_{PH} K_2 (H^+)}{1 + K_2 (H^+)} \quad (12)$$

The concentration of acid in eqs 7–12 is what is left free by the ionic association process produced in the Stern layer of the micelle. However, under the working conditions employed, the concentration of acid adsorbed in the micellar Stern layer must be very small,[19] such that the concentration of free acid can be considered practically equal to the total concentration of HCl added.

Even though eq 6 could give a more complete interpretation of the kinetics of the hydrolysis in the micellar medium,

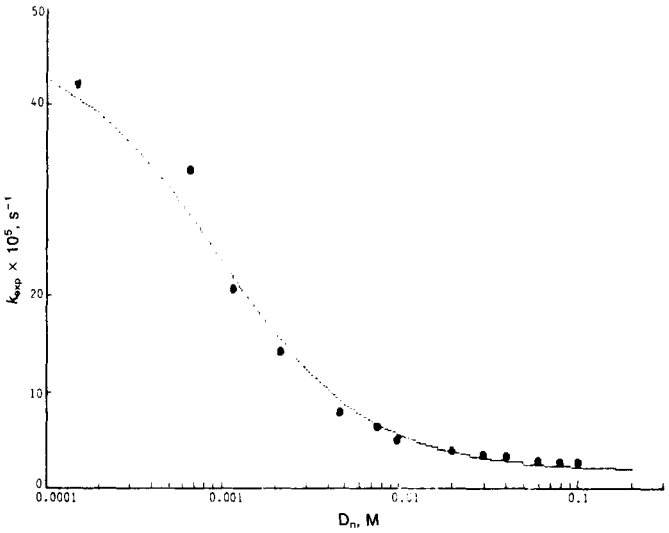


Figure 8—Pseudo first-order experimental rate constant versus SDS concentration for the hydrolysis of flunitrazepam at an HCl concentration of 0.218 M. The curve has been calculated according eq 13 and Table VI.

experimental results do not account for it in an unambiguous way. As in the case of aqueous hydrolysis, the best unambiguous fit is obtained by an equation of the following reduced type:

$$k_{exp} = \frac{k_W + k_M K_D (D_n)}{1 + K_D (D_n)} \quad (13)$$

where:

$$k_W = k_{FW} + k_{PW} \quad (14)$$

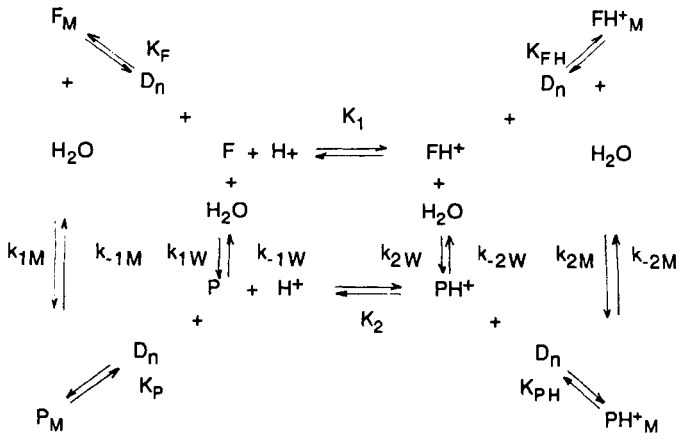
$$k_M = k_{FM} + k_{PM} \quad (15)$$

where k_W and k_M represent the rate constants for the reaction in the aqueous and micellar phases, respectively, and K_D is a fitting parameter averaging kinetic and binding constants in some complicated way. Fits of experimental results to eq 13 were carried out with a least-squares iterative grid method.[15] The best values for the different parameters involved are shown in Table VI. As would be expected, the rate constant in the absence of surfactant obtained from the fit (k_W) is in agreement with the experimentally observed result when the reaction occurs in the absence of surfactants at the same acid concentration.

Regarding parameters k_M and K_D, there seems to be two kinds of behavior. The values corresponding to the hydrolysis of bromazepam do not change appreciably with the concentration of acid, while in the case of diazepam and flunitrazepam, they decrease as the concentration of acid rises. Concerning k_M, numerical extrapolation of these data gives some values for the acid-independent hydrolysis rate constant in micellar medium, (k_{0M}) which, according to eqs 9 and 10 may be identified as follows:

$$k_{0M} = k_{1M} + k_{-1M} \quad (16)$$

The acid-independent hydrolysis rate constants are as follows: $(90 \pm 5) \times 10^{-5}$, $(0.13 \pm 0.01) \times 10^{-5}$, and $(3.7 \pm 0.3) \times 10^{-5}$ s^{-1} for bromazepam, diazepam, and flunitrazepam, respectively. By comparison of these values for the rate constants of the hydrolysis of the nonprotonated species in the micellar phase (k_{0M}) to the ones in the aqueous phase (k_{0W}; Table IV), it is seen that for diazepam and flunitrazepam the reaction in the micellar phase undergoes a strong inhibition, whereas in the case of bromazepam, the reaction is unaffected by the presence of surfactant. This can be explained in terms of the notion that the hydrolysis reaction takes place in two different parts of the molecule. For diazepam and flunitrazepam, the breakage of the ring is caused in the 4,5-azomethine bond and for bromazepam in the 1,2-amidic bond. The stronger hydrophobicity of the azomethine group would carry it towards the more internal zones of the micelle, thus protecting it from hydrolytic degradation. Conversely, in the case of bromazepam, since the reaction takes place in the amidic group, it would be located at the water–micelle interphase, such that the reaction takes place under conditions very similar to those of the aqueous phase and no modifications occur in the rate constant of the process.



