Kinetic Studies on the Influence of Micellar Aggregates upon the Hydrolysis and Transnitrosation Reactions of *N*-methyl-*N*-nitrosotoluene-*p*-sulphonamide

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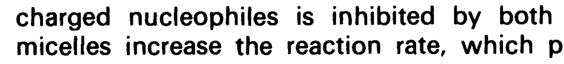
The basic hydrolysis of *N*-methyl-*N*-nitrosotoluene-*p*-sulphonamide and its transnitrosation reaction with secondary amines (sarcosine and dimethylamine) have been studied in the presence of cationic, anionic, and non-ionic micelles. The results show that the electric charge of the nucleophile is essential for the understanding of the micellar effects, which can be quantitatively rationalized on the basis of the pseudophase kinetic model. Thus the reaction with negatively charged nucleophiles is inhibited by both anionic and non-ionic surfactants, whereas cationic micelles increase the reaction rate, which passes through a maximum. The reaction between the substrate and a neutral nucleophile is, however, inhibited by all three classes of surfactant.

N-Methyl-*N*-nitrosotoluene-*p*-sulphonamide (MNTS) has proved to be a highly interesting substrate as regards its behaviour in basic or neutral media both from biomedical and chemical points of view. MNTS reacts with nucleophiles such as OH^- or EtO^- to produce an intermediate that breaks down to afford alkylating species, a traditional method of obtaining diazomethane, in a pattern of behaviour similar to that of other nitrosamides. The carcinogenic properties of this substrate are probably related to the alkylating ability of the products thus formed. Besides this reaction, in the presence of nitrosatable substrates—amines,[1] thiols [2]—a transnitrosation reaction takes place, the centre which receives the nucleophilic attack being the -N=O group (see Scheme 1). The efficiency of this reaction as a source of *N*-nitrosamines under alkaline conditions has been discussed.[1] This pathway allows the formation of carcinogenic *N*-nitrosamines under conditions in which the usual nitrosating agents (NO^+, N_2O_3, nitrosyl halides) do not exist.



Scheme 1.

This interesting behaviour has prompted us to carry out a study of these reactions in media closer in conditions to those found in biological systems. In spite of the vast amount of literature concerning catalysis or inhibition of organic reactions by micelles, there is an obvious lack of information on the reactivity of nitroso compounds in these media. In this paper we report the effect of anionic, cationic, and non-ionic surfactants upon the alkaline hydrolysis of MNTS and upon the transnitrosation reaction from MNTS to secondary amines.

Experimental

All reactants used were of the maximum commercially available purity, supplied either by Merck or Sigma. The low solubility of MNTS in water made it necessary to prepare its solutions by dissolving known weights of MNTS in ethanol–water mixtures. The percentage of ethanol in the different working conditions is indicated in the Results section and is quoted as ml of ethanol per 100 ml of reaction mixture. The reactions were followed spectrophotometrically at 25 °C by recording the decrease in absorbance at 250 nm due to the disappearance of MNTS. The initial concentration of MNTS employed (between 1.6×10^{-5} and 5×10^{-5} mol dm^{-3}) was always much smaller than that of the other reagents used. Kinetic analysis was carried out by using the integration method, the absorbance A_∞ at time $t = \infty$ being optimized by the algorithm of Davies, Swann, and Campey as indicated by Casado *et al.*[3] Graphs of $\ln(A_t - A_\infty)$ *vs.* time were linear for at least 85% reaction and the values of A_∞ optimized in each case agreed perfectly well with the experimental ones. All kinetic experiments were reproducible to within 3%.

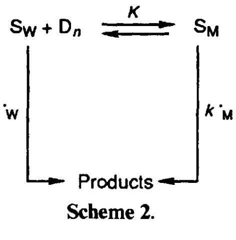
Results and Discussion

1. *Micellar Effects upon the Alkaline Hydrolysis of MNTS.*—(a) *Hydrolysis in the presence of anionic micelles.* The effect of sodium dodecyl sulphate (SDS) upon the hydrolysis of MNTS was studied at $[OH^-]$ 0.0171 mol dm^{-3} and 12% (v/v) ethanol and the concentration of SDS was varied between 3.43×10^{-3} and 4.8×10^{-2} mol dm^{-3}. The results (Figure 1) show the pseudo-first-order rate constant k_0 to be inhibited by SDS.

