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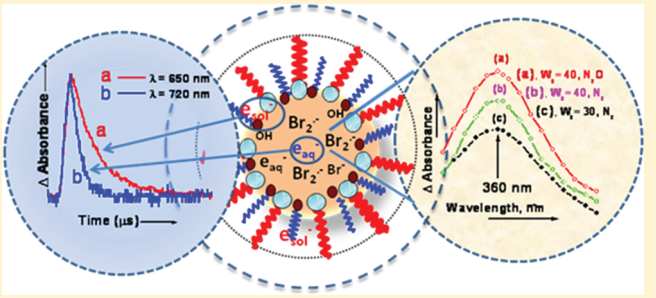
Generation of Counter Ion Radical ($Br_2^{\bullet-}$) and Its Reactions in Water-in-Oil (CTAB or CPB)/*n*-Butanol/Cyclohexane/Water) Microemulsion

Apurav Guleria, Ajay K. Singh, Sisir K. Sarkar, Tulsi Mukherjee, and Soumyakanti Adhikari*

Radiation & Photochemistry Division, Bhabha Atomic Research Centre, Mumbai 400085, India

ABSTRACT: Herein we report the generation of counterion radicals and their reactions in quaternary water-in-oil microemulsion. Hydrated electrons in the microemulsion CTAB/H_2O/*n*-butanol/cyclohexane have a remarkably short half-life (~1 μs) and lower yield as compared to that in the pure water system. Electrons are solvated in two regions: one is the water core and other the interface; however, the electrons in the water core have a shorter half-life than those in the interface. The decay of the solvated electrons in the interface is found to be water content dependent and it has been interpreted in terms of increased interfacial fluidity with the increase in water content of the microemulsion. Interestingly another species, dibromide radical anion ($Br_2^{\bullet-}$) in CTAB and CPB microemulsions have been observed after the electron beam irradiation. Assuming that the extinction coefficient of the radicals is the same as that in the aqueous solution, the yields of the radicals per 100 eV are 0.29 and 0.48 for the $Br_2^{\bullet-}$ radical in CTAB and CPB containing microemulsions (W_0 = 40), respectively, under N_2O saturated conditions. Further, we intended to study electron transfer reactions, which occur at and through the interface. The reaction of the $Br_2^{\bullet-}$ radical anion with ABTS [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] has been studied to generate the ABTS radical in the water core, and further, its reaction has been investigated with the water-insoluble molecule vitamin E (tocopherol) and water-soluble vitamin C (ascorbic acid). In the present study, we were able to show that, even for molecules which are completely insoluble in water, ABTS scavenging assay is possible by pulse radiolysis technique. Furthermore, these results show that it is possible to follow the reaction of the hydrated inorganic radical with solutes dissolved in the organic phase in a microemulsion without use of a phase transfer catalyst.



1. INTRODUCTION

In four component water-in-oil microemulsions, the surfactant and cosurfactant (commonly medium chain linear alcohols) molecules form a spherical shell with a polar core where water can reside at various concentrations. These core shell structures are dispersed homogeneously in liquid hydrocarbons. As a result, the whole system behaves as a transparent, isotropic, and thermodynamically stable solution of water-in-oil. The water, confined in the cavity controls the size and shape of the micelle, and thus, the reverse micelles or microemulsions are often characterized by W_0, which is defined as the mole ratio of water to surfactant, $W_0 = [H_2O]/[\text{surfactant}]$.

Because of the compartmentalization of reactants into polar and nonpolar regions, yet within the reaction zone, the microemulsions have become important in its wide applicability in synthesis. Detailed reviews on organic synthesis[1] and nanoparticle synthesis[2,3] in microemulsions are available in the literature. Numerous examples are on hand in this regard including very recent work on synthesis of specialized materials in microemulsions.[4,5] In two phase reactions, the ionic reactants are extracted by a phase transfer catalyst from the aqueous phase to the organic phase where the reactions take place.[6] The phase transfer catalysts are of environmental concern, and carrying out these sorts of reactions in microemulsions is convenient and more environment friendly. It has been pointed out earlier that the water pools in the reverse micelles mimic the water pockets that are often found in various bioaggregates such as proteins, membranes, and mitochondria.[7] A variety of molecules have been studied in such systems, and it had been shown that the enzymes incorporated in the aqueous core of the reverse micelles were protected against denaturation.[8−10] Free radical reaction of β-carotene in the microemulsion system was found to generate retinol which otherwise is an enzymatic process.[11] Recently, it has been reported that DNA can be readily dissolved into microemulsions stabilized by an anionic surfactant (AOT).[12] These authors found that for W_0 = 44.4, with the increase in DNA concentration, the water droplets become progressively more spherical and monodisperse. The local DNA concentrations in this condition reach values as high as those naturally observed in biological organelles.