Scheme III

Table VI—Kinetic and Binding Parameters for the Acid Hydrolysis of 1,4-Benzodiazepines in Aqueous Sodium Dodecyl Sulfate Solutions

Drug	HCl, M	$k_W \times 10^5$, s^{-1}	$k_M \times 10^5$, s^{-1}	K_D, M^{-1}	$k_w \times 10^5$, s^{-1} (exp)	CMC $\times 10^3$, M
Bromazepam	0.0109	106 ± 2	86.7 ± 1.0	120 ± 3	106 ± 1	4.70 ± 0.20
	0.109	115 ± 1	90.5 ± 1.5	105 ± 2	117 ± 0.3	0.50 ± 0.08
	0.218	130 ± 2	92.8 ± 1.2	112 ± 2	129 ± 0.4	0.40 ± 0.07
Diazepam	0.0109	7.28 ± 0.05	0.10 ± 0.01	8500 ± 50	7.15 ± 0.01	2.17 ± 0.01
	0.109	3.02 ± 0.01	0.079 ± 0.005	4800 ± 10	3.05 ± 0.01	0.80 ± 0.02
	0.218	2.14 ± 0.02	0.041 ± 0.002	3200 ± 30	2.11 ± 0.01	0.47 ± 0.01
Flunitrazepam	0.0109	94.0 ± 1	3.4 ± 0.3	3250 ± 30	95.0 ± 0.4	2.87 ± 0.01
	0.109	67.6 ± 0.5	2.4 ± 0.1	1750 ± 25	66.8 ± 0.2	0.48 ± 0.01
	0.218	48.1 ± 0.3	2.3 ± 0.1	1270 ± 15	47.5 ± 0.2	0.34 ± 0.01

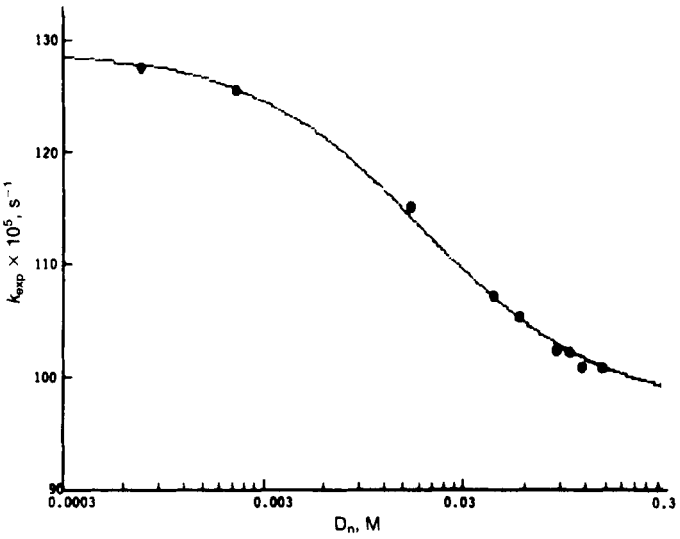


Figure 9—Pseudo first-order experimental rate constant versus SdeS concentration for the hydrolysis of bromazepam at an HCl concentration of 0.218 M. The curve has been calculated according to eq 13 and Table VII.

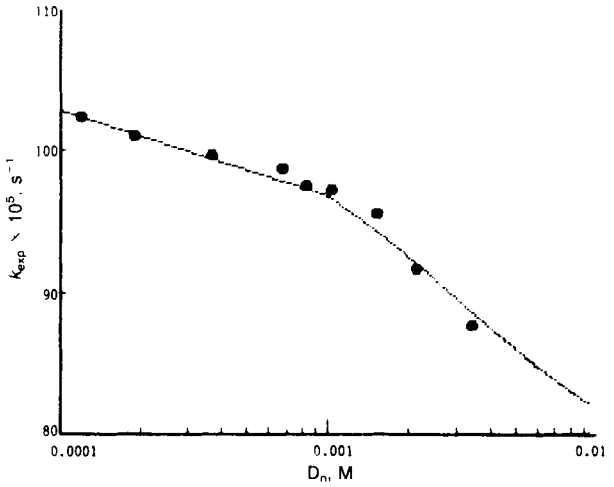


Figure 10—Pseudo first-order experimental rate constant versus STS concentration for the hydrolysis of bromazepam at an HCl concentration of 0.0085 M. The curve has been calculated according to eq 13 and Table VII.