This behaviour can be rationalized in terms of the simple pseudophase model,[4] which considers the total volume of the micelle as a separate phase uniformly distributed in the bulk aqueous phase. Provided that equilibrium is maintained between the substrate in both phases, the overall reaction rate will be obtained easily from Scheme 2, as the sum of rates in water and in the micellar phase.

In Scheme 2, the subscripts M and W denote the micellar and aqueous phases respectively, S is the substrate, and D_n is the micellized surfactant, whose concentration is that of total surfactant less that of the monomer which is assumed to be given by the critical micelle concentration (cmc). The first-order rate constants for processes in both phases will be k'_W and k'_M.

Simple electrostatic considerations[5] predict that, owing to



Scheme 2.

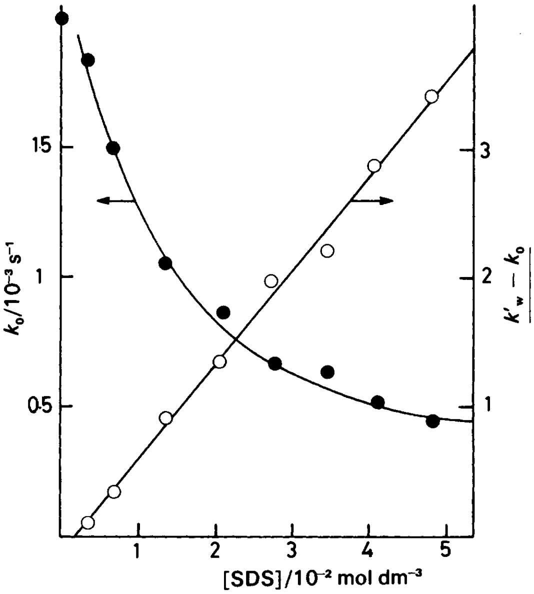


Figure 1. Influence of the concentration of SDS upon the first-order rate constant for the hydrolysis of MNTS. [NaOH] 0.0171 mol dm^{-3}, $[MNTS]_0$ 4.9 × 10^{-5} mol dm^{-3}, 12% ethanol. Linearization in the form of equation (2) is also shown.

the anionic character of the micelle, the concentration of OH^- (or EtO^-) ions in the Stern layer will be very low. This exclusion of hydroxide ions inhibits the reaction, *i.e.* k'_M will be very low. In fact, Scheme 2 leads to equation (1) and if $k'_M \simeq 0$, this equation can be rearranged in the form of equation (2).

$$k_0 = \frac{k'_W + k'_M K([SDS] - cmc)}{1 + K([SDS] - cmc)} \quad (1)$$

$$(k'_W - k_0)/k_0 = K([SDS] - cmc) \quad (2)$$

According to equation (2) a plot of $(k'_W - k_0)/k_0$ *vs.* [SDS] should yield a straight line. In fact, considering that $[OH^-]_{total} = [OH^-]_W$, it is possible to draw such a plot with k'_W taken as the value of k_0 in the absence of surfactant. This plot is shown in Figure 1 and leads to a value of K 73 ± 2 dm^3 mol^{-1}. The excellent fit of the experimental data to equation (2) confirms the model of reactivity proposed with k'_M 0, which is based upon the exclusion of the reactive anions from the Stern layer of the anionic micelle.

