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Impairment of ascorbic acid's anti-oxidant properties in confined media: Inter and intramolecular reactions with air and vanadate at acidic pH

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Abstract

The anti-oxidant properties of L-ascorbic acid were investigated in the confined medium produced by a sodium bis(2-ethylhexyl)sulfosuccinate (aerosol-OT, AOT) self-assembled reverse micelle. Using ^1H–^1H NOESY (proton-proton 2D nuclear overhauser enhancement correlation spectroscopy) NMR spectroscopy, the location of ascorbic acid was investigated and found to be at the AOT-interface in contrast to earlier studies where the ascorbate was assumed to be in the water pool in these microemulsions. The reaction of ascorbic acid with oxygen was investigated using EPR spectroscopy. A delocalized monoanionic ascorbate radical was observed in microemulsions prepared from pH 5.6 stock solutions. This is in contrast to studies carried out in aqueous media where no radical formation was observed. The oxidation of ascorbic acid by aqueous V(V) was investigated in reverse micelles. Modest changes in the kinetic parameters were observed for this system compared to that in water. Details of these reactions were examined and can be summarized as the microemulsion solvating and stabilizing reactive intermediates via rate inhibition or enhancement. The inhibition of the oxidation is due to solvation stabilization of ascorbic acid in microemulsion media. Since ascorbate is a valuable marker of oxidative stress, our results suggest that compartmentization can modify the stabilization of the ascorbate radical and the changes in properties could be important in biological systems.

Keywords: Ascorbate; L-Ascorbic acid; Anti-oxidant; Vanadate; Radical; Redox reactions; Confined media; Sodium bis(2-ethylhexyl)sulfosuccinate; AOT (aerosol-OT); Reverse micelles; Self-assembled structures

1. Introduction

Ascorbic acid (Fig. 1a) is an important biological anti-oxidant [1] that protects the cell from detrimental radicals, particularly those produced during incomplete oxidation by O_2 [2–8]. Along with cysteine, ascorbic acid protects cytoplasmic processes and maintains a reducing environment within the cell cytoplasm [9]. A better understanding of the effect of confinement of these important cellular metabolites and their reactions will assist in the interpretation of the observed changes in these compounds' reactivity in cells and non-aqueous environments. We have initiated a program to explore the effects of confinement on motion of molecules and their reactions. In this work we investigate the impact that confinement has on the properties of ascorbic acid [1] in a simple self-assembled ternary system. Specifically, we investigate two oxidation processes: the air oxidation of ascorbic acid [10–13] and the reaction of ascorbic acid with V(V) [14–16].

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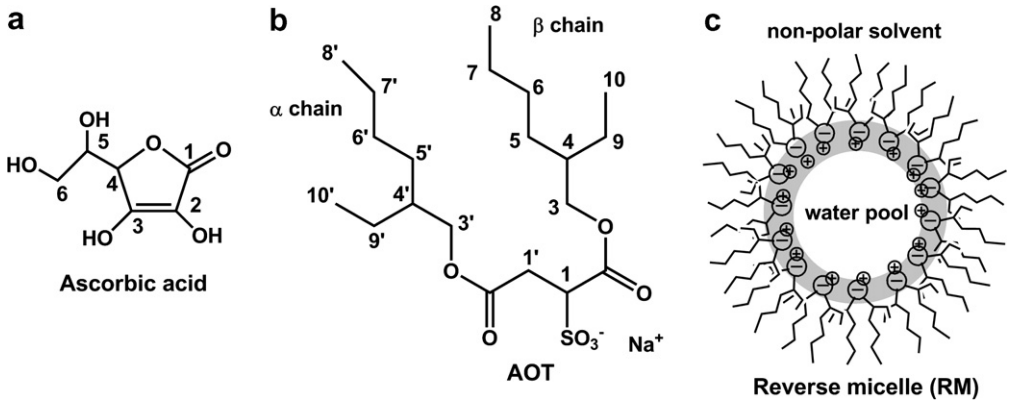


Fig. 1. Molecular structures of ascorbic acid (a), AOT (b) and a cartoon of AOT/isooctane/H_2O reverse micelle (RM) system (c). In this microemulsion system isooctane acts as the non-polar bulk solvent whereas H_2O forms the interior polar water pool and the size of the water pool is defined by, $w_0 = [H_2O]/[AOT]$ [17].

The sodium bis(2-ethylhexyl)sulfosuccinate (aerosol-OT, AOT, Fig. 1b) reverse micelle (RM) (Fig. 1c) is a simple ternary system, in which an exterior organic solvent (isooctane) surrounds the lipid interfacial layer that separates the organic media from the inner polar water pool (Fig. 1c) [17–19]. Depending on the location of a solute, its environment may be hydrophobic in the outer organic layer, or amphiphilic as in the interfacial layer or hydrophilic as in the water pool. The nature of water in the aqueous pool changes in AOT RMs because, in part, confinement disrupts the H-bonding network and changes water's microscopic properties and viscosity [20,21]. The properties of the water also depend on size of the water pool, which can be varied by changing the water to AOT surfactant concentration ratio (w_0). Furthermore, at the interfacial layer near the surfactant, referred to as the Stern layer [22], the aqueous environment differs significantly from that in the water pool in organization and proton-activity [23]. In spherical RMs, the effects of confinement on the oxidation of ascorbic acid can be explained based on the location of the ascorbic acid.

The air oxidation of ascorbic acid has previously been examined in various RMs and micelles [24,25] using a redox sensitive dye to monitor the reaction rate. Confinement in RMs was found to change the oxidation rates due to different interactions of the dye and ascorbate with the surfactant charges [24,25]. For RMs with negative or neutral headgroups, a decreased reaction rate was observed. This was attributed to a decreased encounter probability of the dye with ascorbate since the reactants were assumed to be in different locations within the RM. In contrast, increased reaction rates in RMs possessing positively charged headgroups were observed. This was attributed to increased electrostatic interactions between the reducing dye and ascorbic acid and indicates that both of these components are near the micellar surface [24,25]. Recently, we investigated the location of a polar, charged oxovanadium dipicolinate and found that it penetrates the RM interface [26]. Based on these studies, the possibility that ascorbic acid can also penetrate the lipid surface was investigated since this should affect its reactivity.

The O_2 oxidation of ascorbic acid in aqueous media is a relatively slow process at acidic pHs and involves intermediate radical formation [27,28]. Although oxygen readily dissolves in water, decreased reaction rates in RMs [29,30] have been attributed to the slower O_2 diffusion in a suspension of RMs compared to water [31]. The oxidation of ascorbic acid by vanadate was recently investigated [14], and since vanadium (abbreviated V) is a very sensitive spectroscopic probe, this reaction provided information on confinement on ascorbic acid reactions [24,25]. In addition, the insulin-enhancing properties of vanadium compounds [32–39], makes their study in confined media important because of the potential that their properties can alter cellular absorption and metabolism. In addition, the insulin-enhancing effect of ascorbic acid has recently been examined when co-administered with certain vanadium compounds [39]. These potential changes are relevant to the treatment of diabetes, particularly since one vanadium compound entered Phase 2 clinical trials in the Fall of 2007 [32].

In this work we describe two reactions of ascorbic acid in confined media: the O_2 oxidation of ascorbic acid in AOT RMs and the reaction of ascorbic acid with a mild oxidant, aqueous V(V). These reactions were investigated to gauge the impact on confinement on the anti-oxidant properties of ascorbic acid and the reported increase in insulin-enhancing effectiveness of some V(V)-compounds when co-administered with ascorbate [39].

2. Experimental

2.1. Materials and methods

Ammonium metavanadate, NH_4VO_3 (99%) sodium metavanadate, $NaVO_3$ (99.9%), L-ascorbic acid (99%) and AOT (sodium bis(2-ethylhexyl)sulfosuccinate or Docusate sodium salt) (99%) were purchased from Sigma–Aldrich.

