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Kinetics and Mechanism of the Reaction between Ascorbic Acid Derivatives and an Arenediazonium Salt: Cationic Micellar Effects

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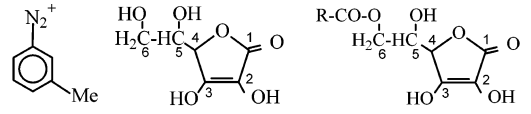
The effects of tetradecyltrimethylammonium bromide, TTAB, and hexadecyl-trimethylammonium bromide, CTAB, micellar systems on the reaction of 3-methylbenzenediazonium, 3MBD, tetrafluoroborate with ascorbic acid, VC, and with the hydrophobic derivatives 6-O-dodecyl-L-ascorbic acid, VC12, and 6-O-palmitoyl-L-ascorbic acid, VC16, were investigated at different pH values by employing a combination of UV−vis spectroscopy and high-performance liquid chromatography, HPLC, techniques. Previous studies in the absence of surfactant showed that the reaction between 3MBD and VC derivatives takes place through a rate-limiting decomposition of a transient diazo ether, DE, formed from reaction between 3MBD and the monoanion form of ascorbic acid, VC^-, in a rapid preequilibrium step. In the presence of a fixed [CTAB], the kinetics of the reaction of 3MBD with VC follows a saturation kinetics similar to that observed in its absence, but for the reaction with VC12 and VC16, only the first linear portions of the saturation profiles could be obtained because k_{obs} values become too large. HPLC analyses of the reaction mixtures show that no unexpected products are detected, suggesting that cationic micelles do not modify the mechanism of the reaction. Analyses of the kinetic data allowed estimations of the rate constant for the decomposition of the diazo ether and of the equilibrium constant for the formation of DE in the presence of CTAB micelles, which is ∼6 times higher than in its absence; this suggests that CTAB micelles promote diazo ether formation. At constant [antioxidant], the variations of k_{obs} for the reactions with VC, VC12, or VC16 follow bell-shaped curves, with rate enhancements of up to 2−3-fold for VC with respect to the value in the absence of surfactant. The rate maximum for the reaction of 3MBD with VC is reached at [CTAB] = 0.02 M suggesting a CTAB-induced rate increase, i.e., micellar catalysis; meanwhile the rate maximum for the reaction with VC12 and VC16, which may behave as amphiphilic compounds, is reached at [CTAB] ∼ 1×10^{-4} M, a concentration about 10 times lower than its critical micelle concentration, cmc, in pure water, but only ∼3 times lower than the cmc of VC16, suggesting the formation of reactive CTAB−VC12 and CTAB−VC16 premicellar aggregates. Kinetic and HPLC results are consistent with the predictions of the pseudophase model and are interpreted in terms of 3MBD ions sampling in the aqueous bulk phase and the micellar effects on the different equilibrium involved. The results should contribute to a better understanding of the role of compartmentalized systems on the efficiency with which hydrophilic and hydrophobic reductants such as ascorbic acid derivatives interact with potentially mutagenic and carcinogenic ArN_2^+ ions.

Introduction

The water soluble vitamin C, VC, or L-ascorbic acid, Scheme 1, is a well-known preventive and chain-breaking antioxidant; for instance, VC recycles vitamin E by reducing α-tocopheroxyl radicals in membranes, in addition to playing an important role in physiological functions.[1−5] In aqueous solution, VC behaves as a weak acid with pK_a values of 4.2 and 11.4 for the hydroxyl groups in the C_3 and C_2 positions, respectively. Hence, at neutral pH, vitamin C exists predominantly as ascorbate monoanion, which is generally accepted to act as a stronger antioxidant than the protonated forms. Nevertheless, applications in the food industry apply to low pH, for example in canned beverages or in some salad dressings.

Scheme 1. Chemical Structures of the Ascorbic Acid Derivatives Employed in This Work and That of 3-Methylbenzenediazonium Ion



3MBD VC R = $CH_3(CH_2)_{10}$, VC12; R = $CH_3(CH_2)_{14}$, VC16

Several studies have shown that antioxidants can partition into different physical locations in emulsions, and this partitioning may dramatically influence antioxidant effectiveness rates.[6−11] In some instances, natural or synthetic hydrophobic antioxidant derivatives can be prepared; for example, the palmitate ester derivative of VC, VC16, is active as an antioxidant, and because of its hydrophobic palmitate side chain the molecule positions

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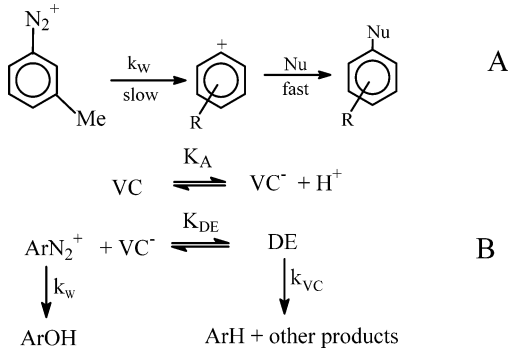
itself into cell membranes, protecting lipids and other components against peroxidation. A number of reports indicate that the antioxidant activity of VC and that of its hydrophobic derivatives are very similar, making them important for a number of applications in the food industry.[2,3,12,13]

Different studies conclude that the activity of a given antioxidant in food systems depends not only on the environmental pH but also on a number of factors including its partitioning between the different regions of the system, making such an evaluation of the antioxidant activity a difficult task.[9,10,14] Micelles and other colloidal systems have been extensively used as models for understanding the effects of heterogeneous environments on reaction dynamics and mechanisms, providing relatively simple models for understanding the complex behavior encountered in food and biological assemblies.[15−19] Micellar systems are usually characterized as "two phase" systems where separation or concentration of the reactants between the aqueous and interfacial regions may occur, allowing one to analyze some of the complexities that arise in real systems in a relatively simpler fashion; for example, partitioning of substrates, local concentration or dilution effects, and so forth. In addition, micelles represent a system in which chemicals equilibrate rapidly from the aqueous phase into the model "interfacial" region.[20−23]

Recently, we studied the reaction between a number of arenediazonium ions with VC and some of its hydrophobic derivatives in aqueous acid solutions, in the presence and absence of anionic micellar aggregates, at different pH values.[24−26] The reactions may have some importance in food and biological systems because antioxidants may be able to directly reduce ArN_2^+ ions via one-electron-transfer processes yielding aryl radicals that, in turn, are believed to be responsible, to some extent, for the carcinogenic properties of arenediazonium ions.[24,27−29]

In aqueous acid solution, in the absence of catalysts or reductants, the reaction between ArN_2^+ and VC takes place through two competitive pathways,[24] Scheme 2: the spontaneous thermal decomposition of the ArN_2^+ ions and an inner sphere mechanism whose slow step is the formation of a kinetically controlled *Z*-diazo ether intermediate, DE, formed from the reaction of ArN_2^+ with the monobasic form of ascorbic acid, VC^-, in a rapid preequilibrium step and that subsequently may either isomerize to yield the thermodynamically stable *E* isomer or may undergo homolytic scission yielding reduction products. Hydrophobic derivatives such as 6-O-octanoyl-L-ascorbic, VC8, acid and 6-O-palmitoyl-L-ascorbic acid, VC16, react in a similar fashion.[25,26]

Scheme 2. Proposed Mechanism for the Reaction between Arenediazonium Ions and Ascorbic Acid Derivatives in the Absence of Surfactant, Where DE Stands for the 3-O-Arenediazo Ether Formed in a Rapid Preequilibrium Step



We also investigated the micellar SDS effects on the reaction between ArN_2^+ ions and ascorbic acid derivatives at different pH values.[25,26] Addition of SDS inhibits the reaction up to a minimum after which further addition of SDS leads to a slight increase in the observed rate constant, k_{obs}. The reaction with VC16 is faster than that with VC at moderate [SDS], but it is also inhibited upon addition of [SDS] at any fixed [VC16], with k_{obs} approaching the value for the thermal decomposition of ArN_2^+. At very high [SDS], $k_{obs}(VC) > k_{obs}(VC16)$; in contrast, k_{obs} values for the reaction between VC8 and ArN_2^+ ions follow bell-shaped curves.[26] In all cases, kinetic data were interpreted in the light of the pseudophase model, and HPLC analyses of the reaction mixtures showed important micellar effects on product distribution.

