Micellar Enhancement of Benzodiazepine Fluorescence

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The interactions that exist between benzodiazepines and surfactants provide micellar enhancement factors for their fluorimetric determination in the range 1.2–6.5, depending on the nature of both the benzodiazepine and the surfactant. A series of benzodiazepines and anionic surfactants were treated topologically to determine the influence of each benzodiazepine substituent on the basic benzodiazepine structure and the influence of both the hydrophobic moiety of the surfactant and its counter ion on the sensitisation process. Sensitisation parameters were used to quantify the effect of the chemical structures of both surfactants and drugs on their interaction.

Keywords: *Benzodiazepines; fluorimetric determination; micellar enhancement; anionic surfactants; topological description*

Benzodiazepines are important psychotherapeutic agents that act on the central nervous system and they are widely used for the treatment of anxiety, insomnia and epileptic convulsions owing to their low toxicity and limited side effects.[1–3] These compounds have been identified and determined in pure drugs, medicines, biological fluids and tissues using a variety of methods.[4–9]

The fluorimetric determination of benzodiazepines can be carried out in one of three ways: (*i*) by direct measurement of their fluorescence[10,11]; (*ii*) by prior hydrolysis[12–14]; or (*iii*) by prior reaction with various substances to form highly fluorescent derivatives.[15,16]

The limited development of simple methods for the fluorimetric determination of benzodiazepines is possibly due to the low sensitivity of their natural fluorescence. For this reason, the fluorimetric study of the interaction of these compounds with micellar media could prove interesting as a means of increasing their fluorescence quantum yield. However, little work has been reported on the fluorimetric determination of psychotherapeutic drugs in micellar media and an initial study concerning anorexics, tranquillisers and other drugs is currently in progress.[17,18]

The addition of a surfactant to a chemical system causes significant changes in behaviour.[19–21] Such changes depend on the surfactant concentration and occur at concentrations higher than the critical micellar concentration (CMC),[22] which indicates that they are the result of micellar formation and not of interactions of the type that occur in co-ordination compounds and ion pairs.

At present there is only limited knowledge of the factors governing the interaction of chemical systems with micelles and, in fact, the only norm that exists is that of charge compatibility between the micelle and the analyte.[23] However, this explains neither the interaction that is possible with non-ionic micelles nor why the same types of surfactant can cause notably different effects. Hence, investigations into the interactions that exist between different surfactants and a particular system, or analogous systems,[24–26] could help in establishing the conditions that result in the best analytical characteristics and a set of rules for predicting the sensitisation conditions of other chemical systems.

The aim of the work described in this paper was to study the interaction of different benzodiazepines with various surfactants in order to establish more sensitive methods for the determination of benzodiazepine tranquillisers using micellar media.

In the fluorimetric investigation of the interaction of benzodiazepines with anionic surfactants, we have tried to establish the influence of both benzodiazepine substituents and surfactant structure on the sensitisation process.

Theory

Topological and group contribution models[27–31] have been used in chemical engineering to establish the laws of variation of the physical and chemical properties of a homologous series of compounds and, more importantly from a technical point of view, to predict the behaviour of unsynthesised compounds and to establish the properties of mixtures of compounds.[32,33]

From a chemical - analytical point of view, we have applied the topological treatment to a series of ethylene oxide condensate surfactants in order to establish the laws of variation of both their physical[34] and spectral[35] properties with the average degree of condensation. Hence, graphs were constructed for the characterisation of these compounds.

In this study a topological treatment was used to quantify the influence of the structures of both anionic surfactants and benzodiazepines on the sensitisation of their fluorescence in micellar media. It was assumed that the molecule of an anionic surfactant consists of two components: a strongly hydrophobic moiety, A, and a counter ion, C.

Any property P_{T_i} of a surfactant T_i, which depends on the surfactant structure, can be separated into two contributions:

$$P_{T_i} = SP_{A_i} + SP_{C_i} \quad . . \quad . . \quad . . \quad (1)$$

where SP_{A_i} and SP_{C_i} are the sensitisation parameters (SPs) corresponding to each part of the molecule.

In order to study the effect of surfactant structure on the fluorescence sensitisation of a benzodiazepine it is necessary to establish a variable that quantifies the process and to establish its value for a sufficiently wide range of systems under the same conditions. For this purpose the micellar enhancement factor (MEF) of the benzodiazepine, defined as the ratio of the slopes of the calibration graphs obtained in the presence and absence of a surfactant, was used.