AOT was purified by dissolving in methanol and stirring it overnight in the presence of activated charcoal. Subsequent filtration and removal of methanol by distillation under vacuum yielded AOT suitable for use. Isooctane was received from Aldrich and cyclohexane, hydrochloric acid were obtained from Fisher Scientific and were used without further purification. D_2O was obtained from Cambridge Isotope Laboratories Inc. Deionized water was used throughout and prepared to a specific resistivity of >18 MΩ cm (Barnstead E-pure system). The pH values of the aqueous solutions were measured using a Orion 420 A pH meter equipped with pH gel semi-micro combination electrode (Cat # 14002-766). The pH values were adjusted to within 0.05 and the drift in pH between preparation and measurement were within 0.1 pH units unless otherwise noted.

2.2. Stock solution preparation

Vanadate and L-ascorbic acid (H_2AA) stock solutions for spectroscopic measurements were prepared by dissolving $NaVO_3$ into water in volumetric flask, adjusting the pH before making up the final volume. A series of 0.200 M V(IV) and V(V) stock solutions were prepared at pH 1.3, 3.8 and 3.9 and a series of ascorbate solutions at pH 1.2, 2.8 and 4.2. Similarly, a series of 0.100 M-ascorbic acid stock solutions were prepared at pH 2.0, 4.4 and 5.6. For all these stock solutions pH was adjusted to required value using 1 M NaOH/1 M HCl before making up the final volume. All solutions were prepared freshly just before the spectroscopic measurements. The pH adjusted 0.200 M vanadate and 0.200 M ascorbic acid stock solutions were mixed in 1:1 ratio just before the EPR measurements. The 1:1 mixture of V(IV) (0.100 M) and ascorbic acid (0.100 M) solutions at pH 2.0, 4.4 and 5.6 were also prepared by mixing the stock solutions immediately before the EPR measurements. The aqueous stock solutions of ascorbic acid for 1D and 2D NMR spectroscopy were prepared in D_2O of concentration 0.100 M at pH 4.5. Stock solutions of ascorbic acid were also prepared in D_2O at pH 2.2 and 5.8 for 1D NMR spectroscopy. The indicated pHs were those measured using a glass electrode, and addition of 0.4 pH units are needed to adjust for the presence of D_2O [40]. For each sample, the pH values were adjusted to within 0.05 and the drift in pH between preparation and measurement were within 0.1 pH units, unless otherwise noted.

2.3. Reverse micelle (RM) preparation

A 0.200 M AOT stock solution was prepared by dissolving AOT in isooctane under ambient conditions. RMs were prepared by pipetting the 1:1 mixtures of V and L-ascorbic acid, at a set pH, into aliquots of the AOT stock solution in isooctane. The w_0 value was adjusted by pipetting a specific volume of pH measured aqueous V(V) and L-ascorbic acid 1:1 mixtures to the AOT stock solution. All samples were mixed by shaking prior to spectroscopic measurements and the resulting solutions were transparent. For the EPR experiments RMs of w_0 equal to 12 were used. In the NMR experiments D_2O was used and for RM with w_0 equal to 20 the pH was 4.5 whereas for w_0 equal to 16 the pH values were either at 2.2 or 5.8.

Dynamic light scattering experiments were performed to demonstrate that RMs form in solution and to measure their size and polydispersity (DynaPro-MSTC). All the measurements were performed at 25 °C and yielded results similar to those reported in the literature [41].

2.4. NMR spectroscopy

All NMR experiments were performed on a Varian Inova 400 MHz NMR spectrometer. Routine parameters were used for the 1D ^{1}H NMR experiments. The ^{1}H chemical shifts were referenced against 3-(trimethylsilyl)propane sulfonic acid sodium salt (DSS) as an external reference [42]. ^{51}V NMR was also recorded using parameters described previously [26,43].

^{1}H–^{1}H-NOESY (proton–proton 2D nuclear overhauser enhancement correlation spectroscopy) NMR experiments were performed using the supplied Varian pulse sequence. The NOESY data were acquired with a 7 kHz window for proton in F2 and F1. The NOESY mixing time, τ_{mix}, was 0.6 s with a total recycle time of 2.1 s between transients. Data sets consisted of 1K complex points in t_2 by 256 complex points in t_1 using States-TPPI. Cosine-squared weighting functions were matched to the time domain in both t_1 and t_2 and the time domains were zero-filled prior to the Fourier transform. The final resolution was 3.5 Hz/pt in F2 and 15 Hz/pt in F1. Spectrometer control was done using Solaris PC versions and data processing was done using the MestReC 4.8.6 for Windows.

2.5. EPR spectroscopy

X-band EPR spectra were recorded on a Bruker EMX 300 spectrometer at ambient temperature. The aqueous solutions were placed in 0.2 mm capillary tubes, which were inserted into 4 mm quartz tubes. The RM samples were placed directly into 4 mm quartz tubes. Spectra of V(IV) species were acquired at 9.82 GHz and 20 mW microwave power with a modulation frequency of 100 kHz, a modulation amplitude of 5.00 G, a time constant of 82 ms, a sweep width of 2000 G, a sweep time of 168 s, a resolution of 1024 points, and four scans with a central field of 3600 G and receiver gain 4.48×10^4. Spectra of C-based radical species were acquired at 9.82 GHz and 2 mW microwave power with a modulation frequency of 100 kHz, a modulation amplitude of 0.7 G, a time constant of 328 ms, a sweep width of 150 G, a sweep time of 83 s, a resolution of 1024 points, and four scans with a central field of 3480 G. A powder sample of 2,2-diphenyl-1-picrylhydrazyl ($g = 2.0036$) [44] was used as an external reference. All

experiments were recorded at ambient temperature. Data analyses were performed with a Bruker WINEPR System.

2.6. Kinetics measurements

Rate measurements were carried out using a Dionex stopped-flow interfaced with an OLIS data collection and reduction program. Pseudo first-order conditions were used throughout this study with the ascorbic acid in large excess over the V(V) concentrations. First-order traces were observed for the redox process and fit well to a single exponential decay to give observed first-order rate constants. These results were in good agreement with those previously reported [14]. The temperature was controlled to 25.0 °C using a constant temperature bath. Spectra of the observed V–ascorbic acid intermediates were taken using an OLIS RSM1000, also temperature controlled to 25.0 °C using a constant temperature bath.

3. Results

3.1. NMR characterization of L-ascorbic acid and AOT

^{1}H NMR spectroscopy can be used to monitor ascorbic acid in RMs when signals are in a different chemical shift range from AOT and organic solvent. In aqueous solution, three distinct chemical shift regions can be identified for AOT: the methylene and methyl groups from 1 to 2 ppm (H5, H5′, H6, H6′, H7, H7′, H9 and H9′ and H8, H8′, H10 and H10′); the CH groups at 1.8 ppm (H4, H4′); and the CH and CH_2 groups adjacent to the negatively charged residues (H1, H1′, H3 and H3′) from 3.0 to 4.5 ppm (Fig. 1SA). The structures and numbering scheme for L-ascorbic acid and AOT are given in Fig. 1 and their spectra are shown in Fig. 1S. The ^{1}H NMR spectrum (Fig. 1SB) for the L-ascorbic acid shows three signals and is pH dependent since ascorbic acid exists in different protonation states ($pK_{a1} = 4.25$, $pK_{a2} = 11.79$ [45–53]). Chemical shifts are given in Table 1 for each species in aqueous solutions at pH 2.2, 4.5 and 5.8 and their corresponding RM solutions.

As ascorbic acid is added to the AOT RM the chemical shifts change due to changes in its environment. The chemical shift changes are sensitive to the w_0 of the specific system, the ascorbic acid concentrations and the effective "pH" of the surrounding medium. Data for w_0 equal to 16 [F1] and 20 are shown in Table 1 for pH 2.2, 4.5 and 5.8. Since two of the three signals for ascorbic acid were distinguishable from AOT protons at the pH values examined, information was obtained on the environment of ascorbic acid in microemulsions using 1D NMR spectroscopy. The chemical shift at pH 2.2 is consistent with H_2AA, whereas at pH 4.5 and 5.8 some deprotonated species exist in aqueous solution. In the RM, both H's on C4 ($H4_A$) and C6 ($H6_A$) shift downfield compared to that of the aqueous solutions (see Table 1 and Fig. 1S). Since the magnitude of this shift for both protons remains constant, these are likely due to changes in the environment and not protonation.