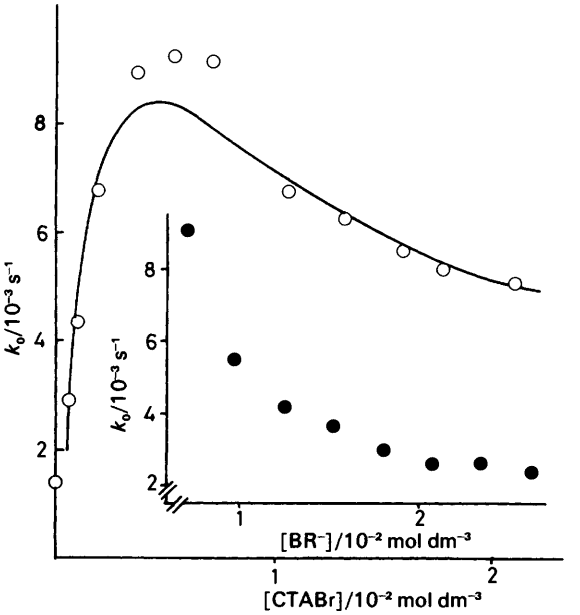


Figure 2. Influence of the concentration of CTABr upon the pseudo-first-order rate constant for the hydrolysis of MNTS. [NaOH] 0.0171 mol dm^{-3}, 4.1% ethanol (v/v), and $[MNTS]_0$ 1.63 × 10^{-5} mol dm^{-3}. In the insert figure, the influence of the concentration of Br^- upon the same constant for hydrolysis of MNTS in the presence of CTABr is shown: [CTABr] 7.06 × 10^{-3} mol dm^{-3}, [NaOH] 0.0171 mol dm^{-3}, $[MNTS]_0$ 1.63 × 10^{-5} mol dm^{-3}, and 4.1% ethanol (v/v). The solid line is calculated using the ion-exchange model.

(b) *Hydrolysis in the presence of cationic micelles.* We have studied the influence that two cationic micelles, cetyltrimethylammonium bromide (CTABr) and tetradecyltrimethylammonium bromide (TTABr), exerted upon the same hydrolytic process. Experiments with CTABr were carried out at 4% ethanol, [NaOH] 0.0171 mol dm^{-3}, $[MNTS]_0$ 1.63 × 10^{-5}, and the concentration of CTABr was changed between 4.4 × 10^{-4} and 2.2 × 10^{-2} mol dm^{-3}. Under these experimental conditions OH^- is likely to be the only effective nucleophile.[1] Figure 2 shows the values obtained for k_0 at different concentrations of CTABr. The k_0 variation with surfactant concentration follows a typical pattern, an initial increase in the concentration of surfactant producing an enhancement of the reaction rate which reaches a maximum and then decreases continuously. This behaviour is typical of other hydrolytic processes and can be explained by means of the pseudophase ion-exchange kinetic model developed by Romsted.[6] According to this model, the initial addition of surfactant leads to catalysis because the relative concentrations of substrate and OH^- in the micellar Stern layer increase rapidly with the amount of surfactant and the reaction is accelerated. Once complete solubilization of the substrate in the micellar pseudophase has taken place, the only effect of increasing the surfactant concentration will be the parallel increase in the concentration of unreactive bromide ions, which displace the micellar-bound hydroxide ion out of the Stern layer. This competition of reactive and inert ions for the micellar surface is the keypoint of the model and a high-charge-density ion such as OH^- will be readily displaced from a cationic micelle by a low-charge-density ion such as Br^-, thus resulting in an inhibitory effect. This point was confirmed by experiments (represented in the insert of Figure 2) in which at fixed concentration of CTABr (7.1 × 10^{-3} mol dm^{-3}) and OH^- (0.0171 mol dm^{-3}) the concentration of Br^- was increased through addition of NaBr. The inhibitory effect of Br^- ions is in agreement with the above mentioned diminution of OH^- in the Stern layer. The model also considers that the fraction β of micellar head group neutralized can be taken as a constant.[6] The ion-exchange equilibrium for our case of reactive OH^- and unreactive Br^- can be expressed by equation (3), with an equilibrium constant K^{OH}_{Br}.

$$OH^-_M + Br^-_W \rightleftharpoons OH^-_W + Br^-_M \quad K^{OH}_{Br} \quad (3)$$

The kinetic data for this reaction were subjected to a treatment using the method described by Rodenas and Vera.[7] The ion-exchange model predicts that the pseudo-first-order rate constant for disappearance of MNTS will follow equation (4), where the rate constants k_W and k_M are related to the

$$k_0 = \frac{k_W[OH^-]_{total} + (k_M K - k_W)m_{OH}[D_n]}{1 + K[D_n]} \quad (4)$$

pseudo-first-order rate constants k'_W and k'_M through equations (5) and (6), and $m_{OH} = [OH^-]_M/[D_n]$ can be obtained from equation (7) (all concentrations are relative to the total volume of solution).