It can be assumed that the MEF obtained in the presence of a given surfactant depends on the hydrophobic moiety and its counter ion. Hence, for a species T_1,

$$MEF_{T_1} = SP_{A_1} + SP_{C_1} \quad . . \quad . . \quad . . \quad (2)$$

For a group of surfactants T_i having x distinct structures of the hydrophobic moiety and y different counter ions,

$$x + y = i \quad . . \quad . . \quad . . \quad . . \quad (3)$$

Hence it can be established that

$$\sum_i MEF_{T_i} = \sum_x SP_{A_x} + \sum_y SP_{C_y} \quad . . \quad . . \quad (4)$$

Equation (4) describes a system of i independent equations with i unknowns when there are i representative surfactants having the same number of possible structures for the hydrophobic moiety and for the counter ion. This system can be described as a combination of matrices consisting of a

vector of the experimental MEF values, a vector of SPs and a topological matrix describing the structure of the different surfactants used:

$$\begin{bmatrix} MEF_{T_1} \\ MEF_{T_2} \\ \vdots \\ MEF_{T_{i-1}} \\ MEF_{T_i} \end{bmatrix} = M \cdot \begin{bmatrix} SP_{A_1} \\ SP_{A_2} \\ \vdots \\ SP_{A_x} \\ SP_{C_1} \\ SP_{C_2} \\ \vdots \\ SP_{C_y} \end{bmatrix} \quad . . \quad (5)$$

where M is a square matrix of i rows and i columns.

The i^2 components comprising the topological matrix correspond to the different hydrophobic groups and counter ions belonging to the group of molecules studied. Using binary notation to indicate either the absence or presence of a determinate part in the molecule, the vectors (the rows of the matrix) describe each of the surfactants in relation to the whole.

Because of the experimental errors inherent in the determination of the MEF values, equation (5) does not have a unique solution as it does not represent a compatible and determinate system. Therefore, it is necessary to adopt some method of rough calculation. In this work the optimum SPs were calculated from the experimental MEF values for a series of well-characterised surfactants in order to minimise the difference, established by a least-squares method, between the MEF values calculated from the SPs and the experimentally obtained values.

To calculate the parameters corresponding to the sensitisation of the benzodiazepine fluorescence by anionic surfactants, a semi-logarithmic method was used to establish the following relationship:

$$\begin{bmatrix} \log MEF_{T_1} \\ \log MEF_{T_2} \\ \vdots \\ \log MEF_{T_{i-1}} \\ \log MEF_{T_i} \end{bmatrix} = M \cdot \begin{bmatrix} SP_{A_1} \\ SP_{A_2} \\ \vdots \\ SP_{A_x} \\ SP_{C_1} \\ SP_{C_2} \\ \vdots \\ SP_{C_y} \end{bmatrix} \quad . . \quad (6)$$

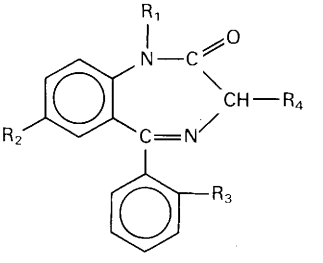
The effect of eight anionic surfactants on the fluorescence of diazepam was investigated. The surfactants, which provided different hydrophobic structures and three different counter ions were the following: lauryl sulphate, A_1; decyl sulphate, A_2; lauryl ether sulphate, A_3; alkyl phenol ether sulphate, A_4; α-olefin sulphonate, A_5; Na^+, C_1; NH_4^+, C_2; and triethanolamine, C_3. The topological description of these surfactants and an expression of equation (6), adapted to the studied population, are given in Table 1.

Table 1. Topological description of the anionic surfactants for determining the influence of their structure on micellar enhancement of diazepam fluorescence