Because of the well-known bioactivity of ArN_2^+ ions and VC derivatives, here we analyze the effects of cationic tetradecyltrimethylammonium bromide, TTAB, and cetyltrimethylammonium bromide, CTAB, micelles on the reaction of 3-methylbenzene diazonium, 3MBD, tetrafluoroborate with ascorbic acid and two of its hydrophobic derivatives, 6-O-lauril-L-ascorbic acid, VC12, and 6-O-palmitoyl-L-ascorbic acid, VC16. The results should allow the determination of the efficiency with which ascorbic acid and its derivatives are able to interact with ArN_2^+ ions in the presence of micellar aggregates and will contribute to a better understanding of the role of compartmentalized systems on such reactions. The structure of 3MBD and those of VC, VC12, and VC16 are shown in Scheme 1. 3MBD was chosen because the kinetics and mechanism of its reaction in aqueous and micellar solutions, either in the presence or absence of VC, is known, showing a higher reactivity toward VC than the other methyl derivatives.[24,25]

On the basis of electrostatic arguments, one would expect an inhibition of the reaction either with VC12 or VC16 in the presence of cationic micellar systems, but as we will show, micellar catalysis is found in all cases with k_{obs} values following bell-shaped curves. The results are

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interpreted in terms of the micellar effects on the different equilibria involved and formation of premicellar VC12−CTAB and VC16−CTAB aggregates and can be perfectly accommodated by common pseudophase models. HPLC analyses of the reaction mixtures show that cationic micelles stabilize the *E*-diazo ether formed in the course of the reaction.

Experimental Section

Instrumentation. UV−Vis spectra and some kinetic experiments were followed on an Agilent HP 8435 diode array UV−vis spectrophotometer equipped with a thermostated cell carrier attached to a computer for data storage. Product analysis was carried out on a Waters HPLC system including a 560 pump, a 717 automatic injector, a 486 UV−vis detector, and a computer for data storage. Products were separated on a Microsorb-MV C-18 (Rainin) reversed-phase column (25 cm length, 4.6 mm i.d., and 5 μm particle size) using a mobile phase of 65/35 v/v MeOH/H_2O containing 10^{-4} M HCl. The injection volume was 25 μL in all runs, and the UV detector was set at 220 nm. The pH was measured by using a previously calibrated Metrhom 713 pH meter equipped with temperature sensors. ^{1}H NMR spectra were obtained on a Brucker ARX 400 spectrometer.

Materials. Reagents were of the maximum purity available and were used as received. The surfactant sodium dodecyl sulfate, SDS (99.9%), L-ascorbic acid, VC, the hydrophilic 6-O-ascorbyl palmitate, VC16, and the reagents used in the preparation of diazonium salts (as tetrafluorborates) were purchased from Aldrich or Fluka. Other materials were from Riedel de Haën.

All solutions were prepared using Milli-Q grade water. 3MBD was prepared under nonaqueous conditions as previously reported[30] and was stored in the dark at low temperature to minimize its decomposition. VC12 was prepared by reaction of the corresponding carboxylic acid with l-ascorbic acid in a concentrated sulfuric acid solution according to a literature procedure.[31] The ^{1}H NMR spectra of VC12 in DMSO is consistent with that described in the literature, confirming the identity of the compound.[31]

Ascorbic acid and its derivatives are highly sensitive to various modes of degradation.[32] Factors that may influence the degradative process include the temperature, pH, oxygen, and metal catalysts. To minimize antioxidant degradation, VC stock solutions were prepared each day by dissolving solid VC in aqueous solutions containing HCl or the universal Britton−Robinson (BR) buffer and citric acid.

VC12 and, especially, VC16 have a limited solubility in water depending on pH; for instance, at pH = 6.8 solutions of [VC16] = 0.15 mM can be prepared,[33] but at lower pH the solubility decreases up to $\sim 8.1 \times 10^{-7}$ mol dm^{-3} at $T = 25$ °C.[34] To minimize this problem, fresh VC12 and VC16 stock solutions were prepared daily by dissolving the appropriate amount of solid VC12 or VC16 in an aqueous CTAB solution of known concentration so that [CTAB] ≫ cmc. Auxiliary spectrophotometric and polarographic experiments demonstrated that VC and its derivatives are stable under such conditions for at least 24 h at $T = 35$ °C. Additional details can be found elsewhere.[24,25]

Methods. Kinetic data were obtained spectrophotometrically by monitoring the disappearance of ArN_2^+ as previously described.[24,25] Observed rate constants were obtained by fitting the absorbance−time data to the integrated first-order eq 1 using a nonlinear least-squares method provided by a commercial computer program

$$\ln\frac{(A - A_\infty)}{(A_0 - A_\infty)} = -k_{obs}t \qquad (1)$$

where A is the absorbance at any time t, A_0 and A_∞ represent the absorbance at zero and infinite time, respectively, and k_{obs} is the measured rate constant. All runs were done at $T = 35° \pm 0.1$ °C, except where otherwise indicated, with the diazonium salts as limiting reagents. The good agreement between the optimized and experimental A_∞ value confirmed that reactions are first order with respect to ascorbic acid. Identification and quantification of reaction products were done by HPLC analyses of the reaction mixtures once the reactions were completed, i.e., at infinite time, by following the procedure described elsewhere.[30]

It is well documented[20,35−37] that micellar systems may shift the pK_a of weak acids with respect to that in the absence of surfactant, and such a micellar-induced change in the ionization constant may have important kinetic effects by lowering or raising the concentration of the reactive entities. To check whether CTAB micelles have an effect on the apparent pK_a of VC and VC16, titration curves (not shown) were obtained by monitoring changes in the absorption of the VC, VC12, and VC16 UV−vis spectra at selected wavelengths by employing buffered solutions of different pH values in the presence of CTAB micelles ([CTAB] = 0.01 M) and by fitting the data to the adapted Henderson−Hasselbach eq 2,

$$pK_a = pH + \log\frac{A_N - A}{A - A_I} \qquad (2)$$

where A_N is the absorbance of the neutral form of the antioxidant, A_I is the absorbance of the ionized form, and A is the absorbance at any pH. Values of p$K_{app} = 4.18 \pm 0.03$, p$K_{app} = 4.11 \pm 0.15$, and p$K_{app} = 4.20 \pm 0.10$ were obtained for VC, VC12, and VC16, respectively. Such values are essentially the same as those reported for VC in aqueous solution, p$K_a = 4.17$ or p$K_a = 4.25$, thus indicating that CTAB micelles have a negligible effect on the acid−base process[38] and that the addition of the alkyl hydrocarbon tail to the ascorbic moiety has an insignificant effect on the ionization constant of the resulting molecules.

