# Effects of Micellar Aggregates on the Kinetics and Mechanism of the Reaction between 4-Nitrobenzenediazonium Ions and Some Amino Acids

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We have explored the kinetics and mechanism of the reaction between 4-nitrobenzenediazonium ions (4NBD), and the hydrophilic amino acids (AA) glycine and serine in the presence and absence of sodium dodecyl sulfate (SDS) micellar aggregates by means of UV/VIS spectroscopy. The observed rate constants $k_{obs}$ were obtained by monitoring the disappearance of 4NBD with time at a suitable wavelength under pseudo-first-order conditions. In aqueous acid (buffer-controlled) solution, in the absence of SDS, the dependence of $k_{obs}$ on [AA] was obtained from the linear relationship found between the experimental rate constant and [AA]. At a fixed amino acid concentration, $k_{obs}$ values show an inverse dependence on acidity in the range of pH 5–6, suggesting that the reaction takes place through the nonprotonated amino group of the amino acid. All kinetic evidence is consistent with an irreversible bimolecular reaction with $k = 2390 \pm 16$ and $376 \pm 7$ $M^{-1}$ $s^{-1}$ for glycine and serine, respectively. Addition of SDS inhibits the reaction because of the micellar-induced separation of reactants originated by the electrical barrier imposed by the SDS micelles; $k_{obs}$ values are depressed by factors of 10 (glycine) and 6 (serine) on going from [SDS] = 0 up to [SDS] = 0.05M. The hypothesis of a micellar-induced separation of the reactants was confirmed by $^1$H-NMR spectroscopy, which was employed to investigate the location of 4NBD in the micellar aggregate: the results showed that the aromatic ring of the arenediazonium ion is predominantly located in the vicinity of the C($\beta$) atom of the surfactant chain, and hence the reactive $-N_2^+$ group is located in the *Stern* layer of the micellar aggregate. The kinetic results can be quantitatively interpreted in terms of the pseudophase kinetic model, allowing estimations of the association constant of 4NBD to the SDS micelles.

**Introduction.** – Micelles are dynamic aggregates of amphiphilic molecules that create highly anisotropic interfacial regions lining the boundary formed by the highly polar aqueous and nonpolar hydrocarbon regions, imparting new chemical and physical properties to the system [1–6]. Micelles, as well as other association colloids, can act as microreactors concentrating, separating, or diluting reactants, and thereby, they may have substantial effects on chemical reactivity [3][4][7]. Because of their peculiar characteristics, micelles have been extensively exploited as models to investigate the effects of heterogeneous environments and microenvironments on a large variety of reactions, providing relatively simple standard systems for understanding processes that are important for grasping the complex behavior encountered in biological assemblies [8–12].

In this work, we have explored the effects of anionic sodium dodecyl sulfate ($NaOS(=O)_2O(CH_2)_{11}Me$; SDS) micelles on the reaction between 4-nitrobenzenediazonium ($4\text{-}NO_2C_6H_4N_2^+$; 4NBD) ions with the hydrophilic amino acids (AAs) glycine and serine, which are basic constituents of proteins and peptides and usually

located in the outermost part of the protein or peptide surface. The study may be relevant to biochemists because of the well-known mutagenic and carcinogenic activity of arenediazonium ions and because they are employed as specific reagents and probes of protein conformation [13]. In fact, the coupling of diazonium ions with free histidine and tyrosine has been studied as a basis for quantitative investigation of the behavior of proteins [14].

The reaction of $ArN_2^+$ ions with amino acids usually yields triazenes [15–17], which are open chain compounds of the general structure $R^1N{=}N{-}NR^2R^3$, where $R^2$ and $R^3$ are either alkyl or aryl groups and $R^1$ is hydrogen, alkyl, aryl, or acyl. Probably the most widely studied triazenes are of the diaryl (ArN=NNHAr), arylalkyl (ArN=NNHR), and aryldialkyl (ArN=NNRR) type [18]. Of these, the latter two have attracted significant attention because of their mutagenic and carcinogenic properties, *i.e.*, they show biological activity derived from their anticancer potential [19] and their ability to form diazonium salts that can alkylate or arylate DNA [20]. Triazenes are also important in organic chemistry [21] because they have been (and still are) used as protecting groups in natural-product synthesis [22] and combinatorial chemistry [22], and they have been incorporated into polymer [23] and oligomer [24] synthesis and used to form novel heterocycles [25]. *Tracey* and *Shuker* [26] characterized azo coupling adducts of benzenediazonium ions with aromatic peptides and proteins, and *Patt* and *Patt* [27] studied the reaction of 4-[$^{18}$F]fluorobenzenediazonium ions with cysteine to prepare radiolabeled peptides.