Surfactant	Hydrophobic part	Counter ion
SDS	A_1	C_1
TLS	A_1	C_3
SLES	A_3	C_1
DSS	A_2	C_1
ALS	A_1	C_2
ALES	A_3	C_2
SAPES	A_4	C_1
Sα-OS	A_5	C_1

$$\begin{bmatrix} \log MEF_{SDS} \\ \log MEF_{TLS} \\ \log MEF_{SLES} \\ \log MEF_{DSS} \\ \log MEF_{ALS} \\ \log MEF_{ALES} \\ \log MEF_{SAPES} \\ \log MEF_{S\alpha\text{-}OS} \end{bmatrix} = \begin{bmatrix} 10000100 \\ 10000001 \\ 00100100 \\ 01000100 \\ 10000010 \\ 00100010 \\ 00010100 \\ 00001100 \end{bmatrix} \cdot \begin{bmatrix} SP_{A_1} \\ SP_{A_2} \\ SP_{A_3} \\ SP_{A_4} \\ SP_{A_5} \\ SP_{C_1} \\ SP_{C_2} \\ SP_{C_3} \end{bmatrix}$$

Table 2. Benzodiazepine structures



Benzodiazepine	R_1	R_2	R_3	R_4
Diazepam	CH_3	Cl	H	H
Prazepam	$CH_2C_3H_5$	Cl	H	H
Nitrazepam	H	NO_2	H	H
Clonazepam	H	NO_2	Cl	H
Temazepam	CH_3	Cl	H	OH
Oxazepam	H	Cl	H	OH
Lorazepam	H	Cl	Cl	OH

A method, analogous to that described above, can be used to quantify the effect of benzodiazepine structures on their interaction with the anionic micelles. In this instance, after determining MEFs for different benzodiazepines in the presence of the same surfactant, equation (5) or (6) can be used to obtain SPs corresponding to each possible structure of the substituents on the benzodiazepine nucleus.

For this study seven benzodiazepines, differing only in the nature of the substituents R_1, R_2, R_3 and R_4, were used (see Table 2). Hence seven SPs were defined corresponding to the R_1 substituents (SP_A), to the R_2 substituents (Cl, SP_B; and NO_2, $SP_{B'}$), to the R_3 substituents (H, SP_C; and Cl, $SP_{C'}$) and to the R_4 substituents (H, SP_D; and OH, $SP_{D'}$).

The three different R_1 substituents are distinguished according to the number of bonds or CH groups present, *viz.*, *a* for H, 2*a* for CH_3 and 5*a* for $CH_2C_3H_5$. This procedure simplifies the situation but can be justified on the basis that all the R_1 substituents are of an analogous type and do not cause an excessive increase in molecular polarity.

Table 3. Topological description of the benzodiazepines for determining the influence of their structure on fluroresecence sensitisation in micellar media

Benzodiazepine	R_1	R_2*	R_3†	R_4‡
Diazepam (D) . .	2*a*	*b*	*c*	*d*
Prazepam (P) . .	5*a*	*b*	*c*	*d*
Nitrazepam (N)	*a*	*b*′	*c*	*d*
Clonazepam (C)	*a*	*b*′	*c*′	*d*
Temazepam (T)	2*a*	*b*	*c*	*d*′
Oxazepam (O) . .	*a*	*b*	*c*	*d*′
Lorazepam (L) . .	*a*	*b*	*c*′	*d*′

$$\begin{bmatrix} MEF_D \\ MEF_P \\ MEF_N \\ MEF_C \\ MEF_T \\ MEF_O \\ MEF_L \end{bmatrix} = \begin{bmatrix} 2101010 \\ 5101010 \\ 1011010 \\ 1010110 \\ 2101001 \\ 1101001 \\ 1100101 \end{bmatrix} \cdot \begin{bmatrix} SP_A \\ SP_B \\ SP_{B'} \\ SP_C \\ SP_{C'} \\ SP_D \\ SP_{D'} \end{bmatrix}$$

* b = Cl; b' = NO_2.
† c = H; c' = Cl.
‡ d = H; d' = OH.

Hence, it can be assumed that the effect of each of these substituents on the sensitisation of the fluorescence is defined by an SP and that the over-all effect of a molecule on the sensitisation process is described by the sum of the contributions of each factor. This assumption is reasonable as it was not intended to establish the contribution of each substituent to the fluorescence quantum yield of the molecule (*viz.*, the activity of the fluorogenic groups) but rather to establish their contribution to the interaction between micelles and benzodiazepine molecules. In addition to being dependent on the respective charges,[23] this interaction is also highly conditioned by steric factors.[25] Accordingly, the benzodiazepine MEF (MEF_B) in the presence of a determinate type of micelle for a particular compound is given by:

$$MEF_B = aSP_A + (SP_B \text{ or } SP_{B'}) + (SP_C \text{ or } SP_{C'}) + (SP_D \text{ or } SP_{D'}) \quad (7)$$

where a corresponds to the number of groups in the substituent R_1. The remaining parameters depend on the structure of the benzodiazepine studied.

