Micellar Effects on the Reaction between an Arenediazonium Ion and the Antioxidants Gallic Acid and Octyl Gallate

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The effect of sodium dodecyl sulfate (SDS) micelles on the reaction between the 3-methylbenzenediazonium (3MBD) ion and either the hydrophilic antioxidant gallic acid (GA) or the hydrophobic analogue octyl gallate (OG) have been investigated as a function of pH. Titration of GA in the absence and presence of SDS micelles showed that the micelles do not alter the first ionization equilibrium of GA. Analysis of the dependence of the observed rate constant (k_{obs}) with pH shows that the reactive species are GA^{2-} and OG^{-}. Kinetics results in the absence and presence of SDS micelles suggest that SDS aggregates do not alter the expected reaction pathway. SDS Micelles inhibit the spontaneous decomposition of 3MBD as well as the reaction between 3MBD and either GA or OG, and upon increasing the SDS concentration, with k_{obs} approaching the value for the thermal decomposition of 3MBD in the presence of SDS. Our results are consistent with the prediction of the pseudophase model and show that the origin of the inhibition for the reaction with GA is different to that for the reaction with OG; in the former case, the observed inhibition can be rationalized in terms of the micelle-induced electrostatic separation of reactants in the micellar *Stern* layer, whereas the observed inhibition in the reaction with OG is a consequence of the dilution effect caused by increasing SDS concentration, decreasing the local OG^{-} concentration in the *Stern* layer.

1. Introduction. – Reactions of natural reducing agents with arenediazonium ions, ArN_2^+, have attracted substantial attention in recent years from a biochemical point of view, because antioxidants reduce ArN_2^+ ions *via* one-electron-transfer processes [1–4] to aryl radicals, which are thought to be tumorigenic or to react with important cellular constituents to generate mutagenic and carcinogenic products, or by undergoing O-coupling reactions leading to highly unstable diazo ethers, Ar–N=N–OR, which may split homolytically [5–7]. Other studies, however, point to the genotoxicity of ArN_2^+ as a whole, or a combination of ArN_2^+ and $Ar^{\bullet}$ [1–3][8–12].

Among others, gallic acid (= 3,4,5-trihydroxybenzoic acid; GA), which can be considered as the simplest prototype of vegetable tannins, is habitually used as a natural reducing agent [13–15]. Tannins constitute a class of natural polyphenolic compounds widely distributed in fruits and plants. Their chemical and biochemical properties have been investigated in detail, because they are significantly present in human diets [16–20]. Esters of GA are also widely used as antioxidant additives in both food and pharmaceutical industry, *e.g.*, in the form of E310 (propyl gallate), E311 (octyl gallate), and E312 (dodecyl gallate), to protect important organic and biological molecules against oxidative degradation responsible for a number of chronic health problems such as aging, cancer, atherosclerosis, *etc.*, as well as to prevent lipid peroxidation

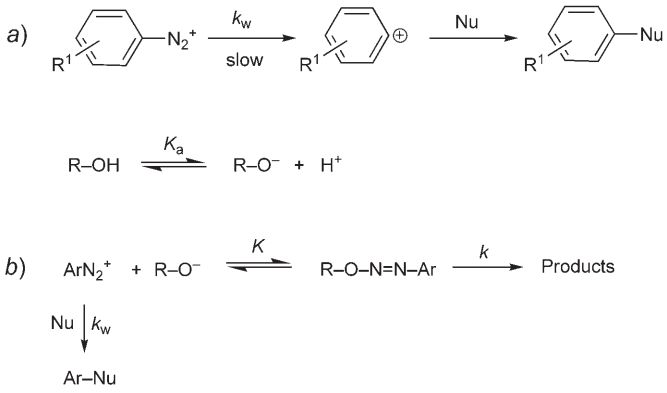
generating rancid odors and flavors in food systems, producing a significant decrease in food quality and safety [21–27]. In addition to the protection against lipid peroxidation, GA may also react with other components in the system such as trace-metal ions to yield active oxygen species that are dangerous pro-oxidants [19].

The biological activity and the efficiency of a particular antioxidant in multiphasic food or biological systems depends on a variety of factors, including the location of the antioxidant in the different phases [23][24][26][28]. The multifunctional environment created by micellar solutions has been used in many areas of chemistry for analysis, control of reactivity, and as model for biological membranes or other biological systems [29–31]. Among them, sodium dodecyl sulfate (SDS) micelles are often used as model system for testing antioxidant activity [21][24][32][33].

In this work, we will explore the effects of substrate compartmentalization on the kinetics of the reaction between 3-methylbenzenediazonium ions (3MBD) and either hydrophilic GA or hydrophobic octyl gallate (OG) in SDS micellar aggregates as a simple membrane-mimetic system.

3MBD was chosen because a substantial knowledge on its reactions in acidic alcohol/H_2O mixtures is available; its spontaneous decomposition takes place through a $D_N + A_N$ mechanism, *i.e.*, rate-limiting formation of a highly reactive aryl cation that immediately reacts with any nucleophile available in its solvation shell (*Scheme 1*,*a*) [34][35]. In addition, we recently investigated the reaction with methyl gallate, concluding that this reaction takes place through the formation of an unstable O-coupling adduct that further decomposes (*Scheme 1*,*b*) [36]. Similar O-coupling mechanisms have been proposed before for the reaction between a number of ArN_2^+ ions with neutral nucleophiles such as MeOH [7] and EtOH [37], cationic nucleophiles such as ascorbate ions [4][6][38][39], and with polyalcohols in systems of restricted geometry [5]. The results of the present study should contribute to bridging the gap between the chemical and biological antioxidant activity by exploring the effects of the

Scheme 1. a) *Currently Accepted $D_N + A_N$ Mechanism for the Spontaneous Dediazoniation of Arenediazonium Ions.* b) *Competitive $D_N + A_N$ and O-Coupling Mechanisms Leading to the Formation of Unstable Diazo Ethers.* The O-coupling reaction may also take place with neutral R–OH nucleophiles followed by loss of H^+ [7][37].



micro-environment in which the antioxidant is located, as studied by means of membrane-mimetic systems.