The micelle–drug average binding constant (K_D), at any particular acid concentration, increases in the following order: bromazepam < flunitrazepam < diazepam. This is in agreement with an increase in the hydrophobic nature of the drugs that favors their penetration into the micellar aggregate, hence corroborating the aforesaid conclusions.

Effect of the Length of the Hydrocarbon Chain—When the reaction of the acid hydrolysis of bromazepam is carried out in the presence of the surfactants SdeS and STS and at different acid concentrations, similar behavior to that produced by SDS is observed. Examples of this kind of behavior, shown in Figures 9 and 10, can be explained in terms of the same scheme as mentioned before. The experimental rate constant would also have the form expressed in eq 13. The values obtained from the fitting of the experimental results to this kind of equation are shown in Table VII, together with those corresponding to SDS.

As in previous cases, there is good agreement between the values obtained for k_W by data fitting and the experimentally obtained value when the reaction is carried out in the absence of surfactant. The rate constant in the micellar phase (k_M) seems to be independent of the concentration of HCl and of the chain length of the surfactant used. Thus, the obtained value of k_{0M} for bromazepam is similar for all three surfactants; that is, $(95 \pm 7) \times 10^{-5}$, $(90 \pm 5) \times 10^{-5}$, and $(80 \pm 8) \times 10^{-5}$ s^{-1} for SdeS, SDS, and STS, respectively. This behavior has been observed in most reactions carried out in micellar systems[20] and shows that the micellar effect on the chemical reactivity is fairly independent of the size and volume of the aggregate when the reaction occurs at the level of the micellar interphase.

The micelle–drug average binding constant (K_D) also seems to be independent of the concentration of acid, with values of 51 ± 2, 111 ± 6, and 306 ± 12 M^{-1} for SdeS, SDS, and STS, respectively. In this sense, a considerable increase is observed in the average binding constant as the hydrocarbon chain of the surfactant becomes longer. This suggests that an increase in the hydrophobic nature of the surfactant would facilitate the penetration of the drug into the micelle.

Glossary

F, P: nonprotonated species of the nonhydrolyzed and hydrolyzed drug, respectively.

FH^+, PH^+: protonated species of the nonhydrolyzed and hydrolyzed drug, respectively.

K_1, K_2: protonation constants of the nonhydrolyzed and hydrolyzed drug, respectively.

W: aqueous phase.

k_{1W}, k_{-1W}, k_{2W}, k_{-2W}: rate constants for reaction steps in aqueous medium.

M: micellar phase.

D_n: micellized surfactant whose concentration amounts to the total surfactant concentration exceeding the CMC.

k_{1M}, k_{-1M}, k_{2M}, k_{-2M}: rate constants for reaction steps in micellar medium.

K_F, K_{FH}, K_P, K_{PH}: micelle–drug binding constants for F, FH^+, P, and PH^+ species, respectively.

Table VII—Kinetic and Binding Parameters for the Acid Hydrolysis of Bromazepam in Different Aqueous Surfactant Solutions

Surfactant	HCl, M	$k_w \times 10^5$, s^{-1}	$k_M \times 10^5$, s^{-1}	K_D, M^{-1}	$k_w \times 10^5$, s^{-1} (exp)	CMC $\times 10^3$, M
Sodium decyl sulfate	0.0109	105 ± 3	91.2 ± 1.0	50 ± 3	106 ± 1	2.20 ± 0.30
	0.109	115 ± 2	97.5 ± 2.0	52 ± 4	117 ± 0.3	2.16 ± 0.25
	0.218	129 ± 1	97.2 ± 1.2	50 ± 4	129 ± 0.4	1.50 ± 0.20
Sodium dodecyl sulfate	0.0109	106 ± 2	86.7 ± 1.0	120 ± 3	106 ± 1	4.70 ± 0.20
	0.109	115 ± 1	90.5 ± 1.5	105 ± 2	117 ± 0.3	0.50 ± 0.08
	0.218	130 ± 2	92.8 ± 1.2	112 ± 2	129 ± 0.4	0.40 ± 0.07
Sodium tetradecyl sulfate	0.0085	104 ± 1	75.0 ± 2.5	318 ± 13	103 ± 1	0.08 ± 0.02
	0.0109	105 ± 1	88.0 ± 1.5	300 ± 12	106 ± 1	0.07 ± 0.01
	0.0218	107 ± 1	78.0 ± 2.0	299 ± 15	107 ± 0.3	0.02 ± 0.004

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Acknowledgments

Thanks are given to Laboratorios Prodes, Barcelona, and to Laboratorios Roche, Madrid, Spain, for generous gifts of drugs samples. The authors would also like to thank Dr. F. González-Lopez, from the Departamento de Farmacia y Tecnología Farmacéutica, Universidad de Salamanca, for his assistance in the HPLC study.