Based on previous considerations, a topological description of each of the compounds studied was established that made use of binary notation to indicate the presence or absence of determinate substituents R_2, R_3 and R_4 and a relative criterion to ascertain the number of associated groups on the R_1 (a) substituent.

A procedure, similar to that used for considering the contribution of the surfactant structures to the MEF (Table 1), was used to define the topological matrix given in Table 3. The product of this matrix and the SP vector is the set of MEFs corresponding to each of the benzodiazepines in the presence of each surfactant.

Experimental

Apparatus

A Shimadzu RF-520 dual-beam spectrofluorimeter equipped with a xenon lamp and a U 135 S recorder was used for the spectrofluorimetric study and a Shimadzu UV-240 spectrophotometer equipped with a Model PR-1 recorder for the absorbance measurements. In both instances 1-cm quartz cells were used.

Values of pH were determined using a Crison Model 501 digital pH meter with a precision of ±0.01 unit and an HP 83 computer was used to carry out data adjustments.

Table 4. Effect of anionic surfactant on the fluorescence of diazepam

Surfactant	MEF*	SD†
SDS	6.2	0.2
TLS	6.5	0.1
SLES	4.8	0.4
DSS	4.8	0.3
ALS	4.3	0.2
ALES	3.1	—
SAPES	2.5	—
Sα-OS	2.1	—

* The MEF is determined from the quotient of the experimental graphs obtained for the fluorescence of solutions containing 1–10 p.p.m. of diazepam both in the presence and absence of each surfactant assayed.
† SD = standard deviation for three independent determinations of MEF.

Reagents

Solutions of the following surfactants were used: Triton X-100 (a *tert*-octylphenol - ethylene oxide condensate) purchased from Probus; Nemol K-38 and Nemol K-3030 (nonylphenol - ethylene oxide condensates) purchased from Massó y Carol; cetyltrimethylammonium bromide (CTAB) purchased from Merck; sodium lauryl sulphate (SDS) purchased from Fluka; and sodium decyl sulphate (DSS), ammonium lauryl sulphate (ALS), triethanolamine lauryl sulphate (TLS), sodium lauryl ether sulphate (SLES), ammonium lauryl ether sulphate (ALES), sodium alkyl phenol ether sulphate (SAPES) and sodium α-olefin sulphonate (Sα-OS) donated by Molins-Kao.

Solutions of diazepam, prazepam, nitrazepam, clonazepam, temazepam, oxazepam, lorazepam and medazepam in sulphuric acid were used.

Diazepam was supplied by the pharmaceutical service of the Hospital of Valencia University, oxazepam from the MADAUS laboratory and the remainder of the benzodiazepines were supplied by the Bromatology Department of the Faculty of Pharmacy. All these products were pure and used without further purification.

General Procedure

Calibration graphs were constructed under the optimum excitation and emission conditions for each system, in both the presence and absence of each surfactant.

The benzodiazepine solutions (1–10 p.p.m.) were prepared by dilution of a stock solution of the drug with 1 M H_2SO_4.

The pK_a of diazepam was determined spectrophotometrically by measuring the absorbance of solutions of equal concentration and different pH (adjusted using 0.926 M HCl, 0.5 M $NaHSO_4$ - 1 M Na_2SO_4 and 1 M CH_3COOH - 1 M CH_3COONa buffers).

The surfactant CMCs were determined by measuring the surface tension (σ) of solutions containing different amounts of surfactant. The discontinuity in the graphs of σ *vs.* surfactant concentration in the presence and absence of diazepam could indicate the existence of mixed micelles.

Results and Discussion

Interaction of Diazepam With Surfactants

Diazepam (7-chloro-1,3-dihydro-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one) has an excitation maximum at 360 nm and an emission maximum at 500 nm. The diazepam fluorescence is strongest at acidic pH and decreases as the temperature decreases. Hence the optimum conditions for the fluorimetric determination of diazepam were previously established to be a temperature of 15 °C and a sulphuric acid concentration of 0.5 M.[36] However, for practical reasons a temperature of 20 °C was used in this work.

The addition of non-ionic surfactants, such as ethylene oxide condensates, or of the cationic surfactant CTAB, has no effect on either the intensity or the shape of the diazepam emission band. However, the addition of 1% *m/V* SDS causes an increase in the diazepam fluorescence of the order of 600%, without producing either a bathochromic or a hypsochromic shift of the fluorescence band.