2. Results. – 2.1. *Micellar Effects on the Spontaneous Dediazoniation of the 3-Methylbenzenediazonium Ion in the Absence of Antioxidants.* Previous kinetics and product-distribution studies on the spontaneous decomposition of 3MBD in acidic aqueous solution (0.01M aq. HCl), in the *absence* of micellar aggregates, showed that the reaction follows a $D_N + A_N$ mechanism [40] *via* rate-limiting formation of a highly unstable aryl cation that further reacts with any nucleophile in the solvation shell (*Scheme 1,a*), with a half-life, $t_{1/2}$, of *ca.* 30 min at a temperature of 30°. High-performance-liquid-chromatography (HPLC) analyses of the reaction mixtures showed that *m*-cresol (= 3-methylphenol) is the major product (> 95% yield) of this reaction [34].

Upon addition of SDS, the observed rate constants, k_{obs}, for the spontaneous decomposition of 3MBD are slightly depressed (*Fig. 1*), with k_{obs} decreasing by *ca.* 30% on increasing the SDS concentration from 0 to 0.1M. Because 3MBD ions are cationic and somewhat hydrophobic, they should distribute between the aqueous and micellar pseudophases, as shown in *Scheme 2* (horizontal process). Thereby, the rate of the reaction is equal to the sum of rates of concurrent reactions in each pseudophase [41][42]. In the absence of antioxidant, the observed rate constant, k_{obs}, is given by *Eqn. 1:*

$$k_{obs} = \frac{k_W + k_M K_s D_n}{1 + K_s D_n} \quad (1)$$

where k_W and k_M refer to the rate constants in the aqueous (W) and micellar (M) pseudophases, and where K_s is the association constant of 3MBD; $D_n = [SDS] - cmc$ stands for the micellized surfactant, *cmc* being the critical micelle concentration, which is 8 mM in neat aqueous solution at 25° [43].

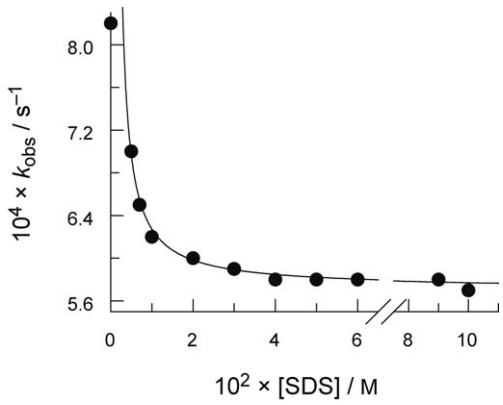
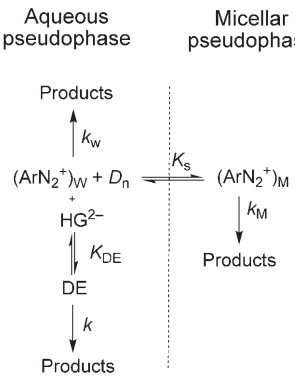


Fig. 1. *Effects of SDS concentration on the spontaneous decomposition of 3MBD.* Conditions: [3MBD] = 10^{-4} M, T = 30°, pH 2.



Scheme 2. *Proposed Mechanism for the Reaction between 3MBD and GA in SDS Micellar Solution under Spontaneous Decomposition of 3MBD, Both in the Aqueous and Micellar Pseudophases, as well as Formation of the Presumed Diazo Ether in the Aqueous Pseudophase.* The ionization equilibrium of GA is not indicated for the sake of clarity.

By fitting the data to *Eqn. 1*, values of $K_s = 200 \pm 12$ M^{-1} and $k_M = (5.7 \pm 0.3) \times 10^{-4}$ s^{-1} for 3MBD were estimated. The K_s value is similar to that obtained previously by other methods [44], and shows that even at low surfactant concentration a significant fraction of 3MBD ions are present in the micellar aggregate. For instance, for [SDS] = 0.1M, *ca.* 95% of the total 3MBD ions are associated to the micellar aggregates.

HPLC Product-distribution analysis of the samples obtained after completion of the reaction indicated that a quantitative conversion to the phenol derivative was achieved; no extraneous chromatographic peaks that could eventually be associated to reduction products were detected [44]. Formation of ArOH in the micellar *Stern* layer is consistent with the current view of micelles as water-permeated structures [45], and is also consistent with results of previous investigations on the location of arenediazonium ions in micellar systems, in which it was concluded that 3MBD is located in the neighborhood of the $C_\alpha - C_\beta$ atoms of the surfactant chain, the reactive $-N{=}N^+$ group being located very close to the micellar surface [44].

2.2. *Effects of Acidity on the Observed Rate Constant for the Reaction of the 3-Methylbenzenediazonium Ion with Gallic Acid and Octyl Gallate.* Gallic acid (GA) behaves as a weak acid, with pK_a(1) and pK_a(2) values of 4.3 and 8.5 for the ionization of the COOH and one of the OH groups of the pyrogallol moiety, respectively. Therefore, electrophilic attack of 3MBD at the O-atom of the carboxylate group of GA is theoretically possible. To test this hypothesis and to analyze the dependence of k_{obs} as a function of the acidity of the medium, we investigated the pH dependence of k_{obs} and the effect of pH on the reaction with GA and octyl gallate (OG).