A literature survey indicates that there are not many kinetic studies on the reaction of arenediazonium ions with amino acids or proteins in aqueous solution [15–17][28]. Most of the kinetic studies have been carried out in alkaline solution, and contradictory reports appeared. For instance, *Howard* and *Wild* [29] reported that the products of the reaction are pentaza-1,4-dienes ($R^1{-}N{=}N{-}NR^2{-}N{=}N{-}R^3$) , but later investigations by *Remes et al.* [30] and *Mejstrik et al.* [31] claimed that at pH *ca.* 8–10, only triazenes are formed.

In the present work, we have employed slightly acidic aqueous solutions (pH 5–6) to minimize kinetic complications derived from the reactions of $ArN_2^+$ with $OH^-$ ions, which yield diazotates [17]. The 4-nitrobenzenediazonium ion (4NBD) was chosen because there is a substantial knowledge on its thermal decomposition in aqueous acid solution and in the absence or presence of nucleophiles [32–34]. In addition, electron-withdrawing substituents at the aromatic ring enhance the electrophilicity of the arenediazonium ions as opposed to electron-donor substituents such as the Me group, for example. In some instances, the formation of transient diazo ethers (*via* O-coupling reaction), which homolytically decompose by loss of $N_2$, is observed [33–36].

**Results.** – 1. *Spontaneous Decomposition of 4NBD in the Absence and in the Presence of Micellar Aggregates.* Previous kinetic and product-distribution studies on the spontaneous decomposition of 4NBD in acidic aqueous solution (0.01M HCl) show that the reaction follows a $D_N + A_N$ mechanism, *i.e.*, the reaction proceeds through the rate-limiting formation of a highly reactive aryl cation which takes up any nucleophile available in the solvation shell [32]. Because $\pi/\sigma$ acceptor substituents in 4-position destabilize the aryl cation more than they destabilize the parent arenediazonium ion [32][37], the thermal decomposition of 4NBD is very slow, with half-life values $t_{1/2} \approx$

5.5 h at 60°, 4-nitrophenol being the major product of dediazoniation [32][38]. At 30°, $t_{1/2}$ is *ca.* 215 h based on reported activation data [32][39].

Preceding work on dediazoniations in surfactant systems shows that micellar aggregates do not change significantly the rate constants for the spontaneous decomposition of micellar-bounded arenediazonium ions but may have a significant effect on the product distribution because local nucleophile concentrations in the micellar *Stern* layer may be much higher than those in bulk solution [5][40]. Preliminary kinetic experiments (not shown), carried out at 60°, confirmed that addition of SDS makes the spontaneous decomposition of 4NBD slightly faster than in its absence; estimated half-lives for the reactions are $t_{1/2}$ *ca.* 5 h when $[SDS] = 2.0 \cdot 10^{-3}$ M and $t_{1/2}$ *ca.* 4.8 h when $[SDS] = 5 \cdot 10^{-2}$ M.

2. *Reaction of 4NBD with Glycine and Serine in the Absence of SDS. Fig. 1* shows the UV/VIS spectrum obtained at different time intervals for the reaction of 4NBD and glycine showing the decrease in the absorbance of the 4NBD absorption band at $\lambda$ 260 nm and the evolution of the absorbance at $\lambda = 370$ nm due to triazene formation; at the latter wavelength, the absorbance decreases slowly after reaching a maximum value. Similar spectral changes were obtained when employing serine instead of glycine. All runs were done under pseudo-first-order conditions, and the observed rate constant $k_{obs}$ for each reaction was obtained by monitoring the disappearance of the absorption at $\lambda$ 260 nm with time and by fitting the corresponding pairs of data to the integrated first-order equation, as indicated in the *Exper. Part.*