Sensitisation of the diazepam fluorescence is achieved by the addition of other anionic surfactants (1% *m/V*). Nevertheless and, in spite of the experimental variation in the calibration graphs obtained for the same system, it can be seen that the degree of sensitisation is different for each of the surfactants studied (Table 4). Sensitisation is highest in the presence of lauryl sulphate, triethanolamine and SDS, whereas an average MEF of 4.8 is obtained by the addition of either sodium lauryl ether sulphate or sodium decyl sulphate. For the remaining surfactant systems progressively smaller increases in sensitivity are found.

Determination of the Sensitisation Parameter Corresponding to Each Anionic Surfactant Structure

Having shown that the surfactant structure influences the sensitisation of diazepam, we tried to establish the contribution to the over-all process of the parts (A and C) comprising the surfactant molecule.

Using the topological model described under Theory, SPs were determined for each of the counter ions and each of the hydrophobic moieties of the surfactants studied.

Two types of data adjustment were used, *viz.*, linear and semi-logarithmic. The SPs obtained for a series of experimental values are given in Table 5 as are the relative errors (E) found between the MEFs calculated using these SPs and those determined experimentally. Clearly, there is less error inherent in a semi-logarithmic adjustment.

The SPs found indicate the greater influence of the hydrophobic moiety, which might be expected if the diazepam micelle interaction is governed by electrostatic and steric factors.

It can be concluded that the structure of lauryl sulphate is the most suitable for the fluorimetric determination of diazepam. The structures of decyl sulphate and lauryl ether sulphate are of comparable suitability, whereas use of the more complex structures is not recommended.

As regards the counter ion, the use of triethanolamine or sodium salts is recommended, whereas the NH_4^+ ion has a negative effect on the micellar interaction process.

Fluorescence of Benzodiazepines in the Presence of Anionic Surfactants

All the benzodiazepines studied gave similar excitation and emission spectra in aqueous sulphuric acid media (Table 6) and the addition of anionic surfactants at concentrations below the CMC caused a fluorescence enhancement of the benzodiazepine solutions although neither hypsochromic nor bathochromic shifts were observed.

The fluorescence of each of the benzodiazepines studied was determined in the presence of SDS, TLS, SLES and DSS micelles. The average MEFs obtained for each system and the standard deviations (SDs) for three independent determinations of the MEF are given in Table 7.

In the presence of 1% of various anionic surfactants the benzodiazepine fluorescence increased and MEFs were obtained in the range 1.2–6.2. Only for temazepam, in micelles of SLES, was a 20% relative decrease in fluorescence obtained.

The influence of the surfactant structure on sensitisation is again confirmed and, in addition, it has been shown that the benzodiazepine structure also affects fluorescence enhancement.

Sensitisation Parameters Corresponding to Benzodiazepine Substituents

Sensitisation parameters were calculated as described under Theory using both linear and semi-logarithmic data adjustments. For each MEF, the corresponding SP was determined and the difference between the experimental MEFs and those calculated using SPs was quantified.

Table 8 gives the SPs corresponding to various benzodiazepine substituents in the presence of sodium lauryl sulphate. The linear data adjustment yields lower average relative errors between the experimental and calculated MEFs and is the more reproducible as far as the SPs are concerned; only in a few instances were low, positive values changed to negative

Table 5. Sensitisation parameters for diazepam in the presence of anionic surfactants obtained by both linear and semi-logarithmic adjustments of a single actual (not average) value of the MEF for each surfactant assayed

Linear	Semi-logarithmic
$SP_{A_1} = 5.17$	$SP_{A_1} = 0.59$
$SP_{A_2} = 3.62$	$SP_{A_2} = 0.45$
$SP_{A_3} = 3.75$	$SP_{A_3} = 0.45$
$SP_{A_4} = 1.62$	$SP_{A_4} = 0.19$
$SP_{A_5} = 1.26$	$SP_{A_5} = 0.13$
$SP_{C_1} = 0.87$	$SP_{C_1} = 0.20$
$SP_{C_2} = -0.83$	$SP_{C_2} = 0.03$
$SP_{C_3} = 1.47$	$SP_{C_3} = 0.24$