In *Fig. 2*,*a* and *Fig. 2*,*b*, the effect of pH on k_{obs} is shown for the reaction with GA, and in *Fig. 2*,*c* and *Fig. 2*,*d* the corresponding plots are depicted for the reaction with OG. In both cases a variation of k_{obs} with pH was found, with a clear upward bend, k_{obs} increasing approximately 10-fold on going from pH 3.5 to 5.1 (GA), and increasing approximately 14-fold on going from pH 4.5 to 6.0 (OG). At pH < 3.5, k_{obs} values approach that for the thermal decomposition of 3MBD: $k_{obs} = (4.0 \pm 0.2) \times 10^{-4}$ s^{-1}. Note that no studies were performed at pH > 6 to minimize complications due to GA and OG autoxidation, which may be important in alkaline solution [27][46], and to minimize side reactions of ArN_2^+ with OH^-, which may lead to the formation of

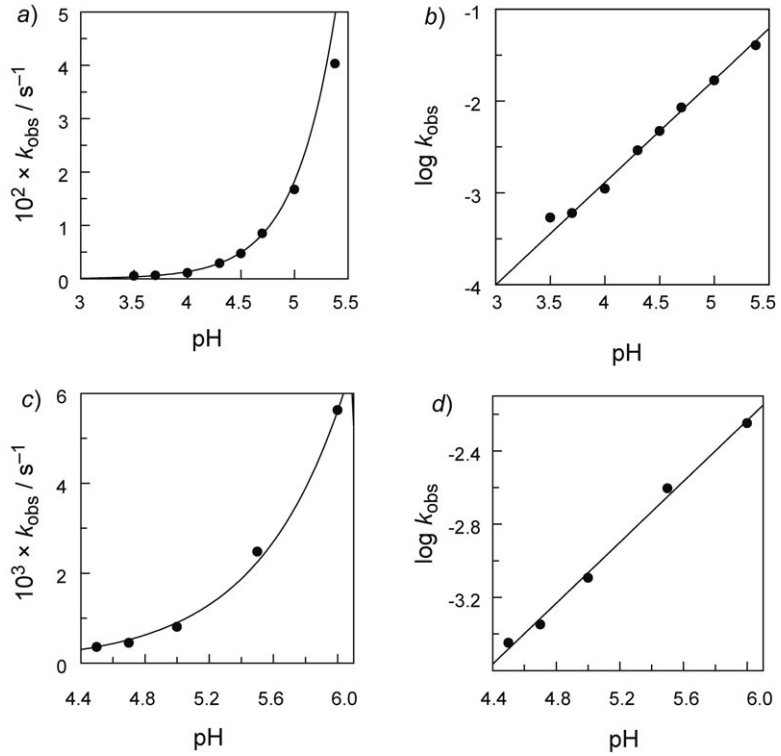


Fig. 2. a) *and* c) *Variation in* k_{obs} *upon changing the pH*. An upward-bent profile for GA (*a*) and OG (*c*) is observed. b) *and* d) *Plot of log* k_{obs} vs. *pH for GA* (*b*) *and OG* (*d*). A linear relationship with slopes close to unity, indicative of a inverse relationship between k_{obs} and $[H^+]$, are found. Conditions: [3MBD] = 0.1 – 0.3 mM, [GA] = 40 mM, [OG] = 20 mM. Reactions with OG were carried out in 500 mM aq. SDS micellar soln. at 30°.

diazotates [10]. The variations of log k_{obs} with pH were found to be linear for both GA and OG, as shown in *Fig. 2*, *b* and *Fig. 2*, *d*, respectively, with slopes of 1.12 ± 0.04 (GA) and 0.87 ± 0.03 (OG), indicating an inverse dependence of k_{obs} with $[H^+]$.

2.3. *Micellar Effects on the First Acidity Constant of Gallic Acid.* Reactions of ArN_2^+ with $RCOO^-$ are known, and they proceed, as most O-coupling reactions, through an equilibrium step. However, this equilibrium lies very much on the side of the starting ions ($K = 10^{-5}$ M^{-1}) [10][47][48]; therefore, a hypothetical O-coupling reaction should be negligible under the present experimental conditions. This point was, nevertheless, investigated, because micellar solutions may shift the acid – base equilibrium of dyes and weak acids, and an eventual shift in the pK_a values of GA may lead to significant changes in the concentration of the reactive species.

The method of choice was spectrophotometric titration, by monitoring the pH-induced change in the absorbance of GA at 271 nm. As can be seen in *Fig. 3*, a typical sigmoidal profile was obtained by fitting the data to the *Henderson – Hasselbach* equation, giving rise to a pK_a(1) value of 4.21 ± 0.03 at 30° in the absence of SDS, in

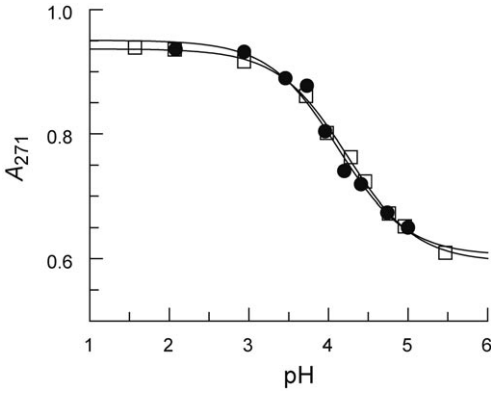


Fig. 3. *Determination of the* pK_a *of GA in aqueous acid* (□) *and in micellar solution* (●). Conditions: [SDS] = 0.25M, [GA] = 10^{-4} M, $T = 30°$. The solid lines were obtained by fitting the exper. data to the *Henderson – Hasselbach* equation.

close agreement with literature data: p$K_a(1) = 4.4$ [49] or 4.16 [50] at 25°. In the *presence* of 0.25M SDS, a p$K_a(1)$ value of 4.12 ± 0.09 was obtained, indicating that the micellar aggregates exhibit only a negligible effect on the acidity constant.

2.4. *Micellar Effects on the Reaction between 3-Methylbenzenediazonium Ions and Gallic Acid or Octyl Gallate.* The effects of SDS micelles on the reaction between 3MBD and GA were determined at different acidities, and those for the reaction with the OH group at a single pH. *Fig. 4,a* shows the effect of increasing SDS concentration on k_{obs} at selected pH values for the reaction with GA. In all cases, the reaction was inhibited, k_{obs} decreasing by factors of *ca.* 18 (pH 5), 13 (pH 4.5), and 7 (pH 4.1), for [SDS] = 0.3M. The k_{obs} values obtained at high SDS concentrations seem to be independent of the pH, approaching the value obtained for the spontaneous decomposition of 3MBD in SDS micellar solution: $k_{obs} = 5.7 \times 10^{-4}$ s^{-1} [44].