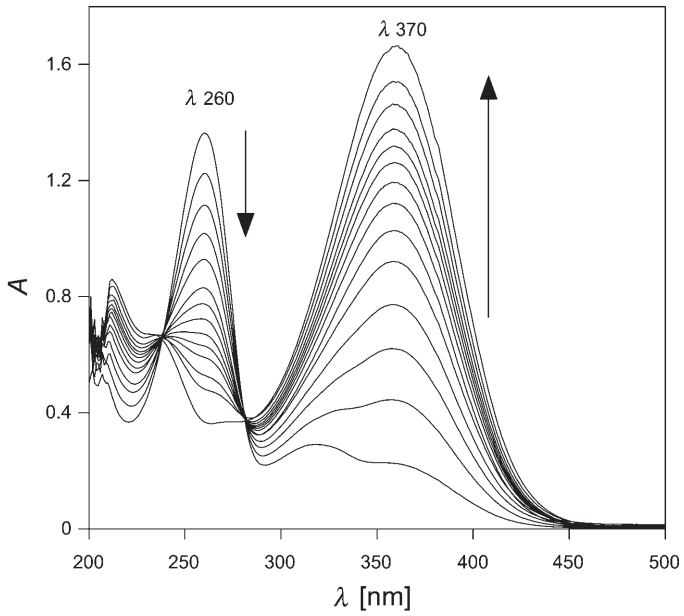



Fig. 1. *Representative Spectra Obtained at Different Times for the Reaction between 4NBD and Glycine in Aqueous Buffered Solution*. The arrows show the disappearance of the main absorption band of 4NBD ($\lambda$ 260 nm) and the new band, associated to the formation of the corresponding triazene ($\lambda$ 370 nm). $[4NBD] = 1.26 \cdot 10^{-4}$ M, $[glycine] = 2 \cdot 10^{-3}$ M, *Britton–Robinson* buffer (pH 7.03), 30°.

The effects of acidity on the reaction were analyzed in aqueous buffered solutions by determining the effects of acidity on $k_{obs}$ at constant 4NBD and amino acid concentrations. Upon increasing the acidity, $k_{obs}$ values are depressed, and upward bent profiles were found. The plots of $\log(k_{obs})$ *vs.* pH are linear (*Fig. 2,a*), with slopes of $1.03 \pm 0.05$ (glycine) and $1.13 \pm 0.05$ (serine), reflecting an inverse dependence of $k_{obs}$ with $[H^+]$. Changes in buffer concentration or buffer nature did not result in significant variations of $k_{obs}$, suggesting the possibility of a specific-acid-catalyzed reaction to be unlikely.

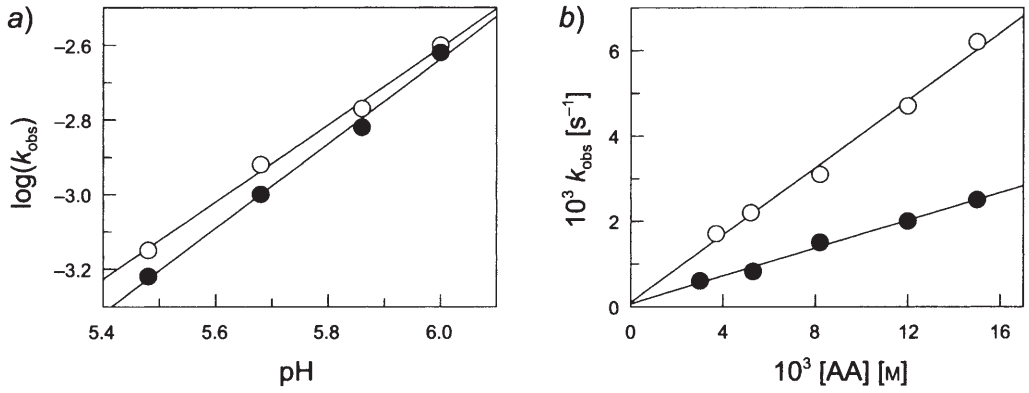



Fig. 2. a) *Change of log($k_{obs}$) with pH for the reaction between 4NBD and glycine* (○) *and serine* (●) *in buffered aqueous solutions.* Linear equations obtained by fitting the experimental data: $\log(k_{obs}) = (-8.8 \pm 0.3) + (1.03 + 0.05)$ pH for glycine, and $\log(k_{obs}) = (-9.4 \pm 0.3) + (1.13 + 0.05)$ pH for serine. b) *Effects of amino acid concentration on $k_{obs}$ for the reaction with 4NBD in aqueous buffered solution* (pH 5.86). ○ Glycine, ● serine. $[4NBD] \approx 2 \cdot 10^{-4}$ M, $[glycine] = 3.7 \cdot 10^{-3}$ M, $[serine] = 8.2 \cdot 10^{-3}$ M, 30°.