3.2. NOE effects of solutes in microemulsion environments

^{1}H–^{1}H homonuclear NOESY experiments were performed on three different RM samples with aqueous solutions of the L-ascorbic acid at pH 4.5. In Fig. 2 the portion of the NOESY spectrum showing interaction (NOE) of the L-ascorbic acid (pH 4.5, w_0 equal to 20) methylene proton (CH_2, ($H6_A$)) with AOT methylene (H3′) is presented where the off diagonal cross-peaks indicate that the respective protons are near each other. Likewise, a signal labeled AOT–AOT in Fig. 2 shows that the respective two protons on AOT are also in close proximity. The off diagonal cross-peaks between AOT and ascorbic acid indicate that the H_2AA proton is near the proton on AOT. These are labeled in Fig. 2 as AOT–AA or as AOT–AOT, respectively. The protons on the AOT that exhibit strong NOEs with ascorbic acid are near the polar region of AOT (H1, H1′, H3, H3′) and not the surfactant tail-group. The observed NOE between the L-ascorbic acid and the AOT molecules show that the ascorbic acid, on average, resides in the hydrophobic part of the interface in the vicinity of the AOT head-groups.

Table 1
The ^{1}H NMR chemical shifts of ascorbic acid and AOT signals between 3 and 5 ppm in AOT microemulsion solutions in isooctane and in aqueous solution[1]

pH	w_0	Ascorbic acid			AOT			
		$H4_A$	$H5_A$	$H6_A$	H1	H3	H3′	H1′
2.2 (aq)[a]		4.95 ± 0.01	4.05 ± 0.02	3.73 ± 0.02				
2.2 (RM)[b]	16	5.09 ± 0.01		3.98 ± 0.01	4.41 ± 0.02	4.33 ± 0.02	4.17 ± 0.02	3.34 ± 0.01
4.5 (aq)[a]		4.63 ± 0.01	4.00 ± 0.01	3.72 ± 0.01				
4.5 (RM)[b]	20	4.76 ± 0.01		3.87 ± 0.02	4.36 ± 0.02	4.28 ± 0.01	4.13 ± 0.02	3.28 ± 0.02
5.8 (aq)[a]		4.50 ± 0.01	4.00 ± 0.01	3.72 ± 0.01				
5.8 (RM)[b]	16	4.74 ± 0.01		3.96 ± 0.01	4.41 ± 0.02	4.34 ± 0.01	4.17 ± 0.02	3.34 ± 0.02

[a] For aqueous solution.
[b] For reverse micelles.
[1] Attempts were made to prepare microemulsions at $w_0 = 20$ for samples at pH 2.2 and 5.8. However, the ionic strength increased at these very low and high pH values making the microemulsions less stable at $w_0 = 20$. Studies were therefore carried out on $w_0 = 16$ where samples are stable.

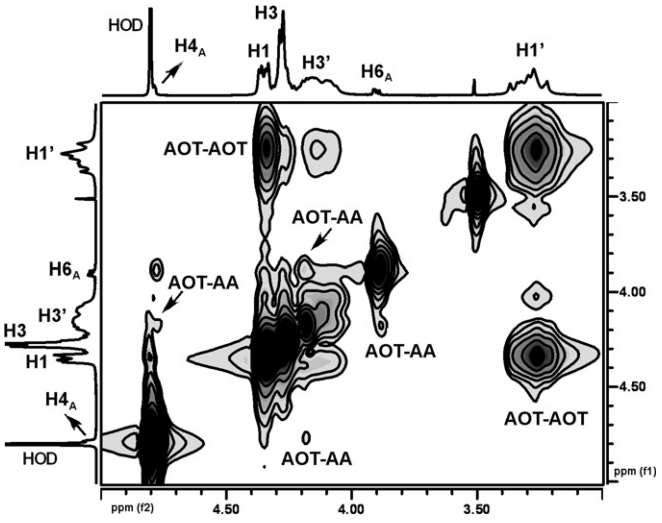


Fig. 2. Detail of the 2D NOESY spectrum recorded for the ascorbic acid in AOT microemulsions in the region showing the NOEs arising from interactions between AOT H3′ and $H6_A$ and $H4_A$ protons in ascorbic acid. Microemulsion samples prepared from 1.0 M AOT stock solution in isooctane with the 0.100 M in 100% D_2O, pH at 4.5 stock solution at $w_0 = 20$, resulting in an overall concentration of 29.0 mM ascorbic acid. The pH indicated is that measured and has not been adjusted for the presence of D_2O.

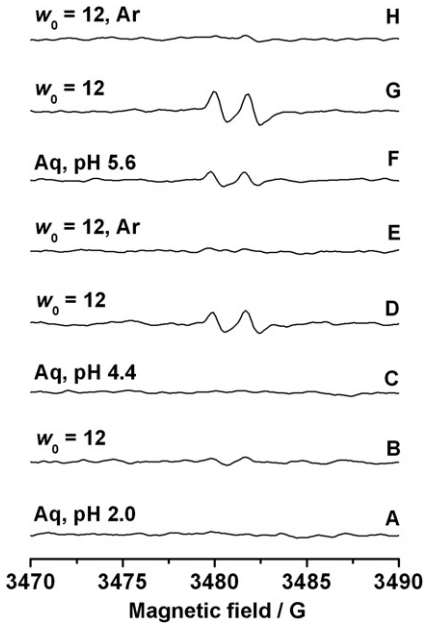


Fig. 3. Ambient-temperature EPR spectra of 0.100 M L-ascorbic acid at pH 2.0, 4.4 and 5.6 in aqueous solutions (A, C and F) and respective RM solutions (B, D, E, G and H). The spectra E and H were collected in presence of argon. The spectra were recorded using the following parameters: number of scans = 4, center of field = 3480 G, sweep width = 150 G, resolution = 1024 points, microwave frequency = 9.82 GHz, microwave power = 2 mW, receiver gain = 4.48×10^4, modulation frequency = 100 kHz, modulation amplitude = 0.7 G, time constant = 328 ms and sweep time = 83 s. All RMs solutions were of 0.200 M AOT/isooctane/H_2O with $w_0 = 12$ and the pH indicated is that measured and has not been adjusted for the presence of D_2O.

3.3. EPR characterization of ascorbic acid in aqueous and RM solutions

Formation of a delocalized carbon(C)-based radical of L-ascorbic acid in aqueous and RM solutions was investigated using EPR spectroscopy at pHs 2.0, 4.4 and 5.6. The parameters used for these studies were a microwave frequency of 9.82 GHz with a modulation frequency of 100 kHz, a spectrometer sweep width equal to 150 G and modulation amplitude equal to 0.7 G [54–56] which deviate from those we commonly used to observe EPR spectra of V-centered radicals [14,57]. The EPR spectra of 0.100 M L-ascorbic acid solution do not show any signal for the C-centered ascorbate radical at low pH (Fig. 3). Only at pH 5.6 was sufficient amount of radical formed for detection by EPR spectroscopy in aqueous solution. The EPR spectra of the microemulsions formed by these stock solutions at acidic pH and added to suspensions of AOT in isooctane showed a C-centered radical at all pH values; however, only trace amounts in the RM prepared from pH 2.0 (Fig. 3, spectrum B) was observed. The characteristic hyperfine splitting patterns and parameters observed in these spectra are identical to those of the ascorbate monoanionic radical [54–56] (Table 2) both in aqueous and RMs suspensions. These studies demonstrate that the microemulsion environment stabilize the ascorbate radical.

An EPR spectrum of an AOT RM system without ascorbic acid showed no radical formation and ruled out the possibility that the radical observed was due to a reaction with AOT and O_2. The possibility that the reaction could take place in the absence of oxygen was also examined at pH 4.4 and 5.6. Solutions were first purged with argon and then mixed to form AOT RMs. The EPR spectra showed no C-centered radical when oxygen was rigorously excluded (Fig. 3, spectra E and H); a small amount of radical was observed when not all oxygen was removed by incomplete purging with argon (Fig. 3S).