Results

1. Estimation of the Association Constant of VC, VC12, and VC16 to CTAB Micelles. The association constant, K_{VC}, of VC to CTAB micelles, equilibrium A and B in Scheme 3,[39] was estimated spectrophotometrically at constant ionic strength ($I = 0.10$ M) by monitoring the shifts in the absorbance of its UV−vis spectrum at $\lambda =$ 243 nm at two different pH values within the [CTAB] = 0−0.2 M range and by fitting the data to eq 3 using a commercial computer program.

$$\frac{1}{A - A_0} = \frac{1}{(A_\infty - A_0)K_{VC}D_n} + \frac{1}{A_\infty - A_0} \qquad (3)$$

In eq 3, A is the measured absorbance at any surfactant concentration, A_0 is the initial absorbance (no added surfactant), A_∞ is the absorbance value at high [CTAB], i.e., when the substrate has been totally incorporated into the micelle, and D_n is the concentration of micellized

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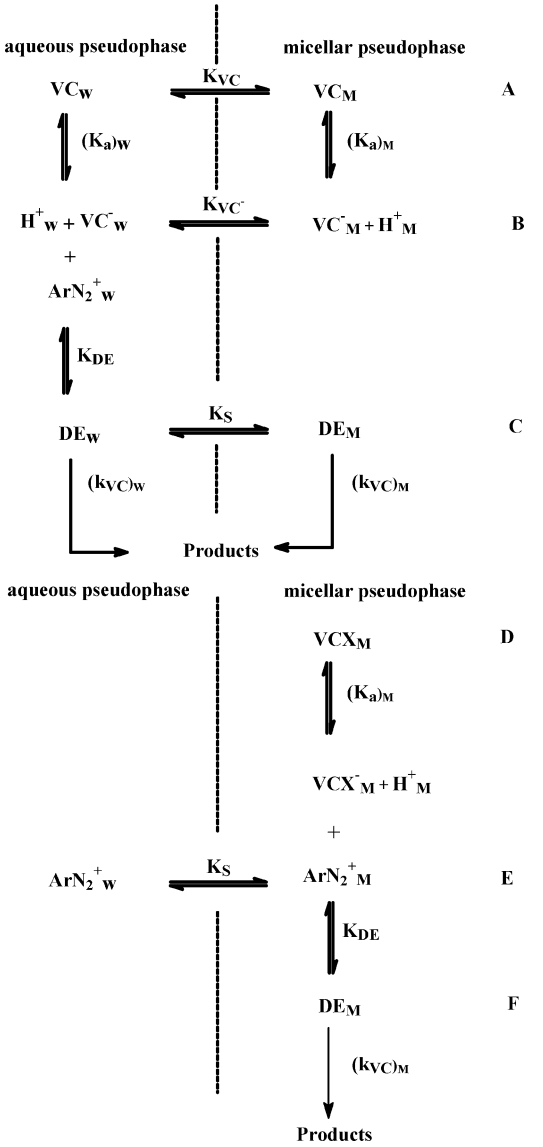
(38) In cationic micellar systems, pK_a shifts of up to 1 unit are usually found. The effect of TTAB and CTAB micelles on the apparent pK_a of VC and its derivatives will depend on their degree of association with the micelles, which is pH dependent (see the Results). Shifts in pK_a also depend on the intrinsic pK_a of the micellar-bound ascorbic acid derivative, so it may happen that binding shifts the pK_a in one direction, and the medium effect of the micellar surface shifts it in the opposite direction, so that the net result is only a small change in the measured pK_a.
(39) Because VC is a weak acid, the observed association constant for VC will be a combination of the binding constant for VC, K_{VC}, and that for its monoanion, K_{VC^-}; the extent of contribution of VC and VC$^-$ to the observed association constant will depend, therefore, on pH.

Scheme 3. Proposed Dediazoniation Mechanism for the Reaction between Arenediazonium Ions and Ascorbic Acid (VC, Top) and that for the Hydrophobic Derivatives (VCX, X = 12, 16, Bottom) in the Presence of Cationic Micelles[a]



[a] In both mechanisms we do not include the equilibrium for ion exchange for the sake of clarity and because it was not studied directly in the present work. In addition, the thermal decomposition reaction of 3MBD is not included. K_a stands for the ionization constant of VC derivatives; K_{VC} and K_{VC^-} symbolize the association constants of the ascorbic and ascorbate derivatives, respectively, to the micelles; K_{DE} stands for the equilibrium constant for the formation of the transient diazo ether DE; K_s is the association constant of DE to the micelles; $(k_{VC})_w$ and $(k_{VC})_M$ stand for the rate constants for the decomposition of DE in the aqueous (W) and micellar (M) pseudophases.

surfactant, i.e., $D_n = [CTAB_T] - cmc$. Linear plots (not shown) were obtained at two different wavelengths from which average values of $K_{VC} = 30 \pm 1$ (pH = 2) and $K_{VC} = 270 \pm 30\ M^{-1}$ (pH = 4) were obtained. The K_{VC} values are higher than those obtained with anionic SDS micelles[25] ($K_{VC} \sim 6$) but still relatively low, reflecting the high hydrophilicity of VC due to the large number of −OH groups in the molecules. The estimated K_{VC} value at pH = 4 is higher than that at pH = 2 probably because of the ionization of VC, which behaves as a diprotic acid with pK_a values of 4.2 and 11.4 for the C_3 and C_2 atoms, respectively. Hence, VC appears to partition between the aqueous and the micellar Stern layer, and about 50% of the total added VC is incorporated into the CTAB micellar aggregates when [CTAB] = ∼0.03 M (pH = 2).

Table 1. Micellar Effects on k_{obs} and Product Distribution for Dediazoniation of 3MBD in the Absence of Antioxidant and Surfactant

10^2 [CTAB] M	$10^4\ k_{obs}\ s^{-1}$	Y_{ArOH}[a]	Y_{ArH}[a]	Y_{ArBr}[a]
0	8.1	93.2		2.5
3.3	8.3	86.3		1.5
6.6	8.3	86.3	1.0	1.2
10.0	8.3	92.0		1.5
13.3	8.3	86.3	1.3	1.6

[a] *Y* stands for yield.

VC12 and VC16 derivatives may behave as surface-active reagents and self-aggregate with the reactive ascorbic ring exposed to the aqueous medium.[13,40] Some of their surfactant properties have been determined; for instance, it has been determined by surface tension measurements that VC12 and VC16 form micelles with cmc = 1.5×10^{-3} M and cmc = 2.6×10^{-4} M,[41] respectively, but other reports indicate that VC16 undergoes self-micellization at a critical micelle temperature of $T = 62.5$ °C.[34] On the basis of the effect of the hydrocarbon chain length on the association constant of the substrates with micelles,[42] it can be expected that the values of K_{VC} for VC12 and VC16 with cationic micelles should be on the order of $\sim 10^5\ M^{-1}$ and probably are pH dependent; hence, both VC12 and VC16 should be totally incorporated into the cationic micellar aggregates, and mixed micelles should be formed. This prediction is confirmed by the results of Wen et al.,[33] who analyzed the micellar effects on the oxidative electrochemistry of lipophilic vitamin C derivatives concluding that premicellar aggregates of CTAB and VC12 and VC16 are formed, with the latter being the main component at low CTAB concentrations.