Amino acids have two ionization constants, the first one ($pK_a(1) \approx 2.3$) corresponds to the ionization of the carboxylic group and the second one ($pK_a(2) \approx 9$) is associated to the ionization of the amino group. At pH 2 and $[AA] \sim 4 \cdot 10^{-3}$ M, $k_{obs}$ values approach that for the thermal decomposition of 4NBD, and thus the observed acid dependence excludes a hypothetical reaction at the O-atoms (O-coupling reaction) of the carboxylic acid, which is partially ionized at pH 2, a reaction which otherwise is usually considered unlikely [15]. Therefore, the reaction between 4NBD and glycine and serine mainly takes place through the nonprotonated amino group of the amino acids, in keeping with previous results from similar reactions [30][31].

At a fixed pH, $k_{obs}$ values increase linearly upon increasing the amino acid concentration, *Fig. 2,b*, with slopes of $0.39 \pm 0.02$ (glycine) and $0.16 \pm 0.01$ (serine). In both cases, the intercepts are negligible, $(9 \pm 20) \times 10^{-5}$ (glycine) and $(20 \pm 6) \times 10^{-5}$ (serine), denoting the irreversibility of the process. In all cases, correlation coefficients higher than 0.998 were obtained.

3. *Location of 4NBD in the SDS Micellar Aggregates.* On the basis of electrostatic considerations, one would expect some incorporation of 4NBD ions into the SDS micellar aggregates because 4NBD ions bear a positive charge but are somewhat hydrophobic, while the surface of SDS micelles is negatively charged. Because arenediazonium ions have been proved to be very sensitive to their environment

[17][34][38][41], proper understanding of their behavior in micellar phases requires knowledge of their location in the micelle. A suitable method for this is $^1$H-NMR spectroscopy, which allows aromatic rings to be located by the upfield shift induced by the ring current (ring-current effect) on the signals of neighboring H-atoms of the surfactant, an effect which depends upon the distance between the ring and the corresponding H-atoms [42]. The method has already been employed to locate diazonium ions [43] and a number of compounds containing benzene rings in micellar aggregates [44]. $^1$H-NMR Signals were assigned on the basis of their position, multiplicity, integration, and previous published data [42]. We found that the greatest upfield shift is observed for the H-atoms at the C($\alpha$) and C($\beta$) atoms of the aliphatic chain of SDS, with $\delta_{SDS} - \delta_{SDS/4NBD} = 0.0680$ (H–C($\alpha$)), 0.2005 (H–C($\beta$)), and 0.0201 (H–C($\gamma$)). It follows, therefore, that the incorporation of 4NBD in the SDS micelles takes place in a way that the aromatic ring of the arenediazonium is predominantly located in the neighborhood of these C-atoms, and thus the reactive $-N_2^+$ group is expected to be close to the micellar surface (*Fig. 3*).

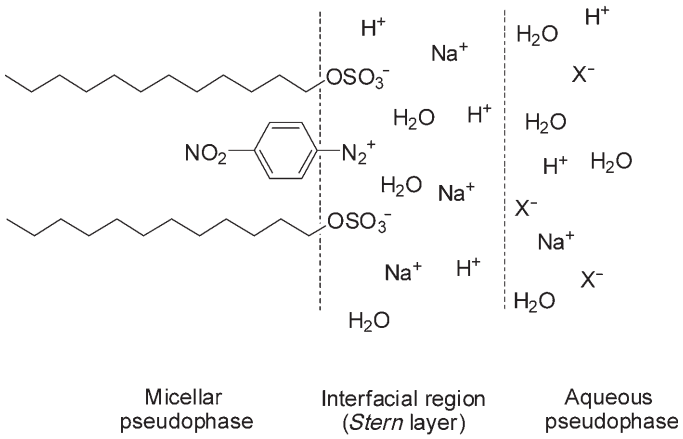



Fig. 3. *Basic two-dimensional representation of a SDS micelle illustrating the location of 4NBD in the aggregate.* Counter-ions ($H_3O^+$, $Na^+$), neutral ($H_2O$), and ionic ($X^-$) nucleophiles are also shown. Anionic nucleophiles are mostly excluded from the *Stern* layer because of the electrical repulsion due to the sulfate head groups.