The effect of the SDS concentration on k_{obs} for the reaction with OG are shown in *Fig. 4,b*. As can be seen, a bell-shaped profile was obtained (inset). Upon addition of SDS, k_{obs} increased slightly up to a maximum value, after which further addition of SDS led to a decrease in k_{obs} by a factor of 6, for [SDS] = 0.2M, thus approaching the value for spontaneous decomposition of 3MBD in SDS micellar solution (5.7×10^{-4} s^{-1}). Biphasic profiles as that shown in *Fig. 4,b* are typical for bimolecular reactions carried out in micellar systems, where concentration and dilution effects play a significant role [29][45].

3. Discussion. – The results presented in *Fig. 3* suggest that SDS micellar aggregates show a negligible effect on the first ionization equilibrium of GA. Therefore, the inhibition observed in *Fig. 4,a* cannot be rationalized on the basis of eventual changes in reactive concentrations due to micelle-induced variations in pK_a. According to the pseudophase-ion-exchange (PIE) model [45], which is commonly used to interpret micellar effects on chemical reactivity [30][45][51], shifts of pK_a values in micellar media compared to the values in the absence of surfactant may be caused primarily due to the transfer of the indicator (in both neutral and ionic form) and H_3O^+ from the large

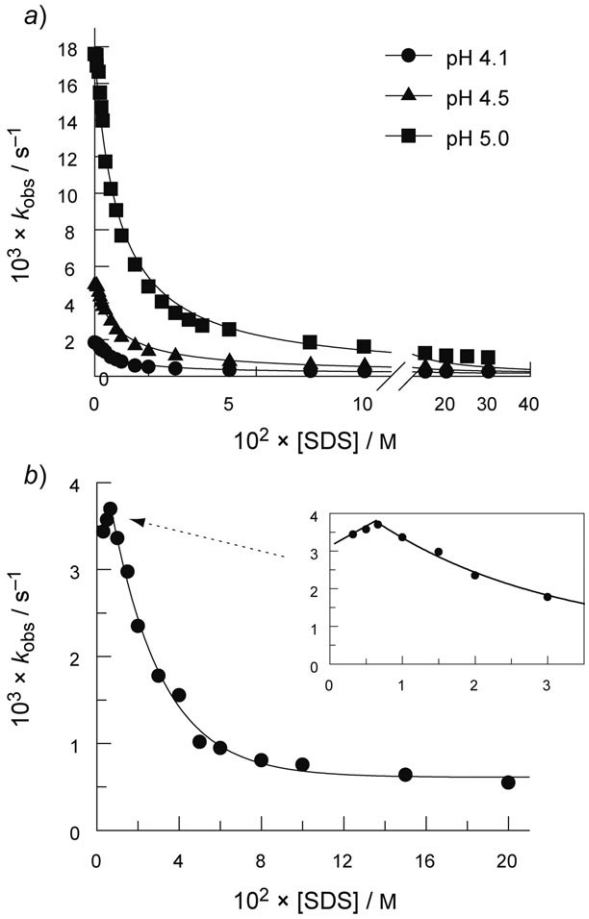


Fig. 4. *Effect of SDS concentration on* k_{obs} *for the reaction of 3MBD with GA* (a) *at different pH values, and with OG* (b) *at pH 5.1.* The inset in *b* is a magnification of the data at low SDS concentration. Conditions: [3MBD] = 0.1 – 0.3 mM, [GA] = 40 mM, [OG] = 1.3 mM, T = 30°.

bulk volume of H_2O into the much smaller volume of the micellar interface. When the indicator is significantly incorporated into the aggregate, then addition of HCl may increase the apparent pK_a of the indicator probe, because addition of HCl into an SDS micellar solution results in the displacement of Na^+ ions from the micellar surface by H^+ ions [52]. The results obtained may, thus, be suggestive of insignificant incorporation of GA into the micellar aggregate, which is consistent with the high water-solubility of GA.

The acid dependence of k_{obs}, shown in *Fig. 2,a*, suggests that the reactive species are the dianionic form of gallic acid (GA^{2-}), and not GA^- or even GA proper. The observed inhibition shown in *Fig. 4,a* can be qualitatively interpreted according to

Hartley's rule, by assuming a micelle-induced separation of reactants originated by the electric barrier imposed by the SDS micelles [51][53][54]. 3MBD is positively charged and bears a hydrophobic aromatic part. It, thus, will be incorporated to some extent into the micellar aggregates upon increasing the SDS concentration, in keeping with the results shown in *Fig. 1* and with ^{1}H-NMR data on the location of 3MBD in micellar aggregates [44]. Alternatively, one would expect that GA is not significantly associated to the micellar aggregates, because it is hydrophilic and, even more, because at the working pH the carboxylic-acid function of GA is completely ionized ($pK_a(1) \approx 4.3$), *i.e.*, negatively charged. Hence, GA^- molecules (and especially the kinetically reactive species GA^{2-}) should be excluded from the *Stern* layer, which should significantly decrease their local concentration in the vicinity of 3MBD.

Quantitatively, the results shown in *Fig. 4*,*a* can be interpreted in terms of the PIE model, which basically assumes that *i*) 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; *ii*) the substrates may be incorporated at nearly diffusion-controlled rates; and *iii*) the micellized surfactant is at thermal equilibrium with solutes throughout the reaction. Further details on the assumptions and limitations of the PIE and related models can be found elsewhere [30][45][51]. Consequently, 3MBD can be distributed between the aqueous and micellar pseudophases, but GA^- and GA^{2-} will be mainly located in the aqueous pseudophase because of the electric repulsion due to the negatively charged micellar surface (*Scheme 2*).