Table 1. Influence of the composition of the medium (dioxane–water) upon the rate of hydrolysis of MNTS. [NaOH] 6.16 × 10^{-2} mol dm^{-3}, [MNTS]$_0$ 3.9 × 10^{-5} mol dm^{-3}.

Dioxane (%v:v)	$10^3\ k_0$/s^{-1}
7.14	5.64
14.3	4.47
21.4	3.69
28.6	3.10
35.7	2.65
40.0	2.35

Table 2. Influence of the concentrations of TTABr and Br$^-$ upon the pseudo-first-order rate constant for the hydrolysis of MNTS. [NaOH] 0.0171 mol dm^{-3}, [MNTS]$_0$ 4.9 × 10^{-5} mol dm^{-3}, 12% ethanol.

[TTABr]/ 10^{-4} mol dm^{-3}	[Br$^-$]$_{\text{total}}$/ 10^{-4} mol dm^{-3}	k_0/10^{-3} s^{-1}
		2
5.83	5.83	1.90
14.6	14.6	1.97
29.1	29.1	3.11
43.7	43.7	4.71
87.4	87.4	5.59
146	146	6.00
204	204	5.60
291	291	5.08
350	350	4.50
408	408	4.04
146	201	4.71
146	228	4.18
146	255	3.74
146	310	2.89
146	364	2.55
146	392	2.34

$$k'_{\mathrm{W}} = k_{\mathrm{W}}[\mathrm{OH}^-]_{\mathrm{W}} \qquad (5)$$

$$k'_{\mathrm{M}} = k_{\mathrm{M}}[\mathrm{OH}^-]_{\mathrm{M}}/[\mathrm{D}_n] \qquad (6)$$

$$m_{\mathrm{OH}}{}^2 + m_{\mathrm{OH}}\left[\frac{[\mathrm{OH}^-]_{\mathrm{total}} + K_{\mathrm{Br}}^{\mathrm{OH}}[\mathrm{Br}^-]_{\mathrm{total}}}{(K_{\mathrm{Br}}^{\mathrm{OH}} - 1)[\mathrm{D}_n]} - \beta\right] - \left[\frac{\beta[\mathrm{OH}^-]_{\mathrm{total}}}{(K_{\mathrm{Br}}^{\mathrm{OH}} - 1)[\mathrm{D}_n]}\right] = 0 \qquad (7)$$