4. *Sodium Dodecyl Sulfate Micellar Effects on the Reaction between 4NBD and Amino Acids. Fig. 4*,*a* and *b* show the effects of [SDS] on the reaction between 4NBD and glycine and serine, respectively. All runs were done in *Britton–Robinson*-buffer aqueous solutions by using a fixed concentration of the amino acid. In all cases, $k_{obs}$ values decrease upon increasing [SDS] by a factor of 10 (glycine) on going from [SDS] = 0 up to [SDS] = 0.15M and by a factor of 6 (serine) on going from [SDS] = 0 up to [SDS] = 0.05M. Data in *Fig. 4* suggets that $k_{obs}$ values approach asymptotically a limiting value which is independent of the employed pH.

**Discussion.** – In the absence of surfactants, $k_{obs}$ values increase linearly upon increasing the amino acid concentration with intercepts close to zero (*Fig. 2*,*b*), and its

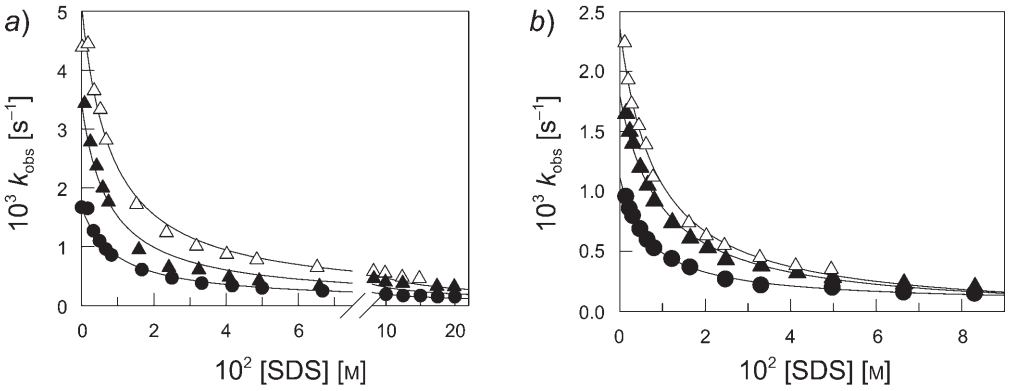
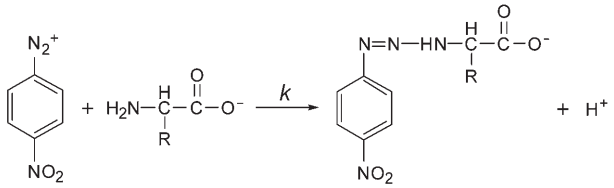



Fig. 4. *Effects of [SDS] on the reactivity of 4NBD with* a*) glycine and* b*) serine at different acidities.* ● pH 5.5, ▲ pH 5.86, △ pH 6.0. [4NBD] $\approx 2 \cdot 10^{-4}$ M, [glycine] = [serine] = $1.7 \cdot 10^{-2}$ M. Solid lines are the theoretical curves obtained by fitting the experimetal data to *Eqn. 4.*

variation with the acidity shows an inverse relationship with $[H^+]$ (*Fig. 2,a*). These results can be accommodated by assuming a bimolecular reaction between 4NBD and the non-protonated amino group of the amino acid (*Scheme 1*). Neglecting the spontaneous decomposition of 4NBD, the rate of disappearance of 4NBD is given by *Eqn. 1*, where $k$ stands for the second-order rate constant for the N-coupling reaction, and $[RNH_2]$ represents the concentration of $\alpha$-amino carboxylate. Bearing in mind the corresponding mass balance for the amino acid and the fact that first-order conditions were applied, the observed rate constant for 4NBD loss is given by *Eqn. 2*, wherein $K_a(2)$ represents the second ionization constant of the amino acid in the zwitterionic state, $[H^+]$ the proton concentration, and $[AA]_T$ the stoichiometric concentration of the amino acid in solution.