2. Effects of Antioxidant Concentration on k_{obs} for Dediazoniation of 3MBD at Fixed Surfactant Concentration. The effects of CTAB and antioxidant concentration were analyzed by using buffer-controlled pH both at a fixed [CTAB] and variable [antioxidant] and by varying [CTAB] at selected antioxidant concentrations. Initially, we investigated the effects of CTAB on the spontaneous decomposition of 3MBD in the absence of antioxidant within the [CTAB] = 0−0.02 M range; the results are displayed in Table 1 showing that, upon increasing [CTAB], k_{obs} values remain essentially equal to that in its absence, the average being $k_{obs} = 8.2 \times 10^{-4}\ s^{-1}$. This k_{obs} value is equal to that previously determined in aqueous acid solution,[43] but the constancy in k_{obs} values upon changing [CTAB] contrasts with the behavior found when employing SDS micelles, where a decrease close to 50% was found.[25]

Figure 1A shows the effects of increasing antioxidant concentration on k_{obs} in the absence and in the presence of a fixed [CTAB]. VC12 and VC16 derivatives are virtually water insoluble, and so no comparative studies in aqueous acid solution could be done. The k_{obs} values for VC in the absence of CTAB, Figure 1A, follow a saturation kinetics profile, in agreement with previous results.[24] Upon

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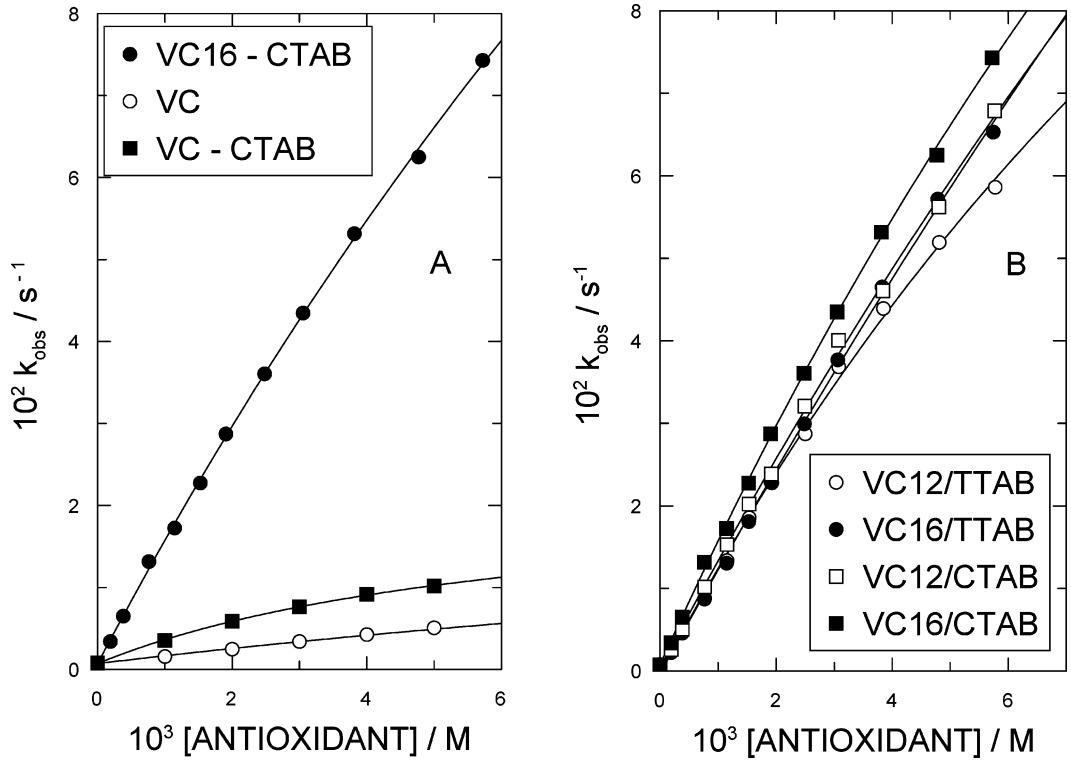

Figure 1. (A) Effects of antioxidant concentration on k_{obs} for dediazoniation of 3MBD in the presence and absence of CTAB. ○ VC, [CTAB] = 0 M; ■ VC, [CTAB] = 0.02 M; ● VC16, [CTAB] = 0.02 M. (B) Effects of antioxidant concentration on k_{obs} for VC12 and VC16 in the presence of TTAB and CTAB. ○ VC12/TTAB; ● VC12/CTAB; □ VC12/CTAB; ■ VC16/CTAB. [TTAB] = [CTAB] = 0.02 M, [3MBD] $\sim 2 \times 10^{-4}$ M, pH = 2 (buffered), T = 35 °C. Solid lines were obtained by fitting the data to eq 4.

addition of a fixed [CTAB] = 0.02 M, a similar saturation kinetic profile is observed, but k_{obs} values are somewhat higher than those in its absence depending on [VC]. For instance, when [VC] = 5×10^{-3} M, $k_{obs} = 5.09 \times 10^{-3}$ s^{-1} ([CTAB] = 0 M); meanwhile $k_{obs} = 10.17 \times 10^{-3}$ s^{-1} when [CTAB] = 0.02 M, i.e., k_{obs} values are higher by a factor of ~2.

Figure 1A also shows that the reaction of 3MBD is strongly accelerated with VC16 in the presence of CTAB micelles compared with that of VC. For example, when [VC] = [VC16] = 3×10^{-3} M, k_{obs}(VC) = 7.66×10^{-3} s^{-1}; meanwhile k_{obs}(VC16) = 4.35×10^{-2} s^{-1}, and only the initial linear region of the presumed saturation profile is observed. It was not possible to use higher VC16 concentrations because of solubility problems and because k_{obs} values become too high to be measured accurately. The finding of saturation kinetic profiles either in the presence or absence of CTAB strongly suggests that CTAB micelles do not change the mechanism of the reaction, as was found when employing SDS micelles and other ascorbic acid derivatives.[26] This assumption was confirmed by HPLC analyses of the reaction products (see below).

Figure 1B shows the variation in k_{obs} upon increasing [VC12] and [VC16] in the presence of TTAB and CTAB micelles. The kinetic profiles are very similar, independent of which antioxidant or surfactant employed; in all cases only the first linear portion of the presumed saturation kinetics profile is shown because k_{obs} values become too large to be measured accurately and because of antioxidant solubility problems. At low antioxidant concentrations, k_{obs} values appear to be independent of the antioxidant and surfactant employed, but deviations become important at increasing antioxidant concentrations. Curiously, k_{obs} values for VC12 in CTAB are very similar to those for VC16 in TTAB at any antioxidant concentration.

3. Effects of [CTAB] on k_{obs} for Dediazoniation of 3MBD at Fixed [Antioxidant]. Figure 2 illustrates the variation in k_{obs} upon increasing CTAB for the reaction of 3MBD with VC at pH = 3. At the same pH but with VC16, the reactions were too fast to be measured, and for this reason experiments with hydrophobic antioxidants were done at pH = 2, Figure 3.