According to the pseudophase model, the rate of the reaction will be the sum of the rates in each pseudophase (*Eqn. 2*):

$$v = k_W[3MBD_W] + k_M[3MBD_M] + kK_{DE}[HG_W^{2-}][3MBD_W] \quad (2)$$

where K_{DE} stands for the equilibrium constant for the formation of the diazo ether, and the subscripts 'W' and 'M' stand for the water and micellar pseudophases, respectively. By considering the corresponding mass balances, and by recognizing that the reactions were carried out under pseudo-first-order conditions, *Eqn. 3* can be derived, where K_s stands for the association constant of 3MBD to the micellar aggregate, and D_n represents, as indicated above, the concentration of micellized surfactant:

$$k_{obs} = \frac{k_W + k_M K_s D_n + kK_{DE}[HG_W^{2-}]}{1 + K_s D_n} \quad (3)$$

The results presented in *Fig. 2* show that at the k_{obs} value for the spontaneous decomposition of 3MBD in aqueous solution is negligible compared to those with GA^{2-}. Moreover, *Fig. 1* indicates that, on increasing the concentration of SDS, k_{obs} for the spontaneous decomposition of 3MBD is even lower than that in acidic aqueous solution. Consequently, *Eqn. 3* can be simplified to *Eqn. 4*:

$$k_{obs} = \frac{kK_{DE}[HG_W^{2-}]}{1 + K_s D_n} = \frac{k^o}{1 + K_s D_n} \quad (4)$$

Thus, k_{obs} should decrease upon increasing D_n, as illustrated in *Fig. 4*,*a*. Note that *Eqn. 4* also predicts that when $D_n = 0$, $k_{obs} = k^o = kK_{DE}[GA_W^{2-}]$, where k^o stands for the

observed rate constant in the *absence* of micelles. *Eqn. 4* can now be rearranged to *Eqn. 5*:

$$\frac{k^{o} - k_{obs}}{k_{obs}} = K_s[SDS]_T - K_s \cdot cmc \quad (5)$$

This latter equation predicts that a plot of $(k^{o} - k_{obs})/k_{obs}$ *vs.* $[SDS]_T$ should be linear, with a negative intercept equal to the product $K_s \cdot cmc$, which, indeed, is the case, as shown in *Fig. 5* and the *Table*, which shows the corresponding K_s and *cmc* values for the three pH values investigated. The K_s values are similar to that obtained by monitoring the effects of SDS micelles on the spontaneous decomposition of 3MBD (*Fig. 1*). The obtained *cmc* values are lower than that in pure aqueous solution (8 mM at 25°), which is consistent with the well-known effects of electrolyte concentration on *cmc* [51][52].

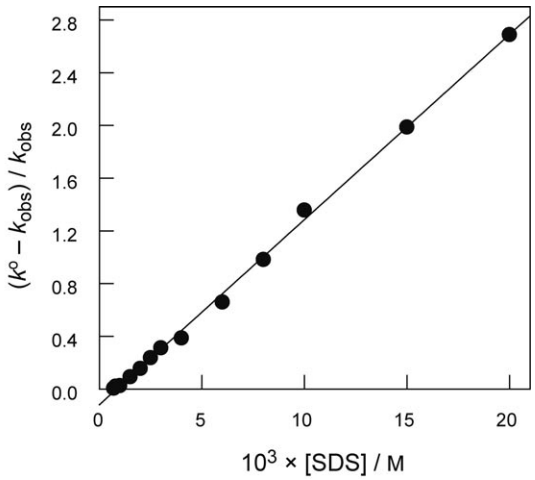


Fig. 5. *Linear plot according to* Eqn. 5. The data were taken from *Fig. 4,a* (pH 4.5).

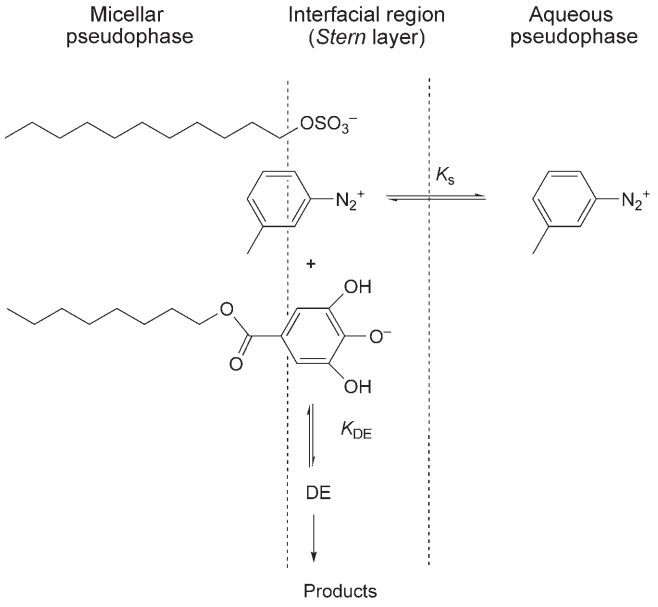
Table. *Binding Constants for the Association of 3MBD with SDS Micelles.* Also given is the kinetically determined critical micelle concentration (*cmc*) obtained by fitting the data of *Fig. 5,a* to *Eqn. 6* (see *Exper. Part*).

pH	K_s [M^{-1}]	*cmc* [M]
4.1	159 ± 4	$(9 \pm 2) \times 10^{-4}$
4.5	140 ± 2	$(8 \pm 1) \times 10^{-4}$
5.0	138 ± 1	$(9 \pm 1) \times 10^{-4}$

The solubility of OG in H_2O is extremely low [55], and because of its hydrophobic tail, it is likely to behave as a surfactant. On the basis of the effect of the hydrocarbon chain length on the association constant of the substrates and micelles [56], it can be expected that the value for the association constant of OG with SDS should be *ca.* 10^3 M^{-1}, similar to that of 6-*O*-octanoyl-L-ascorbic acid [38]. Auxiliary experiments showed that its water-solubility does not increase significantly up to pH > 6 (reported pK_a = 7.82), and even when ionized, it is still sparingly water-soluble. Therefore, both OG

and OG^- ions should be completely bound by the micelles, giving rise to mixed OG–SDS micelles. The reaction between 3MBD and OG^- takes place exclusively in the micellar *Stern* layer, as shown in *Scheme 3*. Similar situations have been described in micellar systems where hydrophobic ascorbic acid esters were dissolved [6][38][39].