Scheme 1. *Proposed Reaction between Arenediazonium Ions and Amino Acids*




$$-\frac{d[4NBD]}{dt} = k[4NBD][RNH_2] \quad (1)$$

$$k_{obs} = \frac{kK_a(2)[AA]_T}{K_a(2) + [H^+]} \quad (2)$$

*Eqn. 2* predicts that, for a given $[H^+]$, the change of $k_{obs}$ with $[AA]_T$ should be linear with intercept at zero and a slope equal to $kK_a(2)/(K_a(2) + [H^+])$ as found experimentally, see *Fig. 2,b*. By fitting the experimental data in *Fig. 2, b*, to a linear

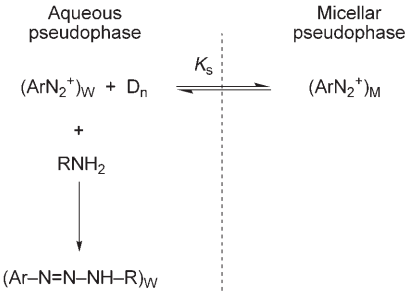
equation, $k = 2390 \pm 16$ and $376 \pm 7$ $M^{-1}$ $s^{-1}$ for glycine ($pK_a(2) = 9.60$) and serine ($pK_a(2) = 9.15$), respectively, can be obtained.

Alternatively, *Eqn. 2* predicts that for those cases where $[H^+] >>> K_a(2)$, $k_{obs}$ should decrease upon increasing $[H^+]$, *i.e.*, the variation of $\log(k_{obs})$ with pH should be linear with a slope of unity, as it was found experimentally, *Fig. 2,a*. Thus, in the absence of SDS, all kinetic evidence is consistent with a nucleophilic attack of the nonprotonated amino group of the amino acid on 4NBD.

Micellar aggregates can modify chemical reactivity essentially in two ways, namely *i*) by governing the contact of reactants with other substrates incorporated in the micelle, with water or with ions present as counterions and co-ions in the *Stern* layer, and *ii*) by governing the polarity of their immediate vicinity [22][23].

Since 4NBD ions are cations with a hydrophobic part, they will be distributed between the aqueous and micellar pseudophases (*Scheme 2*). This assumption is consistent with the $^1$H-NMR results showing that the associated 4NBD ions have their reactive $-N_2^+$ group located close to the micellar surface, with the aromatic residue predominantly located in the vicinity of the C($\alpha$) and C($\beta$) atoms of the surfactant chains. On the other hand, heterolytic dediazoniations are rather insensitive to changes in solvent polarity, and thus no significant changes in $k_{obs}$ are expected on going from the bulk aqueous environment to the micellar environment as it was found experimentally [17][43][45].

Scheme 2. *Proposed Reaction between 4NBD* ($ArN_2^+$) *and Amino Acids* ($RNH_2$) *in the Presence of Anionic SDS Micellar Aggregates Showing the Distribution of 4NBD between the Aqueous* (W) *and Micellar* (M) *Pseudophases.* $D_n$ ($[D_n] = [SDS_T] - CMC$) stands for the micellized surfactant [1][4] ($CMC$ = critical micelle concentration), $K_s$ is the association constant of 4NBD to the micellar aggregate, and $RNH_2$ stands for the carboxylate form of the amino acid. For the sake of simplicity, the different ionization equilibriums of the amino acid are not indicated as well as the spontaneous decomposition reaction of 4NBD in the aqueous and micellar pseudophases.




Qualitatively, the observed inhibition shown in *Fig. 4* can be explained according to *Hartley*'s rules [1][3][46]. As 4NBD is positively charged and bears a hydrophobic aromatic part, it will be incorporated into the micellar aggregates upon increasing the SDS concentration, while one would expect that the amino acids will not significantly associate to the micellar aggregates because they are hydrophilic and, moreover, because at the working acidities, the carboxylic group of the amino acid is completely ionized ($pK_{a1} \approx 2.3$) and thus negatively charged. Hence, the amino acids are almost

excluded from the *Stern* layer, decreasing significantly their local concentration in the vicinity of 4NBD.