3.4. Probing formation of V(IV)–ascorbate adduct from samples added V(V) or V(IV) in aqueous solutions and RM suspensions using EPR spectroscopy: V-centered radical

Mixing V(V) and ascorbic acid leads to formation of a complex ion which subsequently undergoes electron transfer to produce a V(IV) complex that is observable by EPR spectroscopy and no longer observable by ^{51}V NMR spectroscopy [38]. Formation of the ascorbate V(IV) adduct varies with the pH of the solution as depicted in Fig. 4a. At pH 2.0 the EPR parameters are consistent with vanadyl ion (VO^{2+}) (see Table 3) [58]. At pH 4.4 a 1:2 complex [59] forms along with a trace amount of VO^{2+} [58]. At pH 5.6 the 1:2 complex [59] was observed (with g_0 equal to 1.97, A_0 equal to 102.9) as well as a second complex with parameters g_0 equal to 1.97, A_0 equal to 86.3. Two species formed at pH 2.0, 4.4 and 5.6 aqueous solutions and only one species is observable in the microemulsions prepared from these solutions.

Table 2
The EPR parameters (g_0 and A_0) observed for the C-centered radical in the aqueous and microemulsions made from 0.2 M AOT/isooctane

pH	AA/Aq[a]		AA/RMs[b]		1:1 AA + V(V)/Aq[c]		1:1 AA + V(V)/RMs[d]		1:1 AA + V(IV)/Aq[e]		1:1 AA + V(IV)/RMs[f]	
	g_0	A_0 (G)	g_0	A_0 (G)	g_0	A_0 (G)	g_0	A_0 (G)	g_0	A_0 (G)	g_0	A_0 (G)
2.0	–	–	2.006	1.80 ± 0.10	–	–	–	–	–	–	–	–
4.4	–	–	2.006	1.84 ± 0.03	–	–	–	–	–	–	2.005	1.79 ± 0.03
5.6	2.006	1.84 ± 0.02	2.006	1.81 ± 0.04	–	–	2.006	1.62 ± 0.02	–	–	2.006	1.84 ± 0.03

[a] 0.1 M of ascorbate in aqueous solution.
[b] 0.004 M of ascorbate in reverse micelle.
[c] 0.1 M of V(V) and 0.1 M of ascorbate in aqueous solution.
[d] 0.004 M of V(V) and 0.004 M of ascorbate in reverse micelle.
[e] 0.1 M of V(IV) and 0.1 M of ascorbate in aqueous solution.
[f] 0.004 M of V(V) and 0.004 M of ascorbate in reverse micelle.

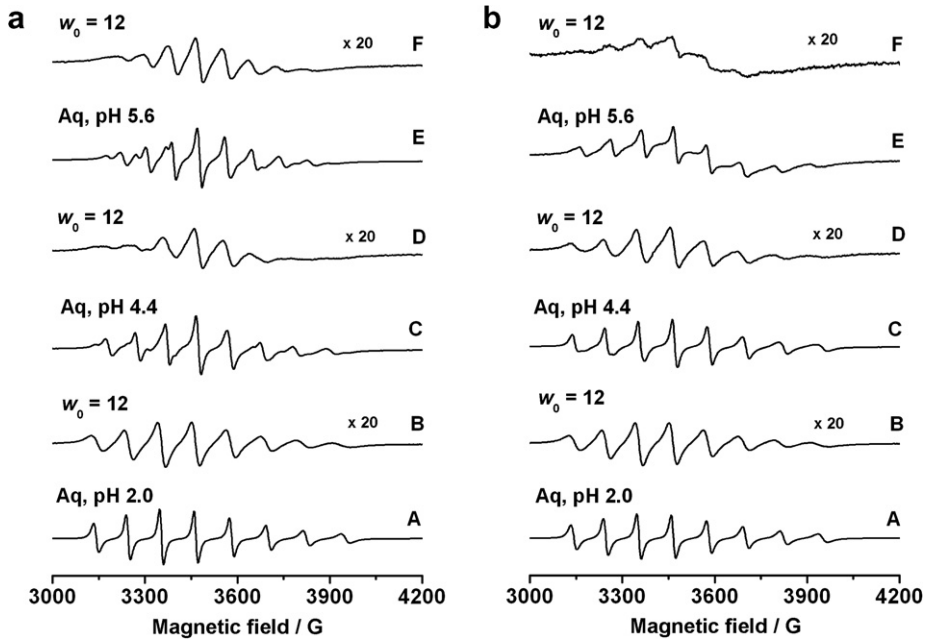


Fig. 4. Ambient-temperature EPR spectra of: (a, left) a 1:1 mixture of 0.100 M L-ascorbic acid and 0.100 M V(V) at pH 2.0, 4.4 and 5.6 in aqueous solutions (A, C and E) and respective RMs solutions (B, D and F); (b, right) a 1:1 mixture of 0.100 M L-ascorbic acid and 0.100 M V(IV) at pH 2.0, 4.4, and 5.6 in aqueous solutions (A, C and E) and respective RM solutions (B, D and F). The spectra were recorded using the following parameters: number of scans = 4, center of field = 3600 G, sweep width = 2000 G, resolution = 1024 points, microwave frequency = 9.82 GHz, microwave power = 20 mW, receiver gain = 4.48×10^4, modulation frequency = 100 kHz, modulation amplitude = 5.0 G, time constant = 82 ms and sweep time = 168 s. All RM solutions were of 0.200 M AOT/isooctane/H_2O with $w_0 = 12$. The pH indicated are those measured and are not adjusted for the presence of D_2O.

A 1:1 mixture of 0.1 M V(IV) and 0.1 M ascorbic acid was also studied in aqueous and RM solutions at pHs 2.0, 4.4 and 5.6 using EPR spectroscopy, Fig. 4b. At pH 2.0 and 4.4 only the VO^{2+} species was observed in aqueous solutions whereas at pH 5.6 a 1:2 complex [59] with g_0 equal to 1.97 and A_0 equal to 106.9 was observed. In the corresponding microemulsions for the pH 4.4 and 5.6 solutions, species with g_0 equal to 1.98 and A_0 equal to 108.7 and g_0 equal to 1.98 and A_0 equal to 101.1, respectively, were observed (see Table 3).

Since the EPR spectra observed with both V(IV) and V(V) solutions containing L-ascorbic acid were the same, V(IV)–ascorbate 1:2 complex [59], the solution containing V(V) must undergo reduction (see Fig. 4). These studies thus demonstrate that redox chemistry does take place in samples containing ascorbic acid and V(V). Kinetic studies of this system will help elucidate the nature of ascorbic acid reactions in further detail.

3.5. Probing formation of V(IV)–ascorbate adduct from samples added V(V) or V(IV) in aqueous solutions and RM suspensions using EPR spectroscopy: C-centered radical

As shown in Fig. 3, L-ascorbic acid forms a radical in microemulsions [60] due to aerobic oxidation. To further investigate this system we examined how V(V) and V(IV)

impact ascorbate radical formation by determining if the C-centered radical forms in solutions of ascorbic acid and V. Because the electron transfer reaction forms both a V-centered and a C-centered radical, two different products can be monitored by EPR spectroscopy, the V(IV)–ascorbate complex and the deprotonated ascorbate radical anion. Observation of both these species shows that the complex undergoes the electron transfer reactions as discussed below in reactions (1)–(3).

The EPR spectra shown in Fig. 5a of the aqueous solutions containing a 1:1 ratio of ascorbic acid and V(V) at pH 2.0, 4.4 and 5.6, demonstrate that no C-centered radicals were observed in these solutions. This is of interest because in these samples the V(IV) radical is observed. Demonstrating that redox chemistry does generate the V(IV)-complex. However, when the pH 5.6 sample was added into RM suspensions a trace of the ascorbate monoanionic radical was observed (Fig. 5a, spectrum F).

The question as to whether an ascorbate radical can be formed in presence of V(IV) was addressed by studying solutions of ascorbic acid added to V(IV) at pH 2.0, 4.4 and 5.6 (Fig. 5b). None of these aqueous solutions showed even trace amounts of the monoanionic ascorbate radical. However, when these solutions are placed in microemulsions the ascorbate radical is observed in trace amounts at pH 4.4 and in significant amount at pH 5.6. Since the radical forms in solutions and suspensions in the absence of V(IV), the presence of V(IV) thus protects oxidation of the ascorbic acid.