Scheme 3. *Basic Two-Dimensional Representation of the* Stern *Layer of an SDS Micelle in which the Reaction between 3MBD and* OG^- *Takes Place.* Note that, in the present case, OG^- is acting as a co-ion. For the sake of clarity, the spontaneous decomposition of 3MBD, the ionization equilibrium of OG, and the counter ions and other co-ions are not included.



The reaction between 3MBD and OG is second-order overall, even though it has been carried out under pseudo-first-order conditions by adjusting [3MBD]≪[OG]. Thus, a bell-type kinetics profile, as that shown in *Fig. 4*,*b*, commonly found in bimolecular processes where both reactants bind to the micelles [30][45][51], was expected. The results can be interpreted in terms of 3MBD and OG reacting at the micellar surface according to *Scheme 3*. The k_{obs} values increased rapidly as long as the concentration of the reactants at the micellar *Stern* layer increased to a maximum, and then decreased sharply due to the 'dilution' of the reactants within the micellar pseudophase upon increasing the micelle concentration in solution [30]. In the present case, the increase in k_{obs} due to this concentration effect was observed in a very narrow SDS concentration range, because OG is virtually insoluble in H_2O, giving rise to immediate formation of mixed micelles. A complete quantitative treatment was not attempted because a number of approximations are needed, but a comprehensive set of equations for solving *Scheme 3* (or similar reaction schemes) can be found elsewhere [30][51].

4. Conclusions. – In summary, we have shown that, regardless of the hydrophobicity of the gallic acid derivative used, SDS micelles inhibit their reaction with 3MBD, with k_{obs} values approaching that for the spontaneous decomposition of 3MBD in aqueous micellar solutions.

The origin of the inhibition for the reaction with GA is different from that for the reaction with OG. On the one hand, at relatively low acidities, a substantial fraction of GA is ionized, and both GA^- and the reactive GA^{2-} ions are excluded from the vicinity of 3MBD, which is associated to the micellar aggregate, because of the electric barrier imposed by the negatively charged micellar SDS surface. Therefore, the effect of SDS micelles is to decrease the local GA^{2-} concentration in the vicinity of 3MBD. On the other hand, the origin of the observed inhibition in the reaction with OG arises from the dilution of the antioxidant within the micellar pseudophase upon increasing the concentration of SDS, *i.e.*, upon increasing the number of available micelles in the system, because 3MBD, OG, and OG^- are water-insoluble, the OG^- ions being co-ions of the dodecyl sulfate monomers of the SDS micelles.

When cationic micelles such as cetyltrimethylammonium halides (CTAX) are used instead of the anionic ones, both GA^- (or GA^{2-}) and OG should be totally bound to the micelles because of the electrostatic attraction with the ammonium head groups and because of the hydrophobic tail of OG. However, the 3MBD ions will be mainly located in the aqueous pseudophase because of their positive charge, as demonstrated previously by *Cuccovia et al.* [57] and by *Bravo-Díaz* and co-workers [58]. Thus, one would expect a similar inhibition of the reactions when employing CTAX micelles.

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Experimental Part

General. UV/VIS Spectra and kinetics experiments were followed on an *Agilent HP-8453* UV/VIS spectrophotometer equipped with a thermostated cell carrier attached to a computer for data storage. Kinetics were performed at a const. temp. of $30 \pm 0.1°$. Reagents were of maximum purity available and used without further purification. Gallic acid (GA) and the reagents used in the preparation of 3-methylbenzenediazonium (3MBD) tetrafluoroborate and in the preparation of *Britton–Robinson* (*BR*) buffer were purchased from *Fluka* or *Aldrich*, and were used as received. Other materials employed were from *Riedel de Häen*. All solns. were prepared with *Milli-Q*-grade H_2O. 3MBD was prepared under non-aqueous conditions as described elsewhere [59], and stored in the dark at low temp. to minimize decomposition. The UV/VIS spectrum of an aq. *ca.* 10^{-4} M soln. of 3MBD in buffer (pH 3) showed one main absorption band centered at 250 nm, with a shoulder at 310 nm. Variations in absorbance as a function of the concentration of 3MBD were linear up to 2×10^{-4} M, with ε_{250} and ε_{310} values of 4700 ± 90 and 1773 ± 19 l mol^{-1} cm^{-1}, in accord with lit. values [34]. The UV/VIS spectrum of a 10^{-4} M aq. soln. of GA in buffered H_2O (pH 1.6) showed two absorption bands at 222 and 271 nm. The wavelength of the absorption at 222 nm remained constant upon changing the pH in the range 1.6–5, but that of the absorption band at 271 nm showed a hypsochromic shift of *ca.* 10 nm at pH 5.0, the exact position depending on the ionization state of GA. Reported acidity constants for GA are a $pK_a(1) = 4.4$ (ionization of the COOH group) at 25° [49] and, upon alkalinization, $pK_a(2) = 8.55$ (deprotonation of a phenolic OH group) [50].

Methods. Reactions were followed by UV/VIS spectroscopy. *Fig. 6, a* displays a number of UV/VIS spectra obtained at different times in the course of the reaction between 3MBD and GA, showing a

decrease in the absorbance at 260 and 310 nm due to 3MBD consumption, and an increase in the absorbance at 416 nm due to product formation. Similar results were obtained for the reaction between 3MBD and OG in 0.5M SDS soln. (data not shown).

In previous studies on the reaction between 3MBD and methyl gallate under acidic conditions [36], a similar increase in the absorbance was detected at 440 nm. Electrochemical results confirmed that such an increase corresponds to a transient diazo ether, which is formed in the course of the reaction, rather than to the formation of an azo dye. The saturation-kinetics profiles attained [36] are also consistent with the formation of a diazo ether, and not with the commonly accepted electrophilic-aromatic-substitution (EAS) mechanism. Moreover, preliminary (unpublished) results on the kinetics of the reaction between 3MBD and GA are also suggestive of saturation-kinetics profiles that are not compatible with the EAS mechanism.