Linear			Semi-logarithmic		
$MEF_{exp.}$	$MEF_{cal.}$	E, %	$MEF_{exp.}$	$MEF_{cal.}$	E, %
6.1900	6.0350	−2.50	6.1900	6.1604	−0.48
6.6400	6.6400	0.00	6.6400	6.6400	0.00
4.4600	4.6150	3.48	4.4600	4.4800	0.48
4.4900	4.4900	0.00	4.4900	4.4900	0.00
4.1800	4.3350	3.71	4.1800	4.2001	0.48
3.0700	2.9150	−5.05	3.0700	3.0553	−0.48
2.4900	2.4900	0.00	2.4900	2.4900	0.00
2.1300	2.1300	0.00	2.1300	2.1300	0.00
Average relative error:		1.84	Average relative error:		0.24

Table 6. Excitation and emission conditions for benzodiazepines in both aqueous solution and in the presence of SDS

	In aqueous medium		In the presence of SDS	
Benzodiazepine	$\lambda_{ex.}$/nm	$\lambda_{em.}$/nm	$\lambda_{ex.}$/nm	$\lambda_{em.}$/nm
Diazepam . .	360	500	360	500
Prazepam . .	360	500	360	490
Nitrazepam . .	350	460	350	450
Clonazepam . .	350	460	350	460
Temazepam . .	360	485	360	485
Oxazepam . .	360	500	360	490
Lorazepam . .	370	500	370	495

Table 7. Average MEF values and SDs of benzodiazepines in the presence of different anionic surfactants

	Surfactant							
	SDS		TLS		SLES		DSS	
Benzodiazepine	MEF	SD	MEF	SD	MEF	SD	MEF	SD
Oxazepam . .	3.3	0.1	2.6	0.7	2.2	0.2	3.0	0.7
Prazepam . .	5.7	0.2	6.2	0.1	4.2	0.4	3.5	0.2
Nitrazepam . .	2.2	0.1	2.3	0.1	1.7	0.2	1.6	0.2
Clonazepam . .	1.6	0.1	1.8	0.1	1.4	0.1	1.2	0.1
Temazepam . .	1.6	0.3	1.2	0.2	0.8	0.0_2	1.2	0.3
Lorazepam . .	2.2	0.1	2.0	0.3	1.2	0.0_2	1.9	0.1
Diazepam . .	6.2	0.2	6.5	0.1	4.8	0.4	4.8	0.3

values. Nevertheless, SPs obtained by the adjustment of the total experimental MEF data were used as a good estimate of the contribution of each substituent to the interaction process.

The SPs and the corresponding errors were of the same order of magnitude for each system and, in general, linear adjustment gave the best results; in all instances average relative errors for the MEFs of less than 20% were obtained. Table 9 summarises the SP data for a variety of substituents in the presence of each surfactant, using linear adjustment. Because the SPs obtained under different conditions are of the same order of magnitude, the validity of the proposed method is confirmed.

From the values of the sensitisation parameters it can be concluded that, typically, the R_1 and R_3 substituents have a negative effect on the sensitisation process and the R_2 substituents, particularly Cl, contribute positively to the sensitisation. As regards the R_4 substituent, its contribution sign inverts on replacing H by OH, except for studies carried out in the presence of triethanolamine lauryl sulphate. In this instance an OH group as the R_4 substituent contributes positively to the sensitisation, although to a lesser extent than the H group.

Table 8. Sensitisation parameters for benzodiazepine substituents in the presence of sodium lauryl sulphate, obtained by both linear and semi-logarithmic adjustments. Numbers and letters in parentheses indicate the different series of data used for the calculation of SPs

	SP(1)	E,* %	SP(2)	E,* %	SP(3)	E,* %	SP(T)	E,* %
Linear adjustment—								
SP_A	−0.41		−0.41		−0.19		−0.34	
SP_B	6.60		4.96		4.81		4.82	
$SP_{B'}$	1.88		−3.50		0.47		0.14	
SP_C	−3.18		−0.75		−1.86		−1.01	
$SP_{C'}$	−3.72	14.5	−1.19	13.7	−2.28	8.7	−1.47	12.8
SP_D	3.85		3.33		3.68		3.33	
$SP_{D'}$	−0.44		−1.20		−1.60		−0.83	
Semi-logarithmic adjustment—								
SP_A	−5.69		−5.53		−2.85		−4.69	
SP_B	1.62		−1.69		0.82		2.46	
$SP_{B'}$	1.02		−2.40		0.28		1.87	
SP_C	−1.01		0.06		−1.07		−0.93	
$SP_{C'}$	−1.10	19.0	−0.00	18.1	−1.14	10.5	−1.01	16.1
SP_D	0.35		2.71		1.13		−0.59	
$SP_{D'}$	0.18		2.17		0.71		−1.09	

* E = average relative error. Given as the difference between MEF values calculated using SPs and the experimental values.