For the reaction with VC, Figure 2, addition of CTAB makes k_{obs} values increase rapidly by a factor of 2−3 up to a maximum at [CTAB] ≈ 0.01−0.03 M after which further addition of CTAB makes k_{obs} to decrease smoothly. The k_{obs} values in the absence of CTAB, which depend on the [VC], are in keeping with those previously reported in aqueous solution under similar experimental conditions.[24]

When employing VC12 or VC16, Figure 3, again similar bell-shaped profiles as those for VC are found, independent of the surfactant employed, but they appear to be sharpened. Higher antioxidant concentrations could not be employed because the k_{obs} values at the maximum were too large to be measured, and values in the absence of surfactant could not be obtained because of the solubility problems mentioned. The k_{obs} values are essentially independent of the ascorbic acid derivative and surfactant employed, as they should be in view of the data in Figure 1B. Bell-like profiles such as those shown in Figures 2 and 3 resemble those typically found for bimolecular reactions in micellar systems where concentration−dilution effects of reactants may play an important role.[20,21,23]

4. Effects of CTAB and Antioxidant Concentrations on the Product Distribution. Prior to determining the effects of CTAB micelles and antioxidant concentration on the reaction with VC and VC16, it was considered appropriate to investigate the effects of CTAB micelles on the product distribution for the spontaneous

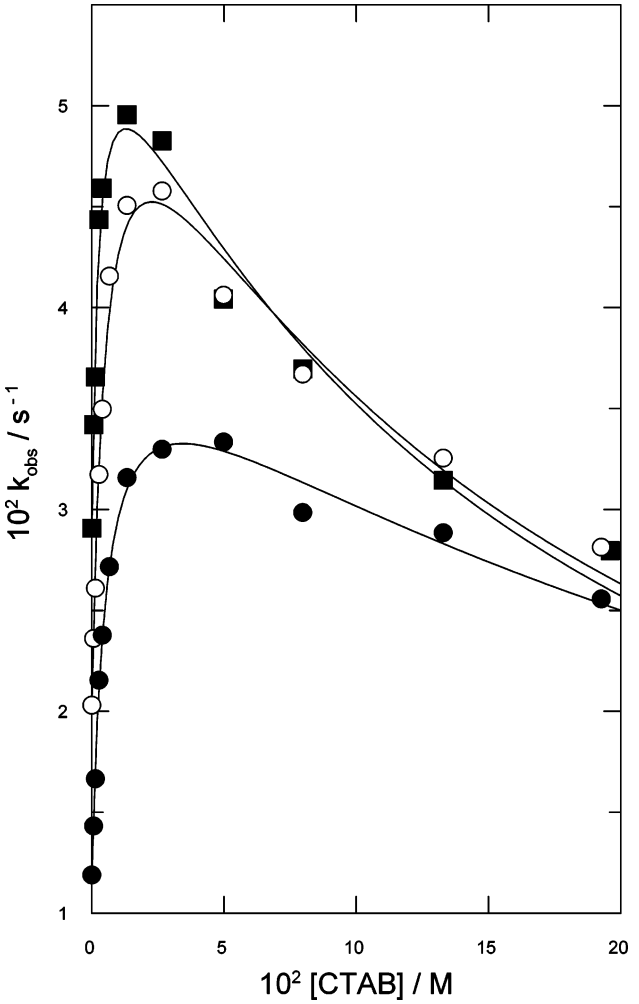

Figure 2. Effects of [CTAB] on k_{obs} for dediazoniation of 3MBD with VC. [3MBD] $\sim 2 \times 10^{-4}$ M, $T = 35$ °C, pH = 3 (buffered). ● [VC] = 5 [3MBD]; ○ [VC] = 10 [3MBD]; ■ [VC] = 20 [3MBD]. Solid lines fitted to aid the eye.

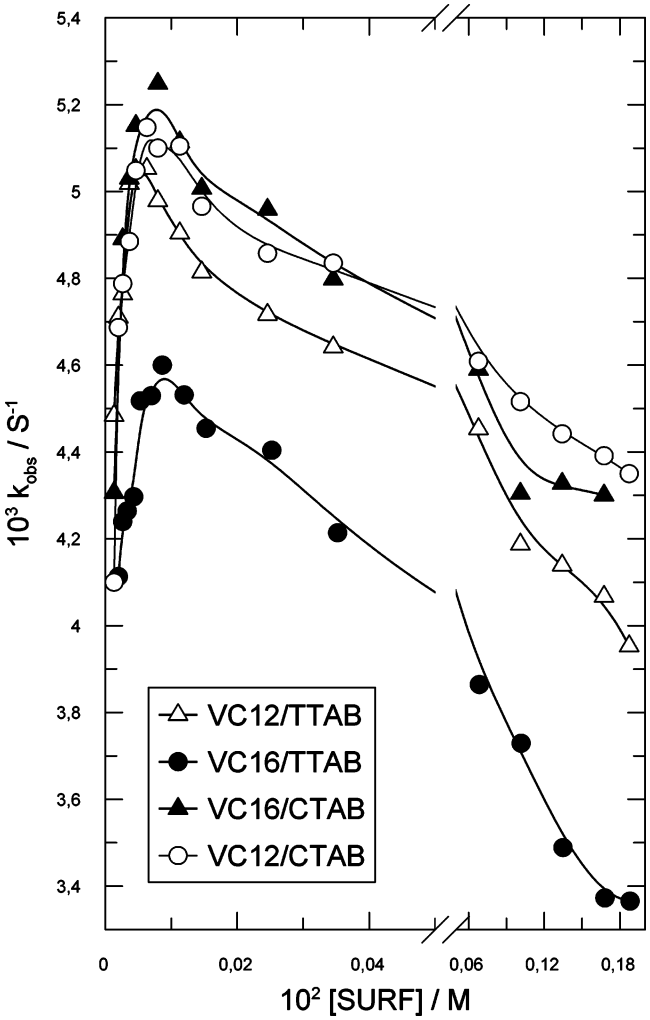

Figure 3. Effects of [CTAB] on k_{obs} for dediazoniation of 3MBD with VC12 and VC16 in the presence of TTAB and CTAB micelles. [3MBD] = 1×10^{-4} M, [antioxidant] = 4×10^{-4} M, pH = 2 (buffered), $T = 35$ °C. Solid lines fitted to aid the eye.

dediazoniation of 3MBD in the absence of antioxidant, Table 1. As shown, only heterolytic products were detected, and quantitative conversion was achieved. ArOH is the major dediazoniation product (~88%), and only very small percentages (<3%) of the ArBr derivative, formed from reaction of 3MBD with nonassociated Br^- ions from CTAB, were detected.

Figure 4 shows the effects of [VC] on the product distribution at pH = 2 in the absence of surfactant. No extraneous peaks other than the front peak and those for the heterolytic ArOH and homolytic ArH derivatives were observed in the chromatograms. The total yield decreases upon increasing [VC] to ~40% at [VC] = 20×10^{-3} M. Similar variations in product yields were previously found and attributed to the formation of the stable *Z*-diazo ether derivative, which elutes with the front peak.[24−26] Figure 5, parts A (pH = 2) and B (pH = 3), illustrates the effects of [VC16], and Figure 5C shows those of [VC12] on product distribution at a fixed CTAB concentration. In all cases, no unexpected HPLC peaks were observed, nonquantitative yields were obtained, and the total yield of homolytic and heterolytic products ranged from 20% to 40%.

Comparison of results in Figures 4 and 5 indicates that at antioxidant concentrations higher than 1×10^{-3} M, total product distribution does not depend on the nature of the antioxidant; however, in the presence of CTAB, much lower amounts of ArH are obtained compared with those in its absence, Figure 4. The yield of ArH is only slightly higher than that of ArOH, suggesting that CTAB micelles promote the formation of the thermodynamically more stable *E*-diazo ether.