3.6. Reduction kinetics of V(V) in aqueous acidic media

The reduction of V(V) in acidic media by ascorbic acid was carried out in water with no ionic strength other than the HCl used to adjust pH. These conditions were defined by the study in AOT–isooctane solution for comparison to the reduction reaction in the RM system. Assuming that at pH 1.5 only the HCl contributes significantly to the ionic

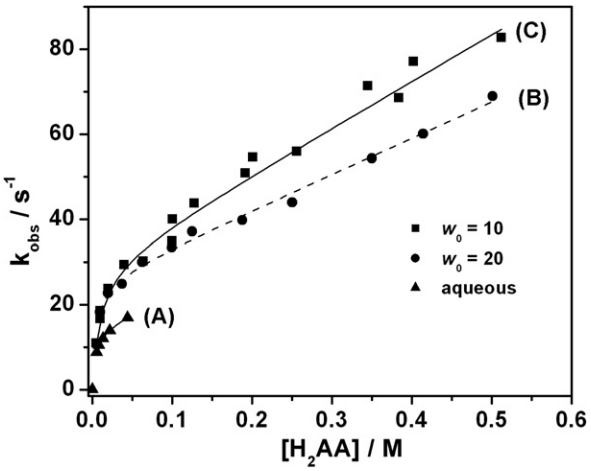


Fig. 6. Observed pseudo-first-order rate constants for the reduction of VO_2^+ (aq) by ascorbic acid in (A) aqueous solution, (B) RMs of $w_0 = 20$ and (C) RMs of $w_0 = 10$. Conditions: [AOT] = 0.20 M in isooctane for RMs, $T = 25.0$ °C, aqueous solution pH 1.5.

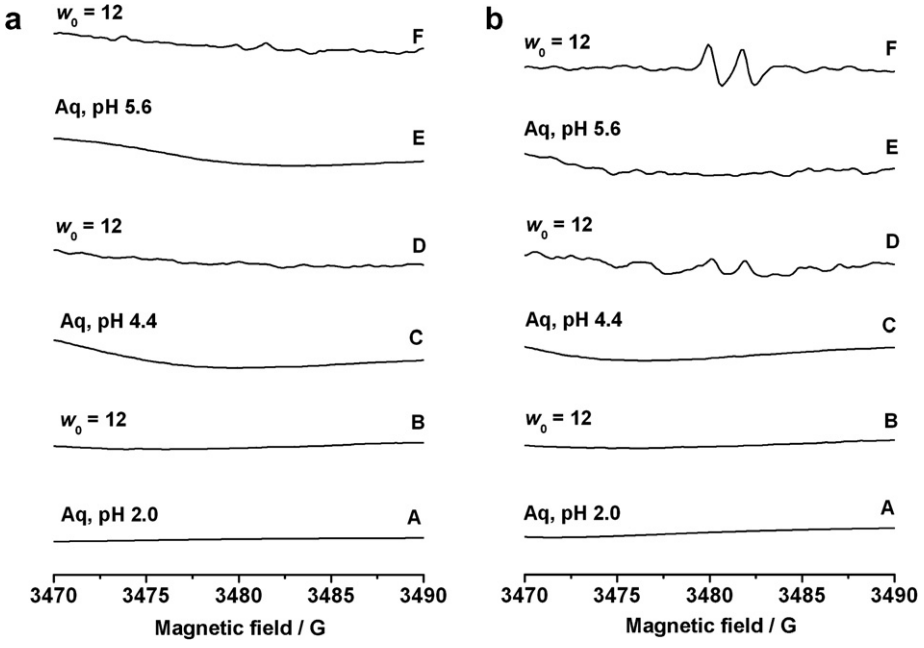
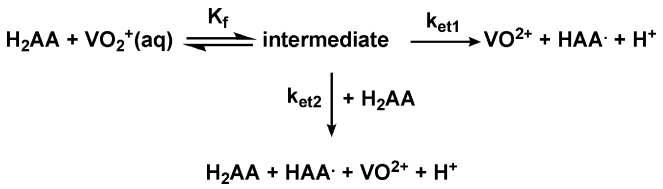


Fig. 5. Ambient-temperature EPR spectra of: (a, left) a 1:1 mixture of 0.100 M L-ascorbic acid and 0.100 M V(V) at pH 2.0, 4.4 and 5.6 in aqueous solutions (A, C and E) and respective RM solutions (B, D and F); (b, right) a 1:1 mixture of 0.100 M L-ascorbic acid and 0.100 M V(IV) at pH 2.0, 4.4 and 5.6 in aqueous solutions (A, C and E) and respective RM solutions (B, D and F). Spectra were also included of samples prepared in the absence of oxygen. The spectra were recorded using the following parameters: number of scans = 4, center of field = 3480 G, sweep width = 150 G, resolution = 1024 points, microwave frequency = 9.82 GHz, microwave power = 2 mW, receiver gain = 4.48×10^4, modulation frequency = 100 kHz, modulation amplitude = 0.7 G, time constant = 328 ms and sweep time = 83 s. All RM solutions were of 0.200 M AOT/isooctane/H_2O with $w_0 = 12$. The pH indicated are those measured and has not been adjusted for the presence of D_2O.



Scheme 1. Detailed mechanism for the ascorbic acid reduction by VO_2^+ presented previously [14].

strength, I equals 0.03 M. Plot (a) in Fig. 6 shows the dependence of the pseudo-first-order rate constant of VO_2^+ (V(V)) on the ascorbic acid concentration.

The curved concentration dependence indicates saturation behavior and supports the formation of an intermediate between the V(V) and ascorbic acid. This adduct subsequently undergoes an intramolecular electron transfer reaction as described previously [14]. At higher ascorbic acid concentrations, deviation from this mechanism was observed and an additional step was required to achieve a satisfactory fit of the data. This additional step involved reduction of the intermediate with an additional molecule of ascorbic acid. The proposed mechanism is shown in Scheme 1.

As described previously [14], the data fit to the following rate law, see Scheme 1 for definition of terms:

$$k_{obs} = (K_f k_{et1}[H_2AA] + K_f k_{et2}[H_2AA]^2)/(1 + K_f[H_2AA]) \quad (1)$$

The kinetic data were analyzed using this equation and both the formation equilibrium constant and the reduction rate constants are shown in Table 4 along with the corresponding values at 0.40 M KCl reported previously [14]. In order to adjust for ionic strength effects, experiments were also carried out at 0.10 M NaCl.

3.7. Reduction of V(V) in reverse micelles prepared from acidic pH solutions

When the reactions were carried out in RMs, an absorbance change comparable to that in water was observed. Specifically, no solution hysteresis was observed immediately after mixing. This indicates that after the rapid mixing in the stopped-flow, the RMs reformed during the "dead time" time of the instrument (3 ms). Therefore, we conclude that the reactions occur within the RM components and not between free redox partners during the mixing process. An absorption spectrum of the V(V)–ascorbic acid intermediate is shown in Fig. 7 and is in excellent agreement with those reported previously [14,15] in aqueous medium.

Since the absorption maxima at 425 nm in both of the RM sizes studied are very similar to that in aqueous media, 430 nm, similar species are formed under both conditions. Unfortunately, the UV–vis spectrum of this complex does not provide further structural detail with regard to the exact nature of the complex in RMs. In addition, its existence is very brief with a lifetime of few seconds, which prevents 2D NMR or other experiments on this system to better define its location and structure.

Plots (B) and (C) in Fig. 6 show the dependence of the observed pseudo-first-order rate constant on the ascorbic acid concentration at w_0's equal to 20 and 10, respectively. While saturation kinetics was noted for these systems at low ascorbic acid concentrations, it is not as pronounced as for the aqueous VO_2^+ species. As the ascorbic acid concentrations are further increased, the dependence becomes linear at the higher reductant concentrations. These two observations indicate that (1) the size of the pre-equilibrium constant is significantly lower in a RM than in water and (2) the squared term in the rate law dominates at high ascorbic acid concentrations with no indication of deviation from the proposed reaction mechanism shown in Scheme 1. Equilibrium and rate constants shown in Scheme 1 were obtained from the non-linear fit of the data using Eq. (1). The calculated lines in Fig. 6 were drawn using the values shown in Table 1 and show excellent correlation with the experimental data obtained over a 100-fold range of ascorbic acid concentrations.