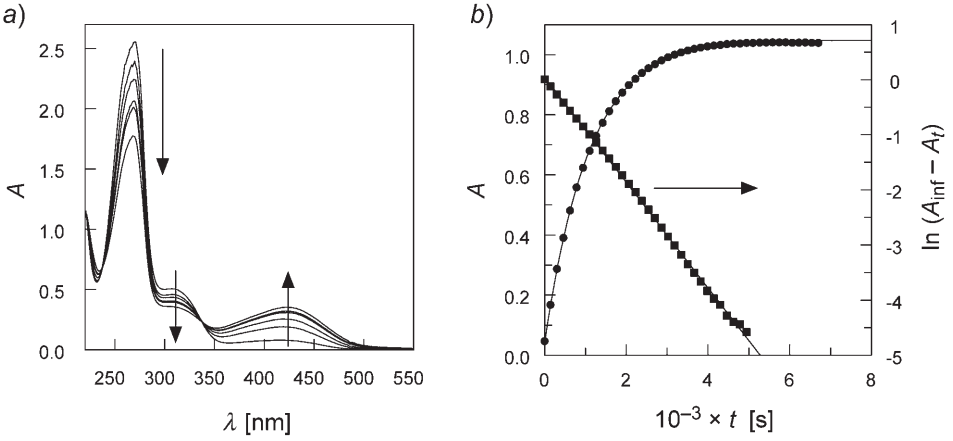

Fig. 6. a) *UV/VIS Monitoring of the reaction between 3MBD and GA as a function of time.* The arrows indicate the trend in the change of absorbance. b) *Typical kinetics run showing the variation in the absorbance at 416 nm and linear fitting to the integrated first-order equation.* [3MBD] = 0.3 mM, [GA] = 80 mM, $T = 30°$, pH 3.7.

The reactions between 3MBD and GA or OG were monitored by measuring the changes in the absorbance at 416 nm with time (product formation), and k_{obs} values were obtained by fitting the time-dependent absorbance data to the integrated first-order *Eqn. 6*, using a non-linear least-squares method provided by a commercial computer program (*Fig. 6,b*):

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

Runs were performed at $30 \pm 0.1°$ under pseudo-first order conditions, and linear plots were obtained for more than 3 $t_{1/2}$. Duplicate or triplicate runs gave identical k_{obs} values within 5% error.

It is worth noting that, after completion of the reaction (*Fig. 6,b*), the absorbance at 416 nm remained practically constant after leveling off (although in some runs a slight decrease was detected), in contrast with the observed behavior in the reaction between 3MBD and methyl gallate, where a subsequent decrease in absorbance was detected [36]. This point may need extra attention because the effects of the substituents in the pyrogallol moiety (*i.e.*, the change of an ester group by a carboxylic one) and the effects of SDS on product stabilization are currently unknown and deserve further investigations. In fact, stabilization of the diazo ethers formed in the course of the reaction between ArN_2^+ and

antioxidants, such as vitamin C (ascorbic acid) and its hydrophobic esters, by SDS micellar aggregates has been pointed out previously, but not sufficiently investigated yet [6][38][39].