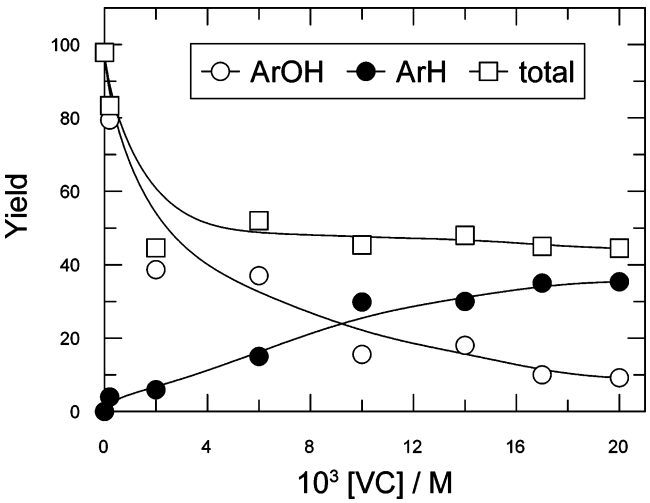

Figure 4. Dediazoniation product distribution for the reaction between VC and 3MBD in the absence of surfactant. [3MBD] = 2×10^{-4} M, pH = 2 (buffered). Reactions were allowed to proceed for at least 8 half-lives, and samples were analyzed within 24 h. Solid lines fitted to aid the eye.

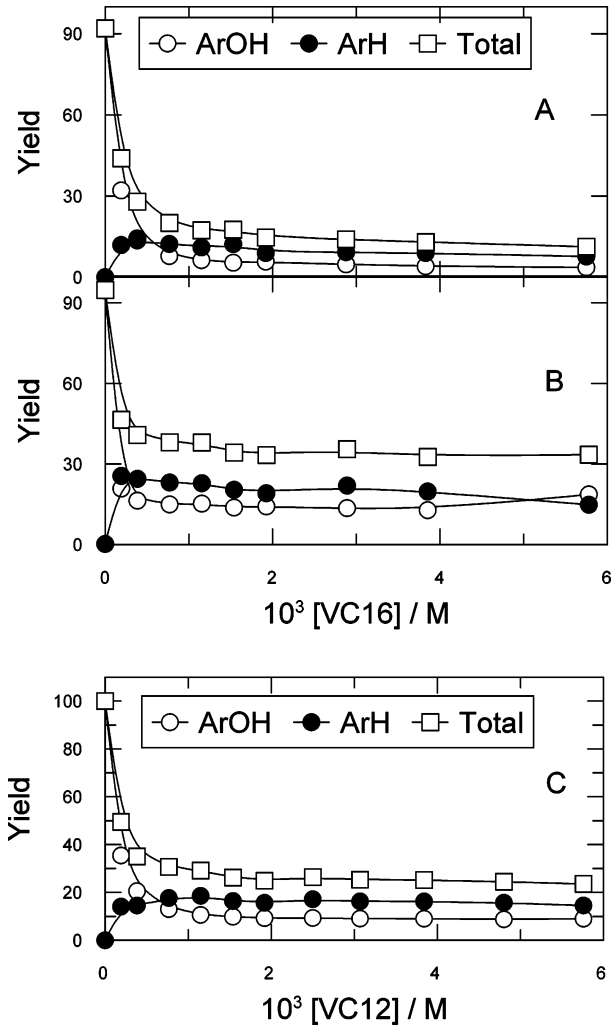

Figure 5. Effects of VC on 3MBD dediazoniation product distribution in the absence of surfactant. [3MBD] = 1×10^{-4} M, pH = 2 (buffered), T = 35 °C. Reactions were allowed to proceed for at least 8 half-lives, and samples were analyzed within 12 h. Solid lines fitted to aid the eye.

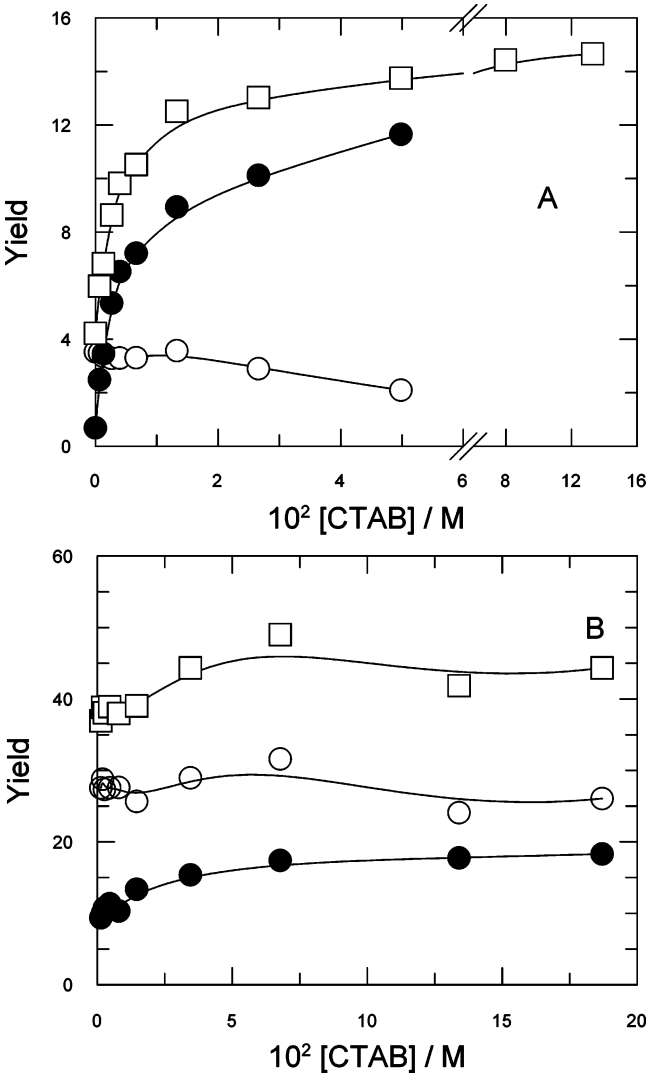

Figure 6. Effects of [antioxidant] on 3MBD dediazoniation product distribution in the presence of [CTAB] = 0.01 M at different buffered pH values. (A) pH = 2. (B) pH = 3. [3MBD] ~ 1×10^{-4} M, T = 35 °C. Reactions were allowed to proceed for at least 8 half-lives, and samples were analyzed within 12 h. Solid lines fitted to aid the eye.

The effects of CTAB micelles on product distribution were determined by HPLC analyses of the reaction mixtures at selected [antioxidant] and fixed pH, Figure 6, parts A (VC) and B (VC16). Again, no extraneous peaks other than that for the front peak and those for the ArOH and ArH derivatives were detected. Note that for both VC and VC16, total yields increase slightly upon increasing [CTAB], mostly due to the increase by a factor of ~2 in ArH yields, and saturation is found when [CTAB] > 5×10^{-2} M either for VC or VC16.