Table 9. Sensitisation parameters for benzodiazepine substituents in the presence of different surfactants

Sensitisation parameter	Surfactant			
	SDS	TLS	SLES	DSS
SP_A	−0.34	−0.25	−0.33	−0.46
SP_B	4.82	4.91	3.93	6.55
SP_B'	0.14	0.09	0.36	2.61
SP_C	−1.01	−3.02	−1.25	−3.20
SP_C'	−1.47	−3.27	−1.64	−3.37
SP_D	3.33	5.35	2.97	2.52
SP_D'	−0.83	0.49	−0.73	−0.62

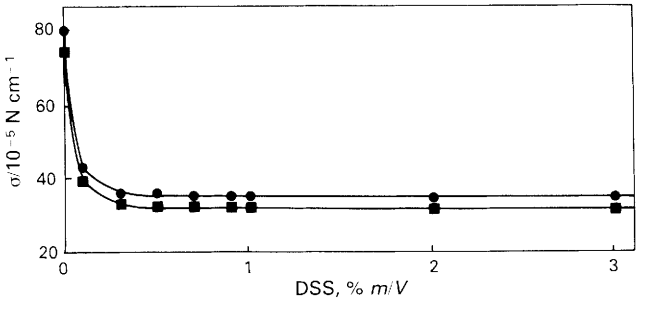


Fig. 1. Effect of DSS concentration in sulphuric acid on the surface tension (■) in the presence and (●) absence of diazepam

Mechanism of Interaction Between Benzodiazepines and Surfactants

Because significant increases in the fluorescence of benzodiazepines have only been obtained in the presence of anionic surfactants, this indicates that the interaction is electrostatic in character.[22] Nevertheless, the different MEFs obtained for each surfactant structure and for each benzodiazepine studied emphasise the importance of steric factors in the process of micellar interaction.

It was assumed that it is known that anionic surfactants affect the dissociation constants of organic molecules by stabilising the protonated forms.[21] Hence, in order to study the interaction mechanism in depth, the pK_a of diazepam in the presence of SDS was determined spectrophotometrically. The value obtained (3.5 ± 0.1) was almost coincident with that found in the absence of a surfactant (3.4 ± 0.1) and it was deduced that the micelles have no influence on the dissociation of benzodiazepines.

The effect of anionic surfactants on the absorbance of benzodiazepine solutions or on the absorption maxima of benzodiazepines in H_2SO_4 media was also studied. The results indicate that the benzodiazepines are scarcely modified in micellar media; only for medazepam was a 25% increase in the molar absorptivity at 453 nm found. This allows the increase in the fluorescence in micellar media to be interpreted on the basis of the increase in the fluorescence quantum yield of the benzodiazepines and the absence of a simultaneous increase in the molar absorption within the excitation bands. Therefore, the origin of the sensitisation process lies in the protection of the singlet excited state of the benzodiazepine in a micellar micro-environment from non-fluorescent de-activation processes.

In order to determine the possible localisation of the benzodiazepine molecules within the micelles, the CMCs of several surfactants were determined in H_2SO_4 both in the presence of 4.8 p.p.m. of diazepam and in its absence. The CMCs were determined from surface tension measurements (see Fig. 1).

Identical CMCs were found in both the presence and absence of diazepam for SDS, SLES and DSS. Hence, it was concluded that mixed micelles do not exist and consequently the benzodiazepine molecules can be expected to remain localised in the micellar surface zone.

Conclusion

The studies described in this paper provided data regarding the influence of the structure of both the surfactant and the sensitised molecule on the sensitisation process.

It has been shown that the determination of SPs is a valid procedure for calculating the influence of each of the benzodiazepine substituents and each of the surfactant component parts on the sensitisation process. Hence, SPs might be used to facilitate the design of surfactant structures that allow a better sensitivity and might therefore contribute to a greater knowledge of micellar interaction processes.

Finally, the results indicate that more sensitive fluorimetric methods for the determination of benzodiazepines can be performed using micellar media.

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