The formation constant for the V(V)–ascorbic acid intermediate, K_f, is diminished by a factor of 3 going from water to the smallest RM. Likewise, the corresponding values for the intramolecular electron transfer rate constant, k_{et1}, increase by approximately 2-fold. A second-order rate constant for the first reduction can be calculated by multiplying K_f and k_{et1} to show a decrease going from water to w_0 equal to 10, from $K_f \times k_{et1} = 4000\ M^{-1}\ s^{-1}$ to $3000\ M^{-1}\ s^{-1}$. This composite change is due to a decrease in K_f. The value calculated from previously reported data at 0.40 M KCl is $K_f \times k_{et1} = 16{,}000\ M^{-1}\ s^{-1}$ and is significantly higher than that at $w_0 = 10$ with 0.40 M NaCl, $K_f \times k_{et1} = 5000\ M^{-1}\ s^{-1}$. The bimolecular electron transfer step between a second ascorbic acid molecule and the intermediate, k_{et2}, showed little difference between any of the aqueous or RM environments.

4. Discussion

4.1. Location of L-ascorbic acid in AOT RMs

Both ^1H and 2D NMR spectroscopy provide information about the location of ascorbic acid at acidic pH. Since ^1H NMR chemical shifts are sensitive to the protonation states of ascorbic acid [48,49,52,53,61–65], the spectra obtained from pH 2.0 to 5.6 show that ascorbic acid is protonated in the aqueous stock solutions and inside the AOT RM ($w_0 = 20$). As the pH is raised ($pK_{a1} = 4.25$ [45–53]) the monoanionic species becomes the major species in solution and the stability of L-ascorbic acid solution decreases, since L-ascorbic acid is readily oxidized in the form of the monoanion [48]. In the presence of the transition metals, rates of oxidation also increase dramatically at low pH which suggests formation of a reactive metal-ascorbate complex [10–13,66]. Most of the studies presented here

Table 3
The parameters (g_0 and A_0) for the species observed for V-centered radical[a] by EPR spectroscopy in aqueous solution and in microemulsions prepared from 0.2 M AOT/isooctane

pH	1:1 V(V) + AA/Aq[b]		1:1 V(V) + AA/RMs[c]		1:1 V(IV) + AA/Aq[d]		1:1V(IV) + AA/RMs[e]	
	g_0	A_0 (G)	g_0	A_0 (G)	g_0	A_0 (G)	g_0	A_0 (G)
2.0	1.97 ± 0.00	114.9 ± 0.2	1.97 ± 0.00	111.8 ± 0.2	1.97 ± 0.00	114.7 ± 0.2	1.97 ± 0.00	111.7 ± 0.3
4.4	1.97 ± 0.00	114.9 ± 0.4	1.98 ± 0.00	94.1 ± 1.0	1.97 ± 0.00	114.0 ± 0.2	1.98 ± 0.00	108.7 ± 1.0
	1.97 ± 0.00	102.5 ± 0.3						
5.6	1.97 ± 0.00	86.3 ± 0.3	1.98 ± 0.00	87.1 ± 1.1	1.97 ± 0.01	106.9 ± 0.3	1.98 ± 0.01	101.1 ± 1.2
	1.97 ± 0.00	102.9 ± 0.2						

[a] EPR parameters of VO^{2+} at pH 2: g0 = 1.964 ± 0.001; A_0 = 116.4 ± 0.2 [58].
[b] 0.1 M of V(V) and 0.1 M of ascorbate in aqueous solution.
[c] 0.004 M of V(V) and 0.004 M of ascorbate in reverse micelle.
[d] 0.1 M of V(IV) and 0.1 M of ascorbate in aqueous solution.
[e] 0.004 M of V(V) and 0.004 M of ascorbate in reverse micelle.

Table 4
Rate constants for the ascorbic acid reduction of cis-dioxoV(V)

Conditions[a]	k_{et1}, s^{-1}	K_f, M^{-1}	k_{et2}, M^{-1} s^{-1}
Aqueous	14 ± 1	280 ± 10	97 ± 5
$w_0 = 20$	26 ± 2	220 ± 70	85 ± 4
$w_0 = 10$	31 ± 3	110 ± 30	110 ± 10
Aqueous, $I = 0.10$ M[b]	36 ± 3	300 ± 20	100 ± 5
Aqueous, $I = 0.40$ M[c]	74	220	Not reported
$w_0 = 10$, $I = 0.40$ M[c]	26 ± 1	190 ± 40	103 ± 4

[a] This study: no ionic strength control $I \sim 0.20$ M AOT, pH 1.5, $T = 25.0$ °C for RM data and 0.03 M for aqueous unless otherwise indicated.
[b] Ionic strength maintained with NaCl. Temperature and pH are the same as in (a).
[c] Kinetic parameters calculated using values in Ref. [37], $I = 0.40$ M KCl. Temperature and pH the same as in (a).

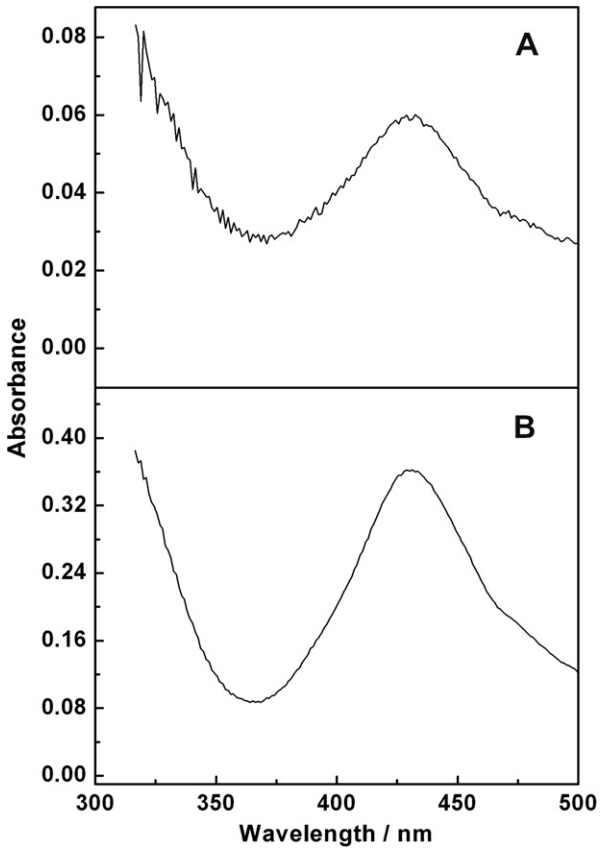


Fig. 7. Spectra of $[VO_2(H_2AA)]^+$ intermediate in (A) $w_0 = 10$ reverse micelle and (B) in aqueous solution. Aqueous pH 1.5, [AOT] = 0.20 M in isooctane. $[VO_2^+] = 1 \times 10^{-3}$M, $[H_2AA] = 0.10$ M, T = 25.0 °C. Spectra recorded approximately 50 ms after mixing.

are under conditions where ascorbic acid exists as a diacid (H_2AA).

The impact of the confined media is sensitive to the matrix size. A solute in a small RM is likely to experience greater interface effects than when it is in a large RM [67]. The ^{1}H NMR data shown in this study for RMs of a size w_0 equal to 20 were prepared from a 0.100 M aqueous stock solution at pH 4.5. A RM of w_0 equal to 20 has an aggregation number (n_{AOT}) of 302 [68] and the number of water molecules per micelle (n_{H2O}) is 6061 [68]. Combining Dynamic Light Scattering measurements and the literature data, the w_0 equal to 20 has a water pool radius (r_w) of about 35 Å [68]. With this size, the stock solution of 0.100 M ascorbic acid added gives RM's that contain ~5 ascorbic acid molecules. For a w_0 equal to 16 these parameters are: r_w of about 29 Å and ~3 ascorbic acid molecules per RM. Both of these RMs are medium sized and, despite the differences in sizes of the RMs, the confinement effects on the reactions should be similar and the ^{1}H NMR spectra support this expectation.

In the past, 1D NMR data have been used to support localization of probes [69,70]. A downfield ^{1}H NMR shift has previously indicated close association with the water side of the interface (Stern layer) of a CTAB-micelle. An upfield shift suggests penetration of the probe into the interface [69,70]. Recently, we have found that this pattern is not strictly followed for dioxovanadiumdipicolinato in AOT RMs [26]. This makes interpretation of the downfield ^{1}H NMR shift of ascorbic acid in AOT microemulsions (Fig. 1S) warrenting 2D ^{1}H–^{1}H NOESY NMR data in order to obtain more information on the location of ascorbic acid.