Discussion

The kinetic and HPLC data in Table 1 confirm that CTAB micelles do not change the mechanism of the spontaneous dediazoniation of 3MBD, Scheme 2A, in keeping with previous reports for other arenediazonium ions in cationic micelles.[44] Figure 1 shows that either in the presence or the absence of CTAB micelles, k_{obs} values follow saturation kinetics upon increasing [antioxidant], and the HPLC product distribution, Figures 5 and 6, shows that no unexpected products other than ArOH, ArH, and the recognized Z-diazo ether are formed. All evidence suggests, therefore, that CTAB micelles do not modify the mechanism of the reaction. From Scheme 2, the observed rate constant for the reaction is given by eq 4, which predicts that k_{obs} is independent of [antioxidant] at high antioxidant concentrations.[24]

$$k_{obs} = \frac{k_w + k_{VC}B[VC]_T}{1 + B[VC]_T} \quad (4)$$

where k_w and k_{VC} are the rate constants for the thermal decomposition of ArN_2^+ in the absence of VC and for the cleavage of the DE diazo ether, respectively. B is given by eq 5

$$B = \frac{K_a K_{DE}}{K_a + [H_3O^+]} \quad (5)$$

where K_{DE} is the equilibrium constant for the formation of the transient diazo ether intermediate DE, and K_a the first ionization constant.

(44) Romsted, L. S. Interfacial Compositions of Surfactant Assemblies by Chemical Trapping with Arenediazonium Ions: Method and Applications. In *Reactions and Synthesis in Surfactant Systems*; Texter, J., Ed.; Marcel Dekker: New York, 2001.

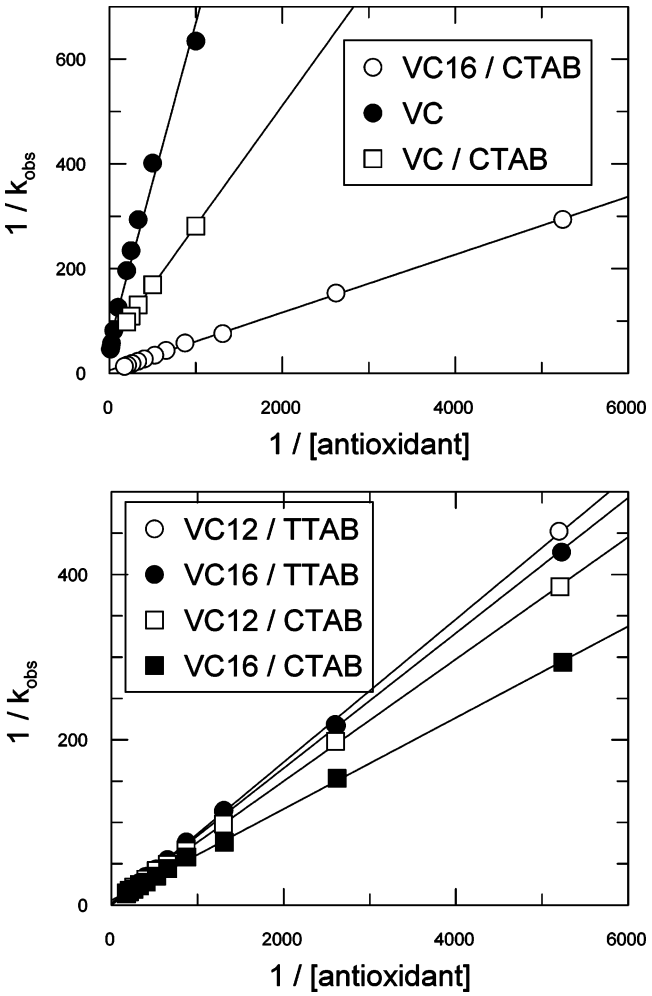

Figure 7. Double reciprocal plots ($1/k_{obs}$ vs 1/[antioxidant]) according to eq 6. Data from Figure 1.

Table 2. Parameters Obtained for VC in the Absence and Presence of CTAB Micelles

	VC	VC/CTAB
pK_a	4.2	4.18 ± 0.03
k_{vc}/M^{-1}		30 ± 1 (pH = 2)
		270 ± 30 (pH = 4)
intercept[a]	34 ± 5	53 ± 1
slope[a]	0.61 ± 0.03	0.23 ± 0.01
$10^2\ k_{vc}/s^{-1}$	2.94 ± 0.02[a]	2.1 ± 0.1[a]
	2.93 ± 0.03[b]	2.3 ± 0.1[b]
K_{DE}/M^{-1}	5750[c]	29200[c]

[a] By fitting the data in Figure 1A to eq 6. [b] By fitting the data in Figure 1A to eq 4. [c] Obtained from eq 6 by using $[H_3O^+] = 0.01$ M and $pK_a = 4.2$.

Assuming that the thermal decomposition is negligible compared with that through the formation of the transient diazo ether, i.e., $k_w \ll k_{vc}B[VC]_T$, eq 4 can be rearranged to eq 6, which predicts that a plot of $1/k_{obs}$ versus 1/[antioxidant] should be linear.

$$\frac{1}{k_{obs}} = \frac{1}{k_{VC}} + \frac{1}{k_{VC}B[VC]_T} \qquad (6)$$

Figure 7 shows such linear plots for VC, VC12, and VC16, providing further evidence for the mechanism of the reaction. Values for k_{vc}, B, and K_{DE} for the reaction with VC are shown in Table 2, but values for VC12 and VC16 could not be determined accurately because saturation is not achieved within the investigated concentration range; nevertheless, the K_{DE} values should be in the range from 10^5 to 10^6 M^{-1} because the diazo ether formed is a neutral molecule with a hydrophobic tail. It is worth noting that the k_{vc} and K_{DE} values obtained for VC in the absence of surfactant are very similar to those previously reported.[24]

Figure 4 shows that, in the absence of surfactant, product yields decrease upon increasing [VC] up to about ~50%. In the absence of VC, the total yield is quantitative in agreement with previous results, and the low yields were interpreted in terms of diazo ether formation.[24,26] The formation of the transient diazo ether was detected electrochemically in aqueous systems when studying the reaction between 3MBD and VC and 6-O-octanoyl-L-ascorbic acid,[26] but unfortunately it was not possible to detect it in the presence of CTAB micelles because the polarographic CTAB signal masks those for the expected diazo ether.

The observed low yields in Figures 5 and 6 can be explained bearing in mind the processes involved in the formation and decomposition of the transient *Z*-diazo ether, which basically occur in two steps.[24,45] The first one is the very rapid formation of a kinetically controlled *Z*-diazo ether which, in a second step, decomposes to yield reduction products (usually hydro-dediazoniation) and the rest isomerizes to the thermodynamically more stable *E*-diazo ether, which elutes with the front peak together with the excess of VC and other ions in the system. The bond-rotating mechanism to transform the *Z* isomer into the much more stable *E* derivative has been described in aqueous solution,[46] but nothing is known about the micellar effects on the reactivity and stability of such diazo ethers. These studies will be part of future reports.

The results in Figures 2 and 3 showing variations in k_{obs} for VC, VC12, or VC16 following bell-like profiles clearly contrast with the expectation of an inhibition of the reaction because of the electrostatic, micellar-induced, separation of reactants as was observed in the presence of anionic SDS micelles.[25,26] Such bell-like profiles are usually explained in terms of co-ion incorporation into the micellar Stern layer,[20,23] i.e., addition of surfactant leads to the binding of both reactants into the micellar pseudophase, and this increased local concentration increases the reaction rate, and eventually, further addition of surfactant dilutes the reactants in the micellar pseudophase and the rate falls.