Quantitatively, the results in *Fig. 4* can be interpreted in terms of the pseudophase model [1][4][47] (*Scheme 2*), which assumes that the micelles are uniformly distributed in the aqueous phase and may act as a separate phase, the so-called pseudophase, where the reaction may take place and where the substrates may be incorporated at nearly diffusion-controlled rates. Assuming that the micellized surfactant is at thermal equilibrium with solutes throughout the reaction, and recognizing that $k_{obs}$ values for the spontaneous decomposition of 4NBD in the absence and in the presence of SDS micellar aggregates are negligible compared to those in the presence of amino acids, the rate of disappearance of 4NBD is given by *Eqn. 3*, where $[4NBD]_w$ represents the concentration of 4NBD in the aqueous pseudophase. Bearing in mind the distribution equilibrium of 4NBD (*Scheme 2*), and that pseudo-first-order conditions were applied, the observed rate constant $k_{obs}$ is given by *Eqn. 4*, where $K_s$ is the association constant of 4NBD to the micellar aggregate, $D_n$ is the micellized surfactant, whose concentration is defined as $D_n = [SDS]_T - CMC$, *CMC* being the critical micelle concentration [1][4]. Note that when no micelles are present (*i.e.*, $D_n = 0$) *Eqn. 4* is reduced to *Eqn. 2*, and thus $k[RNH_2]$ represents the observed rate constant in the absence of surfactant. Denoting $k_w = k[RNH_2]$, *Eqn. 4* can be rearranged to *Eqn. 5*, which predicts that a plot of $(k_w - k_{obs})/k_{obs}$ *vs.* $[SDS]_T$ should be linear with a negative intercept. *Fig. 5* shows that such a prediction is fulfilled, and therefore, the assumption of a micellar-induced separation of reactants seems likely. By linear square fitting of the data, average values of $K_s = 150 \pm 6\ M^{-1}$ and $CMC = (7.0 \pm 0.5) \cdot 10^{-4}$ M can be obtained.

$$-\frac{d[4NBD]}{dt} = k[4NBD]_w[RNH_2] \tag{3}$$

$$k_{obs} = \frac{k[RNH_2]}{1 + K_s[D_n]} \tag{4}$$

$$\frac{k_w - k_{obs}}{k_{obs}} = k_s[SDS]_T - K_s \cdot CMC \tag{5}$$

The $K_s$ values are very similar to those obtained for other arenediazonium ions [43] but lower than that determined by employing a derivatization method [45]. Such a value indicates that, even at low surfactant concentrations (above the *CMC*), a substantial fraction of 4NBD ions are incorporated to the micellar aggregate, for instance when $[SDS] = 0.05$M, *ca.* 80% of the total 4NBD is incorporated in the micellar aggregate, thus decreasing the effective concentration in the aqueous pseudophase, which is the region where the amino acids are mainly located. The obtained *CMC* value is lower than that in pure aqueous solution, $CMC = 8 \cdot 10^{-3}$ M at 25°, consistent with the well-known effects of electrolyte concentration on *CMC* values [1][48].

It is apparent, therefore, that micelles provide an environment that prevents triazene formation because of their ability to separate reactants due to the electrical barrier imposed by the surface of the SDS micelles.

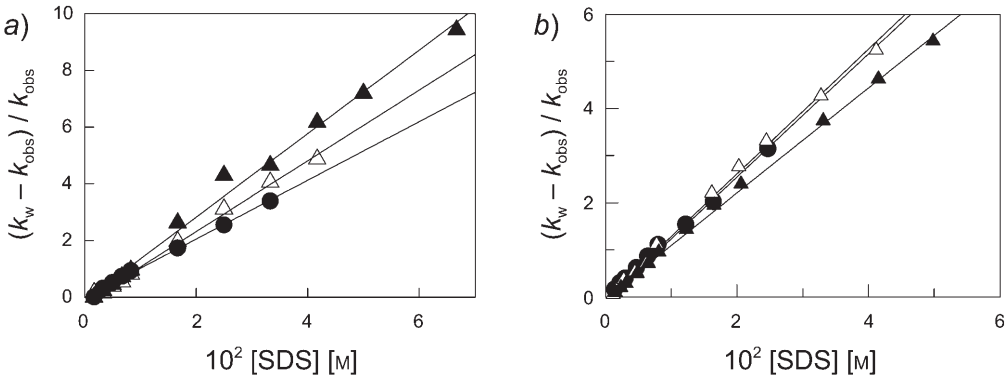



Fig. 5. *Linear plots according to* Eqn. 5 *for* a*) glycine and* b*) serine.* ● pH 5.5, ▲ pH 5.86, △ pH 6.0. Exper. conditions as in *Fig. 4*.