The experimental data can be fitted to equations (4) and (7) using a simulation program to determine the best values of K, $K_{\mathrm{Br}}^{\mathrm{OH}}$, and k_{M}. The kinetic parameter k_{W} corresponds to hydrolysis in water and was given the experimental value of 0.0836 dm^3 mol^{-1} s^{-1}. We gave a value of 0.7 to the parameter β[8] and a value of 3 × 10^{-4} mol dm^{-3} to the cmc of CTABr. This value is sensibly lower than the commonly used value of 8–9 × 10^{-4} mol dm^{-3}, which corresponds to aqueous solutions.[9] Two effects operating in the same direction may help to reduce the cmc of CTABr: on the one hand, Bunton[10] has reported that it is reduced in the presence of NaOH and on the other the presence of a low amount of ethanol (4.1% in our case) also decreases the cmc.[11] Similar values (3 × 10^{-4} mol dm^{-3}) have been used by other authors in hydrolysis reactions.[12] The curve drawn in Figure 2 corresponds to the k_0-values predicted from the following values of the parameters involved: $K_{\mathrm{Br}}^{\mathrm{OH}}$ 23; K 184 dm^3 mol^{-1}; k_{M} 0.104 s^{-1}, although other sets of similar values also give an acceptable fit of the experimental data. In order to compare reaction rates in the micellar and aqueous phases, it is necessary to realize that k_{W} cannot be directly compared with k_{M}, since this second-order rate constant has the dimensions of reciprocal time, because the concentration of OH$^-$ is written as a mole ratio. Comparison could be made considering that the volume of the micellar Stern layer (*ca.* 0.14 dm^3 mol^{-1})[13] is the volume element of the reaction in the micellar pseudophase, thus resulting in bimolecular rate constant in the micellar pseudophase $k_2{}^{\mathrm{M}}$ being given by $k_2{}^{\mathrm{M}} = k_{\mathrm{M}} \times 0.14$. Even though some small amount of ethanol was incorporated into the micelle, numerical calculations show that the magnitude of this volume would not change appreciably.[14,15] Comparison between $k_2{}^{\mathrm{M}}$ and k_{W} shows the reaction rate to be intrinsically faster in water by a factor of *ca.* 6. This can be attributed—at least in part—to a medium effect. In fact, the Stern layer has a dielectric constant of *ca.* 35,[16] markedly lower than that of water. The influence of the lowering of the dielectric constant upon the reaction rate was investigated by studying the hydrolysis of MNTS in dioxane–water mixtures. The results obtained are given in Table 1 and show that, in fact, the effect of decreasing polarity of the solvent is a decrease in the reaction rate.

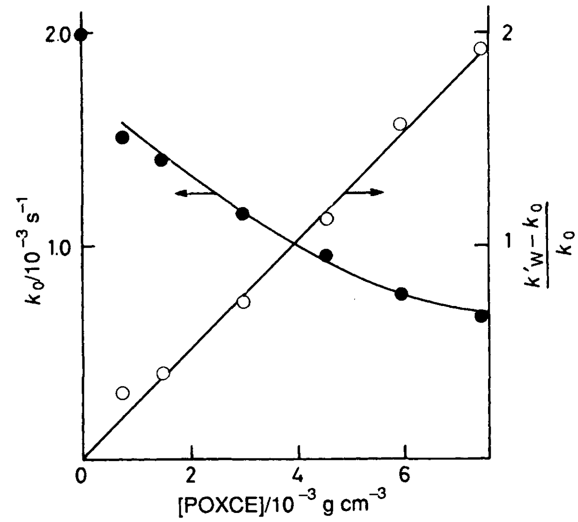


Figure 3. Influence of the concentration of polyoxyethylene 20-cetyl ether upon the pseudo-first-order rate constant for the hydrolysis of MNTS. The linearization of the data according to equation (2) is also shown. [NaOH] 0.0171 mol dm^{-3}, [MNTS]$_0$ 4.9 × 10^{-5} mol dm^{-3}, 12% ethanol (v/v).

Table 2 shows the behaviour found when the surfactant used was TTABr, whose pattern of behaviour is completely analogous to that of CTABr, the reaction rate going through a maximum (at 1.46 × 10^{-2} mol dm^{-3} the rate constant is multiplied by a factor of *ca.* 3). The reason for this behaviour is analogous to that for CTABr and this is again confirmed by the results—also shown in Table 2—in which the effect of increasing bromide concentration at the concentration of TTABr required to achieve the maximum can be seen. These results are again in agreement with the pseudophase ion-exchange model.