The maximum rate constant for the reaction between 3MBD and the lipophilic VC12 and VC16 derivatives, Figure 3, is achieved at CTAB concentrations of 10^{-4} M, a concentration about 10 times lower than the cmc of CTAB in pure water.[15] There is extensive evidence that substrates and surfactants interact at [surfactant] < cmc, even considering the well-known decrease in the cmc induced by ionic and organic compounds.[20,23,47,48] For example, in many reactions, rate constants increase at [surfactant] < cmc, especially with hydrophobic reagents,[20] and in some reactions double rate maxima are observed.[48] Hence, the results in Figure 3 can be rationalized by considering the formation of kinetically active premicellar assemblies of CTAB and VC12 or VC16. This hypothesis is consistent with the results reported by Wen et al.,[33] who were able to demonstrate in a convincing way the formation of such aggregates, with VC12 or VC16 being the main compo-

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(46) Hanson, P.; Jones, J. R.; Taylor, A. B.; Walton, P. H.; Timms, A. W. *J. Chem. Soc., Perkin Trans. 2* **2002**, 1135.
(47) Cerichelli, G.; Mancini, G.; Luchetti, L.; Savelli, G.; Bunton, C. A. *Langmuir* **1994**, *10*, 3982.
(48) Cuenca, A. *Langmuir* **2000**, *16*, 72.

nents. Hence, the increase in k_{obs} can be attributed to a concentration effect. Further addition of CTAB causes formation of aggregates with CTAB monomers being the main component and solubilizing VC12 or VC16 into the micelles, decreasing k_{obs} because of a combination of the dilution effect and the electrostatic, micellar-induced, separation of reactants.

The results with VC could be interpreted in terms of co-ion incorporation, but substantial evidence coming from different directions suggests that the hypothesis of 3MBD ions being incorporated into the micellar Stern layer of CTAB micelles as co-ions does not seem to be likely but insinuates that 3MBD ions are sampling in the bulk aqueous environment, and hence other effects need to be considered.[49]

Cuccovia et al.[50] demonstrated, by employing fluorescence methods, that 2,4,6-trimethylbenzene diazonium ions, which are more hydrophobic than our 3MBD probe, do not associate to the cationic CTAB micellar aggregates. Results in later dediazoniation work, where the short-alcohol-induced micelle breakdown was monitored by chemical trapping of Br^- ions,[51] are also consistent with the assumption that the 3MBD probe does not associate with CTAB micelles. In addition, rate increases in bimolecular reactions due to co-ion incorporation into the micellar aggregate are usually relatively small,[20,23] but those observed here, up to 2−3-fold, see Figure 2, seem too large to be rationalized exclusively in terms of co-ion incorporation. Furthermore, the data in Table 1 also appear to confirm the hypothesis that 3MBD is located in the intermicellar bulk aqueous phase because of the very low yields of ArBr; association of a fraction of the 3MBD probe with CTAB micelles would make a significant contribution to the observed ArBr yields because the concentration of Br^- ions at the surface of CTAB micelles is about 2−3 orders of magnitude greater than the stoichiometric surfactant concentration.[50] Ranganathan et al.[52] recently reported equilibrium and dynamic aspects of the partitioning of organic co-ions between the aqueous and micellar pseudophases of ionic surfactants and concluded that the transition state for organic co-ion incorporation into micelles is located just outside the Stern layer; appreciable micellar incorporation takes place only when the co-ion has sufficient amphiphilic character to counterbalance the electrostatic repulsion.

A more plausible interpretation of the bell-shaped profiles in Figure 2 can be given in terms of the pseudophase model, Scheme 3, by analyzing the effects of micelles on the different equilibrium involved. Kinetic expressions for Scheme 3 may be derived, and the final rate equation will be a composite of expressions used for micellar effects in bimolecular reactions and in acid−base equilibrium;[20] nevertheless, any quantitative treatment is unreliable at the present stage because of the number of variables that need to be determined or assumed.

The data in Figure 2 show that the maximum rate constant for the reaction between 3MBD and VC is achieved at CTAB concentrations much higher than its cmc, thus resulting in micellar catalysis. On the other hand, the data in Table 2 indicate that the K_{DE} value for diazo ether formation in the presence of CTAB micelles is about 6 times higher than that in its absence, and the resulting diazo ether should bind more strongly than the parent compounds to the micelles because of its somewhat hydrophobic nature and because it is neutral. Hence, the observed increase in the rate constant may be explained in terms of cationic micelles favoring the formation of the transient diazo ether by shifting the equilibrium in Scheme 3C and by incorporating it to the micellar aggregate. This would require DE formation to be rapid and reversible, as we have demonstrated,[24] and ion exchange between Br^- ions and the reactive VC^- ions may also have a role (see the footnote in Scheme 3).

As discussed before, the apparent K_{VC} values for the association of VC to CTAB micelles are pH dependent[39] and far from being negligible; K_{VC} for VC at pH = 2 is 30 M^{-1}, see the Results. Hence, the interfacial concentrations of VC increase upon increasing [CTAB]. Conversely, the interfacial concentrations of the monoanions VC^-, which are the reactive species, Scheme 2B, decrease with increasing [CTAB] at constant pH, because the bulk pH is buffer controlled but that of the interfacial region is not; thus, interfacial acidity should increase upon increasing [CTAB], shifting the equilibrium between the antioxidant and its monoanion toward the neutral forms and removing them from the bulk aqueous phase, thereby inhibiting the reaction.

Conclusions

In conclusion, the results shown in this paper indicate that the presence of cationic surfactants has no effect on the pK_a of VC derivatives and does not change the reaction between 3MBD and ascorbic acid derivatives, which proceeds through two competitive pathways, the thermal $D_n + A_N$ mechanism and a rate-limiting decomposition of a transient diazo ether, DE, formed from reaction between 3MBD and the monoanion form of ascorbic acid, VC^-, in a rapid preequilibrium step. The micellar effects on the reaction can be interpreted in terms of the pseudophase model and by taking into consideration the effects of micelles on the different equilibria involved. At a given [CTAB], the reaction with VC16 is much faster than that with VC, but there are no significant differences in the kinetics of 3MBD with VC12 and VC16 either in the presence of CTAB or TTAB. The results in Figures 2 and 3 show that the reactions between 3MBD and the VC derivatives are micelle-catalyzed, with k_{obs} values following bell-shaped curves in clear contrast with those results obtained when employing anionic SDS micelles, where an inhibition of the reaction was found. By fitting the data to the equation derived from the proposed mechanism, values for the decomposition of the diazo ether and for the equilibrium constant in the presence of cationic micelles were obtained. The nonquantitative yields obtained in the presence of micellar aggregates are attributed to the formation of the thermodynamically stable *E*-diazo ether, which elutes with the front peak.

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(49) In previous work with 6-O-octanoyl-L-ascorbic acid, we tentatively hypothesized that 3MBD ions could be partially incorporated into the micellar Stern layer of cationic micelles, behaving as co-ions of the cationic monomers. Results in this work point out that such incorporation cannot completely explain the experimental results, and hence, other phenomena should be taken into consideration.

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(51) Pazo-Llorente, R.; Bravo-Díaz, C.; González-Romero, E. *Langmuir* **2003**, *19*, 9142.

(52) Ranganathan, R.; Okano, L. T.; Yihwa, C.; Alonso, E.; Quina, F. H. *J. Phys. Chem. B* **1999**, *103*, 1977.