The ^{1}H–^{1}H NOESY results in Fig. 2 show that ascorbic acid from a pH 4.5 stock solution in RM microemulsions

of size w_0 equal to 20 is located in the vicinity of the lipid interface of AOT. The cross-peaks between H3′ of AOT and with both (CH_2, ($H6_A$)) and (CH, ($H4_A$)) on ascorbic acid indicate that the molecule is oriented such that the H3′ protons of AOT interact with the protons on C6 ($H6_A$) and C4 ($H4_A$) of ascorbic acid. These results suggest that either the remainder of the ascorbic acid molecule penetrates deeply into the AOT (Fig. 8A) or is oriented near the head group of AOT (Fig. 8B). Since the final ^{1}H NMR signal in ascorbic acid could not be observed because of signal overlap, these two possibilities cannot be experimentally distinguished

$$H_2AA + O_2 \rightarrow AA^{\cdot -} + O_2^{\cdot -} + 2H^+ \quad (2)$$

$$HAA^- + O_2 \rightarrow AA^{\cdot -} + O_2^{\cdot -} + H^+ \quad (3)$$

4.2. Reaction of ascorbic acid with oxygen in confined media

L-Ascorbic acid is a known natural anti-oxidant [1] and forms the ascorbate radical in the presence of air in a pH dependent manner [66], where oxygen is the reactive agent as shown in reactions (2) and (3). The ΔG for reaction (3) is 10.1 kcal/mol, which makes the concentration of ascorbate radical below detection limits [10–13]. While the oxidation of ascorbate by oxygen in acidic medium is slow, the oxidation of ascorbic acid is even slower [10–13]. Both of these reactions are accelerated in the presence of transition metal ions [10–13]. In our EPR studies, the enhanced radical formation observed at neutral pH is consistent with these earlier reports. The parameters (g_0 and A_0) of the monoanionic ascorbate radical (Table 2) generated during the reaction of ascorbic acid with V(V) and V(IV) are consistent with the literature reports [71]. Since the radical is observed at all pH values for solutions added to microemulsions, this phenomenon can be attributed to either the microemulsion causing the reaction, the "pH" of the environment changing to support radical formation, or the environment in the confined media stabilizing the radical.

The oxidizing agent in these reactions is atmospheric oxygen [10–13]. For the oxidation reactions in the microemulsions, the involvement of oxygen is demonstrated by purging the solutions with argon. Radical formation was decreased and ultimately eliminated when oxygen was rigorously excluded. The fact that ascorbate oxidation is observed in microemulsions but not in the aqueous solution does reflect something very different about the microemulsion environment. In solutions containing V(V) and ascorbic acid, the radical formation is also decreased and recycling of V(IV) has been reported in other systems [57]. The possibility that AOT or trace

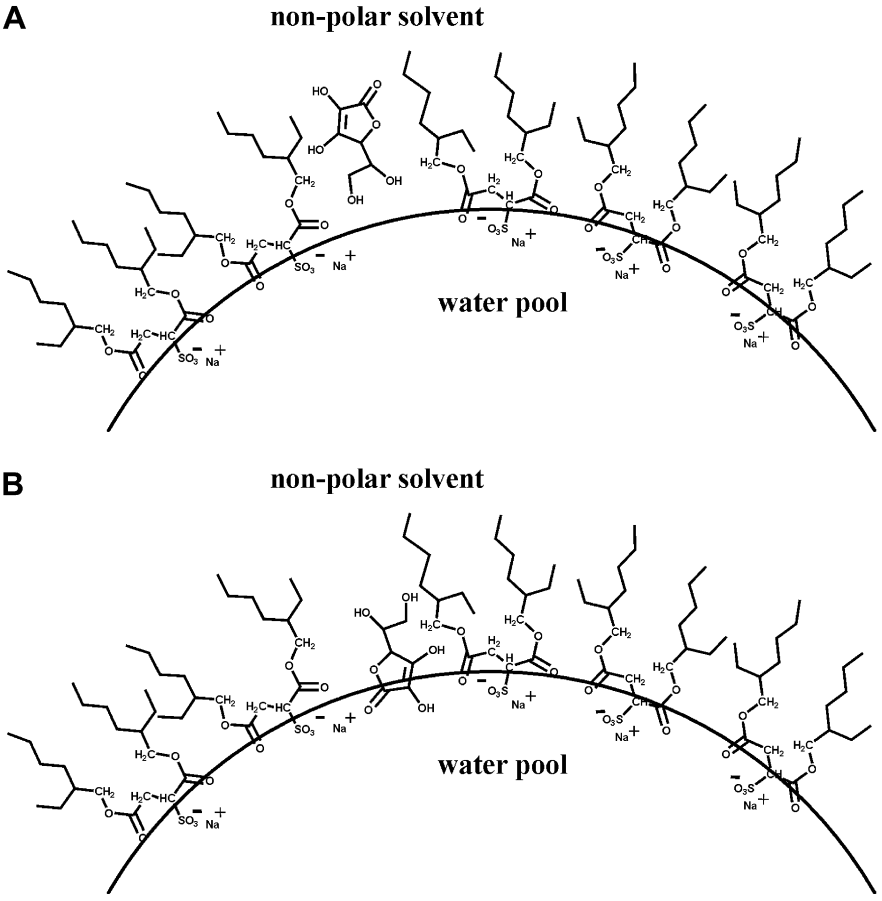


Fig. 8. The location of L-ascorbic acid in AOT RMs at pH 4.5 showing deep penetration into the lipid interface (A) and penetration resulting in surface interaction (B).

metals introduced with the AOT preparation form a radical was ruled out since no radical is formed in a microemulsion containing AOT, isooctane and water in the absence of ascorbic acid.

Since monoanionic ascorbate is more reactive towards oxygen, changes in pH in the microemulsions [72–76] could explain the changes in oxidation rates. We and others have used several techniques to measure the proton activity or the effective "pH" in this environment. In microemulsions the water pool does not contain the sufficient number of water molecules for pH to have the meaning defined by the Sørensen equation [77]. However, probes in such solutions added to microsuspensions do in fact show effects in environments with a wide range of difference in proton acidities as monitored by physical properties and solute reactivities [72,73,78,79]. The above considerations apply to the water pool and not the interface where the ascorbic acid resides. In the event that the reaction takes place at the interface, where the concept of "pH" is even less defined than in the water pool [72,73] changes in proton activities could be responsible for the observed differences.

The final possibility for the observed reactivity change is stabilization of the radical in a confined media. The EPR signals are significantly line broadened when recorded in microemulsions. This broadening presumably reflects the lower mobility of the compounds in this environment and can, in part, contribute to their stability. The observation of a C-centered ascorbate radical in the microemulsions is important and documents that confinement of this reaction stabilizes intermediate steps in the oxidation pathway. Radical stabilization could be accounted for by solute interactions, solvation and isolation of radical species [80–82]. Solute interactions that could stabilize the radical or ascorbic acid include head group or counter ion interactions. In addition to stabilization through polar and hydrophobic interactions, H-bonding seems likely to contribute to the solvation of the ascorbic acid and its radical anion. The microviscosity and fluidity of the aqueous solution change significantly in the RM and that solutes in these environments are responding as if the temperature of the solution is 10–20° cooler [83,84].

4.3. Reaction of ascorbic acid with the dioxovanadium(V) cation at acidic pH in confined media

The reaction between V(V) and ascorbic acid is pH dependent [85–87]. The initial study by Kustin and Toppen of the V(V)–ascorbic acid system was carried out below pH 1 [15]. Wilkins et al. reinvestigated this reaction in acidic and extended it toward neutral pHs to better understand this fundamental redox process [14]. In the present work, our studies were carried out on ascorbic acid aqueous solutions between pH 1 and 6. Table 4 summarizes the kinetic parameters of the oxidation of ascorbic acid by V(V) in aqueous solution and in microemulsions.