(c) *Hydrolysis in the presence of a non-ionic micelle.* The effect of this kind of micelle was studied in the case of polyoxyethylene 20-cetyl ether (POXCE), whose concentration was varied between 7.5 × 10^{-3} and 7.5 × 10^{-4} g cm^{-3}. The addition of surfactant results in a decrease of the reaction rate (see Figure 3) and the experimental data can be satisfactorily fitted to equation (2), with K 243 ± 9 cm^3 g^{-1}, suggesting an effective association of the substrate with the micellar aggregate, the inhibition being due to the absence of reaction of this bound substrate. Since the linearization is good with a value of k'_{W} which corresponds to the reaction in the absence of surfactant, the reason for the inhibition seems to be the absence of

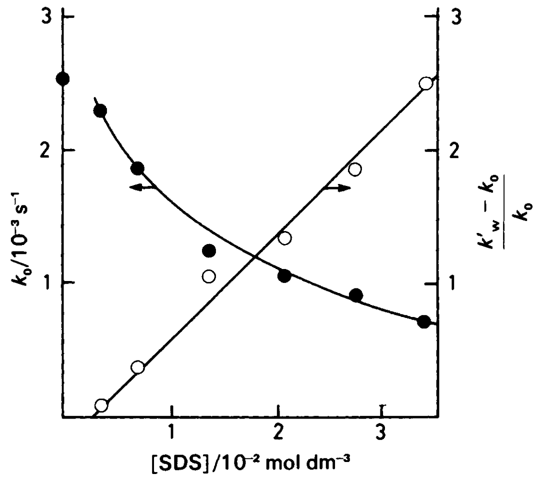


Figure 4. Influence of SDS upon the pseudo-first-order rate constant of reaction between MNTS and SAR. Linearization of the data according to equation (2) is also shown. $[SAR]_t$ 0.0866 mol dm^{-3} (buffer ratio 1.2:1), $[MNTS]_0$ 4.9×10^{-5} mol dm^{-3}, 12% ethanol.

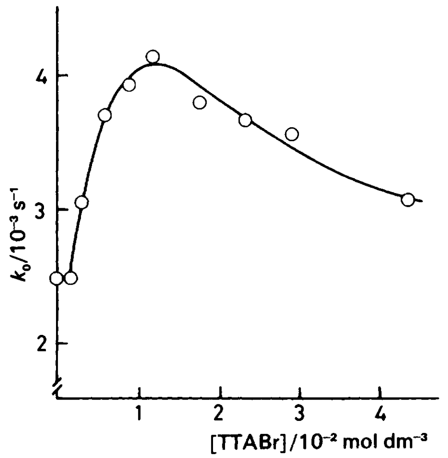


Figure 5. Influence of the concentration of TTABr upon the pseudo-first-order rate constant for the reaction between MNTS and SAR. $[SAR]_{total}$ 0.0866 mol dm^{-3}, $[MNTS]_0$ 4.9×10^{-5} mol dm^{-3}, 12% ethanol.

significant amounts of OH^- bound to the micelle—*i.e.*, $[OH^-]_W = [OH^-]_{total}$.

2. *Micellar Effects upon the Transnitrosation Reaction from MNTS to Sarcosine and Dimethylamine.*—The reaction in which the -N=O group of MNTS is transferred to secondary amines, yielding nitrosamines, was studied using dimethylamine (DMA) and sarcosine (SAR) as the secondary amines. The choice was based upon the different charge of their reactive forms Me_2NH and $MeNHCH_2CO_2^-$, so that the electrostatic effects, essential in the understanding of catalysis or inhibition by micelles, change.

(a) *Reaction with sarcosine* (*SAR*). The reaction between MNTS and SAR was studied with a sarcosine buffer of total concentration 0.0866 mol dm^{-3}, a quotient $[MeNHCH_2CO_2^-]/[MeNH_2^+CH_2CO_2^-]$ 1.2, and 12% ethanol. We studied first the influence of SDS on the reaction rate, and observed that an increase in the concentration of the surfactant resulted in an inhibitory effect similar to that found in the reaction between MNTS and OH^-. This is probably due to the fact that the two nucleophiles bear the same charge, thus leading to the same electrostatic considerations within the pseudophase model. The results obtained are shown in Figure 4, in which we have also drawn the corresponding linearization in the form of equation (2). From this linearization one can obtain a value of $K = 75 \pm 4$ mol^{-1} dm^3, which agrees extremely well with the value of 73 mol^{-1} dm^3 found for the same binding constant obtained from the hydrolysis experiments. It seems therefore that the negative charge of the reactive form of SAR excludes it from the micellar surface, so that the bound substrate cannot react appreciably.