**Conclusions.** – We have analyzed the reaction between 4NBD and the amino acids glycine and serine in the absence and in the presence of an anionic surfactant. In the absence of surfactant, the reaction takes place by nucleophilic attack of the non-protonated amino group of the amino acid on 4NBD, and hence the reaction only takes place in alkaline or slightly acidic solutions, where substantial amounts of the $\alpha$-amino carboxylate form are present. In the presence of SDS micelles, the reactants are separated because of the electrical barrier imposed by the micellar surface which makes the amino acids to be located primarily in the aqueous pseudophase. $^1$H-NMR Experiments showed that the aromatic residue of 4NBD is mainly located in the vicinity of the C($\alpha$) and C($\beta$) atoms of the hydrocarbon chain of the surfactant, while a substantial fraction of 4NBD ions are incorporated to the micellar aggregate.

A similar inhibition is expected if cationic surfactants such as cetyltrimethylammonium halides (CTAX) are employed instead of SDS. The electrical nature of CTAX micelles, whose head groups $-(Me)_3N^+$ are positively charged, makes the $\alpha$-amino carboxylate form of the amino acid to be associated to the micellar aggregates but will exclude 4NBD ions from the *Stern* layer because of the electrical repulsion. The global effect should be again an effective separation of reactants and hence a decrease of the local concentration of the reactive form of the amino acid in the vicinity of the 4NBD ions.

Incorporation of substrates to micellar aggregates is mainly governed by the balance of electrostatic and hydrophobic forces [1][49]. The electrical barrier imposed by the micelles could be overwhelmed, therefore, by increasing the hydrophobicity of the substrates, namely that of the amino acid when using anionic surfactants or that of the arenediazonium ion when employing cationic surfactants. In such a situation, concentration effects may be important leading to micelle-catalyzed reactions. They deserve further investigations which are in due course and will be part of a future report.

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## Experimental Part

*General.* Reagents were of maximum purity available and were used without further purification. The surfactant sodium dodecyl sulfate (SDS; 99.9%), the reagents used in the preparation of the *Britton–Robinson* buffer ($H_3BO_3$, $H_3PO_4$, and AcOH, final concentration 0.05M), and those employed in the preparation of 4NBD were purchased from *Aldrich*. Other materials employed were from *Riedel de Haën*. All solns. were prepared by using *Milli-Q*-grade water. The pH was measured with a previously calibrated *Metrohm 713* pH meter equipped with a temp. sensor. UV/VIS Spectra: *Beckman DU-640-UV-VIS* spectrophotometer, equipped with a thermostated cell carrier attached to a computer for data storage; $\tau_{max}(\varepsilon)$ in nm. $^1$H-NMR Experiments: *Bruker 400* spectrometer; at r.t. with solns. in 99.9% $D_2O$; $\delta$ in ppm; $I$, in Hz.

*4-Nitrobenzenediazonium Tetrafluoroborate* (4NBD · $BF_4^-$). The 4NBD was prepared as tetrafluoroborate under nonaqueous conditions as described elsewhere [50], recrystallized three times from MeCN/cold $Et_2O$, stored in the dark at low temp. to minimize decomposition, and recrystallized periodically. UV/VIS (aq. $2.0 \cdot 10^{-3}$ M HCl): 260 (16450), 310 (sh); the *Lambert–Beer* law for 4NBD was also fulfilled in presence of SDS. $^1$H-NMR ($CD_3CN$, 25°): 8.70 (*d*, $J = 7$, 2 H); 8.86 (*d*, $J = 7$, 2 H); in agreement with [32]. Stock solutions of 4NBD were prepared by dissolving the diazonium salt in aq. HCl soln. to minimize diazotate formation, and they were prepared and used immediately or stored at low temp. in the dark to minimize their decomposition and renewed frequently.

*Kinetics.* Kinetic data were obtained UV/VIS-spectrophotometrically by following the integrated method. Observed rate constants were obtained by monitoring the disappearance of 4NBD at a suitable wavelength. Absorbance/time data were fitted to the integrated first-order *Eqn. 6* by using a nonlinear least-squares method provided by a commercial computer program, where $A_t$, $A_0$ and $A_\infty$ are the measured absorbance at any time $t$, at $t = 0$, and at infinite time, respectively. Runs were done at $30 \pm 0.1°$ under pseudo-first-order conditions ($[ArN_2^+] << [\text{amino acid}]$) at constant ionic strength. The good agreement between the optimized and experimental $A_\infty$ value confirmed that reactions were first-order with respect to the amino acid.

$$\ln \frac{A_t - A_\infty}{A_0 - A_\infty} = -k_{obs} t \tag{6}$$

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