The modest differences in reaction rates between aqueous solutions and microemulsions may be attributed to three possible effects. First, the RM environment favors an altered reaction environment due to the hydrophobicity of the interface. Second, the proton activity in the vicinity of ascorbic acid in the RM is different from the aqueous solution used to form it. Third, the change in the environment of ascorbic acid results from an effective increased ionic strength, from either the substrate organization or from the sodium ions and sulfonate headgroups of the AOT together. Three parameters can be used to explore the source of these differences and are summarized in Table 4: the intramolecular rate constant, k_{et1}, the corresponding complexation constant, K_f and the bimolecular electron transfer rate constant k_{et2}.

To investigate the ionic strength effects on the intramolecular electron transfer rate the rate constants were measured at two higher ionic strengths for the aqueous systems and at w_0 equal to 10 by adding enough NaCl to raise the ionic strength to 0.400 M. Table 4 shows that aqueous k_{et1} increases from 14 s^{-1} to 74 s^{-1} going from ~0 to 0.400 M. In contrast, k_{et1} for the aqueous RM system remained essentially unchanged when these solutions are added to w_0 equal to 10. Similarly there was little change observed when w_0 was changed from 10 to 20. These observations show that the intramolecular electron transfer rate constant is more sensitive to changes in the aqueous media than to changes in the RM. Such observations would be expected if the ascorbic acid or significant part of it is penetrated into the lipid interface, and thus not affected by changes in the ionic strength of the RM water pool or the size of the RMs. In addition to the minor changes in k_{et1}, effect on the pre-equilibrium would impact the overall reaction rate. The value of K_f dropped from 280 in aqueous medium to 100 in w_0 equal to 10 RMs. When 0.40 M NaCl is added to this RM, the value for the equilibrium constant increased to 190 supporting that the observed drop in K_f is not due to ionic strength increases in the water pool of the RM.

The observed invariability of the value for k_{et2}, the bimolecular rate constant for electron transfer, on ionic strength in aqueous solution and placement into RM may seem surprising. Although ascorbic acid and the V(V)-complex may be located in the interface, larger differences would have been anticipated for this bimolecular reaction. At pH 1.5 the ascorbic acid is fully protonated and therefore has no charge. According to Debye–Huckel theory, the reaction would be expected to be insensitive to changes in ionic strength [14]. The invariability of k_{et2} as compared to k_{et1} on environment is surprising since one would anticipate the opposite. This strongly suggests that counteracting effects are governing the k_{et2} rate constant and the kinetic measurements are unable to elucidate them.

The observed differences could result from a change in the proton activity upon placement in the RMs. If the effective "pH" of the interface environment was raised closer to

the first pK_a for ascorbic acid (pK_{a1} = 4.25 [45–53]) an increased reduction rate would be expected since HAA^- is a thermodynamically stronger, and kinetically faster, reducing agent than H_2AA. Since no such increase in rate constant is observed, we conclude that the effective proton activity is not above a pH of approximately 2.5–3.0 [14] as found for aqueous solution. Given these considerations, an appreciable change in the aqueous solution toward the neutral pH range upon placement into the reverse micelle is not likely. These results are consistent with the NMR data listed in Table 2 showing that the ascorbic acid is protonated in these microemulsions.

Based on the measured values for k_{et1} and K_f and those measured in an earlier study in aqueous solution, [14] the results observed in the microemulsions are what would be expected if the effective proton activity of environment of H_2AA is slightly higher than pH 1.5. Based on the experiments at 0.40 M KCl, a doubling of the intramolecular rate constant occurs only when change from pH 1.5 to 2.2 takes place. Using these data we can predict the effective "pH" of 2.1 to be consistent with the observed decrease of K_f at 0.40 M KCl by a factor of ~3 (K_f equal to 100). We cannot rule out the possibility that the kinetic data could be explained by a difference in the pK_a of the V(V)–ascorbic acid intermediate or the pK_a of ascorbic acid itself in the microemulsions.

Since the ascorbate radical is formed in these microemulsions, an alternative pathway may be accessible. For example, the proton from ascorbic acid is not released (see Eq. (3)), or that the local environment can support such a proton associating with ascorbic acid in a complex ion. Such interpretation is consistent with the NMR results presented here. The lack of sensitivity in k_{et2} with respect to aqueous or RM environments does not limit the type of environment around the ascorbic acid. The possibility that ascorbate is oriented as shown in Fig. 8B would place such a proton near the interface of the RM. Several lines of evidence exist to the notion that protons accumulate near the interface of the RM [72,73,88,89].

EPR spectroscopy was used to examine the ascorbic acid oxidation by V(V) at acidic pH. Observation of V-based species in solution with ascorbate added to the V(V) both in aqueous and microemulsion solutions confirms that V(V) is reduced to V(IV). EPR spectroscopy probing C-based radicals showed no evidence of ascorbate radical formation both in aqueous and microemulsion solutions. This is unexpected, because these reactions were anticipated in such solutions. However, these results are consistent with the rapid and complete reaction between V(V) and ascorbate as observed in our kinetic studies, and *reflect complete conversion of* V(V) to V(IV) and not a lack of reaction.

For comparison the EPR spectra were recorded in solutions containing V(IV) and ascorbate. V(IV) does not oxidize ascorbate, unless there is oxygen present in solution. Since much less ascorbate radical is observed in aqueous solution in the presence of V(IV) (Fig. 5) than in its absence (Fig. 3), V(IV) protects the ascorbic acid from oxidation. Since formation of a V(IV)–ascorbate complex is observed, we conclude that this complex inhibits the oxidation of coordinated ascorbic acid in contrast to the complex formation with V(V) that enhances oxidation.

The specific conditions for ascorbate radical formation has previously been somewhat controversial [90]. In aqueous studies, ascorbate radical formation near neutral and in basic solutions was reported. Prolonged storing of ascorbic acid solutions in the presence of oxygen result in formation of oxalate [91]. Oxalate formation required reaction times of at least two weeks and no oxalic acid was observed in the work presented here. The studies described in this work were carried out in acidic solutions and complement the studies carried out at higher pH values. As the pH increases, ascorbic acid deprotonates and undergoes a more rapid oxidation [14,48]. This deprotonation may prevent its penetration into the AOT interface and thus may not be directly comparable to the studies previously reported in micellar and RMs [24,25]. The observation of the ascorbate radical in microemulsions clearly demonstrates how environmental changes can stabilize reaction intermediates. The increased radical formation during the reaction of V(V) and V(IV) in RMs can be attributed to a combination of factors such as solute interaction, solvation, H-bonding, hydrophobic interactions in the RM environment and changes in the microviscosity and microfluidity.

5. Conclusions

Using ^1H–^1H NOESY NMR spectroscopy, the location of ascorbic acid in microemulsions is defined. After L-ascorbic acid penetrates the AOT interface, it is located near the head group of AOT flanking the succinic acid moiety and the diester functionalities. Although previous studies have placed the monoanionic ascorbic acid in the water pool, we show that the protonated ascorbic acid (H_2AA) is located at the interface. Residing at the interface, the ascorbic acid air oxidation generates a C-centered ascorbate radical. This reaction did not take place in the absence of either oxygen, or ascorbic acid, and thus reactions with AOT can be ruled out. The overall ascorbic acid oxidation by V(V) is slower in a AOT RM than in aqueous solution, and the effect is attributed to the different environment and solvation in the RM. Kinetic studies of the V(V) oxidation of ascorbic acid in microemulsions show limited changes in the intramolecular electron transfer reaction between aqueous solution and the microemulsion. This is consistent with a unimolecular process which should be relatively insensitive to large changes in environment. The kinetic data also show that a pre-equilibrium step between VO_2^+ and ascorbic acid to form a V(V)–ascorbate complex is decreased, i.e., a smaller K_f, and therefore responsible for the lower ascorbic acid oxidation rate in AOT RMs. These studies suggest that it is the location of the ascorbic acid and its

solvation in the microemulsion that dictates its reactivity toward oxidation.

6. Abbreviations

AOT aerosol-OT or sodium bis(2-ethylheyl)sulfosuccinate
D_2O deuterium oxide
DSS 3-(trimethylsilyl)propane sulfonic acid sodium salt (or 2,2-dimethyl-2-silapentane-5-sulfonic acid sodium salt)
NOESY nuclear overhauser enhancement spectroscopy
RM reverse micelle

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jinorgbio.2008.01.015.

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