Table 3. Influence of surfactants upon the rate of reaction between MNTS and DMA. $[DMA]_{total}$ 0.0434 mol dm^{-3} (buffer ratio 1:1), $[MNTS]_0$ 4.9×10^{-5} mol dm^{-3}, 12% ethanol (v/v).

Surfactant	[Surfactant]/10^{-4} mol dm^{-3}	$k_0/10^{-3}$ s^{-1}
CTABr	8.83	6.94
CTABr	17.7	6.58
CTABr	35.3	6.06
CTABr	53.0	5.11
CTABr	70.6	4.64
CTABr	88.3	4.26
CTABr	132	3.72
CTABr	166	3.37
SDS	68.2	6.20
SDS	85.7	5.88
SDS	102	5.65
SDS	170	5.59
SDS	239	5.10
SDS	307	4.73
SDS	341	4.86
SDS	511	4.30
SDS	648	4.28
POXCE	7.55 (g cm^{-3})	6.54
POXCE	15.1	5.31
POXCE	30.2	4.68
POXCE	45.3	4.08
POXCE	60.4	3.38
POXCE	75.5	3.22
POXCE	98.1	2.73

On the other hand, when we deal with a cationic surfactant (TTABr) the influence found shows the pattern typical of the reaction between a negatively charged nucleophile and a substrate bound to a cationic micelle. The reaction rate goes through a maximum (at which the reaction rate is increased by a factor of 1.7) and decreases afterwards (see Figure 5) following the characteristic profile predicted by the pseudophase ion-exchange model. The behaviour of MNTS with SAR and OH^- in the presence of micelles is then qualitatively analogous, even though the nucleophiles react at two different sites of the molecule, and is mainly governed by electrostatic considerations.

(b) *Reaction with dimethylamine* (*DMA*). In the case of DMA we worked with a buffer made up with NaOH and dimethylammonium chloride, whose total concentration was 0.0434 mol dm^{-3} and ratio $[Me_2NH]:[Me_2NH_2^+]$ 1:1. The reactive species—unprotonated DMA[1]—is uncharged. The reaction was studied in the presence of anionic (SDS), cationic (CTABr), and non-ionic (polyoxyethylene 20-cetyl ether) surfactants. In all cases, the effect found when increasing the surfactant concentration was inhibitory, as shown in the data collected in Table 3. This behaviour agrees qualitatively, for example, with that found by Behme *et al.*[17] when comparing the effects produced by anionic and cationic micelles upon the aminolysis of *p*-nitrophenyl hexanoate by morpholine (neutral amine) and leucine (negatively charged amine). A quantitative treatment of this inhibition would require a knowledge of the pseudophase partition coefficients for DMA.

Conclusions.—The rate of hydrolysis of MNTS is affected by surfactants in a way that can be understood within the framework of the pseudophase model, non-ionic and anionic surfactants inhibiting the reaction by effectively binding the substrate. This bound substrate does not react appreciably due to the absence of OH^- in the micellar phase. On the other hand, when the surfactant is cationic there is an enhancement of the reaction rate, which passes through a maximum in a pattern of behaviour predicted by the pseudophase ion-exchange model. The transnitrosation reaction from MNTS to secondary amines is also affected by the presence of micelles. A negatively charged amine—sarcosine—shows a pattern of reactivity similar to that of OH^-, whereas all three kinds of surfactant inhibit the reaction between MNTS and a neutral nucleophile—dimethylamine. On the whole, it is important to recognize the important changes in the rate of decomposition of MNTS produced by the presence of micelles in comparison with aqueous conditions and how these changes may be of relevant consequences when estimating the risks associated with nitroso compounds for human beings. Biological membranes and vesicles—similar to micelles—may probably alter the rates of formation and decomposition of these substrates, thus affecting their carcinogenic properties.*

Acknowledgements

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* Tables containing the experimental data corresponding to Figures 1–5 are available from the authors on request.