REFERENCES

[1] K. C. Brown, M. P. Doyle, *J. Org. Chem.* **1988**, *53*, 3255.
[2] K. J. Reszka, C. F. Chignell, *J. Am. Chem. Soc.* **1993**, *115*, 7752.
[3] K. J. Reszka, C. F. Chignell, *Chem.-Biol. Interact.* **1995**, *96*, 223.
[4] U. Costas-Costas, E. Gonzalez-Romeroand, C. Bravo-Díaz, *Helv. Chim. Acta* **2001**, *84*, 632.
[5] E. González-Romero, B. Malvido-Hermelo, C. Bravo-Díaz, *Langmuir* **2002**, *18*, 46.
[6] U. Costas-Costas, C. Bravo-Díaz, E. González-Romero, *Langmuir* **2005**, *21*, 10983.
[7] R. Pazo-Llorente, H. Maskill, C. Bravo-Díaz, E. González-Romero, *Eur. J. Org. Chem.* **2006**, 2201.
[8] C. Galli, *Chem. Rev.* **1988**, *88*, 765.
[9] B. Toth, J. Taylor, B. Mattson, P. Gannett, *In Vivo* **1989**, *3*, 17.
[10] H. Zollinger, 'Diazo Chemistry I: Aromatic and Heteroaromatic Compounds', VCH, Wheinhein, 1994.
[11] B. Quintero, J. J. Morales, M. Quiros, M. Martinez-Puentedura, M. C. Cabeza, *Free Radic. Biol. Med.* **2000**, *29*, 464.
[12] J. H. Powell, P. M. Gannet, *J. Environ. Pathol. Toxicol. Oncol.* **2002**, *21*, 1.
[13] J. Pokorny, N. Yanishlieva, M. Gordon, 'Antioxidants in Food: Practical Applications', CRC Press, Boca Raton, 2001.
[14] T. Basu, N. J. Temple, M. L. Garg, 'Antioxidants in Human Health and Disease', CABI, Wallingford, UK, 1999.
[15] B. Halliwell, M. A. Murcia, S. Chirico, O. I. Aruoma, *Crit. Rev. Food Sci. Nutr.* **1995**, *35*, 7.
[16] C. Capelli, B. Mennuci, S. Monti, *J. Phys. Chem., Sect. A* **2005**, *109*, 1933.
[17] G. C. Yena, P. D. Duhb, H. L. Tsaia, *Food Chem.* **2002**, *79*, 307.
[18] O. I. Aruoma, 'Free Radicals and Antioxidants in Food Science', Chapman & Hall, London, 1998.
[19] O. I. Aruoma, A. Murcia, J. Butler, B. Halliwell, *J. Agric. Food. Chem.* **1993**, *41*, 1880.
[20] I. Kubo, F. K. I. Nihei, K. I. Nihei, *J. Agric. Food Chem.* **2002**, *50*, 6692.
[21] Z. L. Liu, in 'Bioradicals Detected by ESR Spectroscopy', Eds. H. Ohya-Nishiguchi, L. Packer, Birkhäuser, Basel, 1995, p. 259.
[22] E. N. Frankel, *Food Chem.* **1996**, *57*, 51.
[23] E. N. Frankel, *J. Oleo Sci.* **2001**, *50*, 387.
[24] E. N. Frankel, A. S. Meyer, *J. Sci. Food Agric.* **2000**, *80*, 1925.
[25] C. Jacobsen, *Lipid – Fett* **1999**, *101*, 484.
[26] D. J. McClements, E. A. Decker, *J. Food Sci.* **2000**, *65*, 1270.
[27] V. Tulyathan, R. B. Boulton, V. L. Singleton, *J. Agric. Food Chem.* **1989**, *37*, 844.
[28] M. P. Richards, W. Chaiyasit, D. J. McClements, E. A. Decker, *J. Agric. Food Chem* **2002**, *50*, 1254.
[29] J. Fendler, 'Membrane Mimetic Chemistry', J. Wiley & Sons, N. Y., 1982.
[30] G. Savelli, R. Germani, L. Brinchi, in 'Reactions and Synthesis in Surfactant Systems', Ed. J. Texter, Marcel-Dekker, N. Y., 2001, p. 175.
[31] M. P. Pileni, 'Structure and Reactivity in Reverse Micelles', Elsevier, N. Y., 1989.
[32] W. A. Pryor, T. Strickland, D. F. Church, *J. Am. Chem. Soc.* **1988**, *110*, 2224.
[33] W. A. Pryor, J. A. Cornicelli, L. J. Devall, B. Tait, B. K. Trivedi, D. T. Witiak, M. Wu, *J. Org. Chem.* **1993**, *58*, 3521.
[34] R. Pazo-Llorente, M. J. Sarabia-Rodriguez, C. Bravo-Díaz, E. Gonzalez-Romero, *Int. J. Chem. Kinet.* **1999**, *31*, 73.
[35] R. Pazo-Llorente, C. Bravo-Díaz, E. González-Romero, *Eur. J. Org. Chem.* **2003**, 3421.
[36] S. Losada-Barreiro, V. Sánchez-Paz, C. Bravo-Díaz, *Helv. Chim. Acta* **2007**, *90*, 1559.
[37] R. Pazo-Llorente, C. Bravo-Díaz, E. González-Romero, *Eur. J. Org. Chem.* **2004**, 3221.
[38] U. Costas-Costas, C. Bravo-Díaz, E. González-Romero, *Langmuir* **2004**, *20*, 1631.
[39] U. Costas-Costas, C. Bravo-Díaz, E. González-Romero, *Langmuir* **2003**, *19*, 5197.

[40] C. Bravo-Diaz, L. S. Romsted, M. Harbowy, M. E. Romero-Nieto, E. Gonzalez-Romero, *J. Phys. Org. Chem.* **1999**, *12*, 130.
[41] P. LoNostro, G. Capuzzi, P. Pinelli, N. Mulinacci, A. Romani, F. F. Vincieri, *Colloid. Surf., A: Physicochem. Eng. Aspects* **2000**, *167*, 83.
[42] P. LoNostro, G. Capuzzi, A. Romani, N. Mulinacci, *Langmuir* **2000**, *16*, 1744.
[43] J. H. Fendler, E. F. Fendler, 'Catalysis in Micellar and Macromolecular Systems', Academic Press, N. Y., 1975.
[44] C. Bravo-Diaz, M. Soengas-Fernandez, M. J. Rodriguez-Sarabia, E. Gonzalez-Romero, *Langmuir* **1998**, *14*, 5098.
[45] C. A. Bunton, F. Nome, F. H. Quina, L. S. Romsted, *Acc. Chem. Res.* **1991**, *24*, 357.
[46] N. Takenaka, M. Tanaka, K. Okitsu, H. Bandow, *J. Phys. Chem., Sect. A* **2006**, *110*, 10628.
[47] A. F. Hegarty, in 'The Chemistry of Diazonium and Diazo Compounds', Ed. S. Patai, J. Wiley & Sons, N. Y., 1978.
[48] K. H. Saunders, R. L. M. Allen, 'Aromatic Diazo Compounds', Edward Arnold, Baltimore, MD, 1985.
[49] 'CRC Handbook of Chemsitry and Physics', 78th edn., CRC Press, Boca Raton, FL, 1997.
[50] N. Binbuga, K. Chambers, W. P. Henry, T. P. Schultz, *Holzforschung* **2005**, *59*, 205.
[51] L. S. Romsted, in 'Surfactants in Solution', Eds. K. L. Mittal, J. Lindman, Plenum Press, N. Y., 1984.
[52] R. Pazo Llorente, C. Bravo-Díaz, E. González-Romero, *Langmuir* **2004**, *20*, 2962.
[53] C. A. Bunton, G. Savelli, *Adv. Phys. Org. Chem.* **1986**, *22*, 213.
[54] S. Tascioglu, *Tetrahedron* **1996**, *52*, 11113.
[55] L.-L. Lu, X.-Y. Lu, *J. Chem. Eng. Data* **2007**, *52*, 37.
[56] F. H. Quina, E. Alonso, J. P. S. Farah, *J. Phys. Chem.* **1995**, *99*, 11708.
[57] I. M. Cuccovia, I. N. da Silva, H. Chaimovich, L. S. Romsted, *Langmuir* **1997**, *13*, 647.
[58] R. Pazo-Llorente, C. Bravo-Díaz, E. González-Romero, *Langmuir* **2003**, *19*, 9142.
[59] M. C. Garcia-Meijide, C. Bravo-Diaz, L. S. Romsted, *Int. J. Chem. Kinet.* **1998**, *30*, 31.

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