Cetyl trimethyl ammonium bromide (CTAB) has been used as a surfactant for making reverse micelles and microemulsion for several important studies reported earlier. CTAB does not form

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reverse micelles in alkanes in the absence of a cosurfactant, and only in presence of medium chain length primary alcohols, such as 1-butanol, 1-pentanol, or 1-hexanol, is the formation of CTAB microemulsion in alkane possible.[13] The characterization of this microemulsion which includes size and shape,[14,15] water core radius and aggregation number,[16] and thickness of the interface and ion concentration at the interface[16−18] are well studied. The hydrodynamic radius of a system (CTAB/1-pentanol/cyclohexanol/water) similar to the one used in the present study, was reported to vary from 2.4 to11.4 nm as W_0 increases from 5 to 40, and it is not significantly affected by a change in the cosurfactant.[13] Of late, it has been demonstrated that, in the CTAB/1-pentanol/cyclohexane/water system, with the increase in water content, the packing parameter decreases which eventually leads to an increase in interfacial fluidity.[19] In such a situation, reactions which involve both water core and interface might be favored.

A variety of studies in the past[20] have made noteworthy contributions to the understanding of the electron processes in reverse micelles and microemulsions. Kinetic evidence had shown earlier that the location of the probes in reverse micelles plays an important role in their reactivities.[21] In oil-in-water (benzene/water) microemulsions, Wu et al.[22] have observed that the rate constants for the addition of e_{aq}^- and the $^{\bullet}OH$ radical to benzene are in agreement with those in aqueous solutions. The hydrated electrons have been studied earlier using pulse radiolysis and near IR spectroscopic measurements in reverse micellar systems of Brij30, a mixture of AOT and Brij30, and AOT doped with PEG.[23] These authors have demonstrated that, even though hydrated electrons could probe the polar core of the reverse micelles, it is difficult to formulate a simple relation between characteristics of the hydrated electron and the properties of reverse micelles. A report on the yield of hydrated electrons in the cationic microemulsion composed of CTAB/water/1-pentanol/cyclohexane also exists in the literature.[24] However, in a microemulsion, the counterion radical had not been observed earlier, and their involvement in redox processes has not been reported until date.

Thus, in the present work, we attempted to look for three important physicochemical aspects of water-in-oil microemulsions. In addition to revisiting the properties of the hydrated electron and its decay pattern in the CTAB microemulsion system, our intention was to examine whether counterion radicals could be observed in the small water pockets. If observed, what could be their reactivity with molecules dissolved in the aqueous phase of the microemulsion? Finally, we wished to study the two phase reaction between radicals produced in the aqueous core and a molecule that is completely insoluble in water. In the present report, it has been demonstrated that the interface of the microemulsion could compete with the water pool in the solvation of dry electrons produced in the oil phase. The difference in kinetic behavior of the two types of solvated electrons that has been observed in this study could unfold the physical nature of the interface. Further, we have clearly shown that the bromide ions present in the water pocket are capable of interfering in redox processes occurring in this media.

2. MATERIALS AND METHODS

2.1. Chemicals and Microemulsion Preparation. Cetyltrimethylammonium bromide (CTAB), cetylpyridiniumbromide (CPB), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), ascorbic acid, and tocopherol were from Aldrich. All of the surfactants were recrystallized before use. CTAB was recrystallized twice from ethanol.[25] CPB was purified by washing with acetone 5 times.[26] ABTS, tocopherol, and ascorbic acid were used as received. All other chemicals were of spectroscopic grade. Nanopure water (conductivity, 0.06 μS cm^{-1}) was used for preparing the solutions. High purity (>99.9%) N_2 and N_2O were used for purging solutions as per requirement.

To prepare the microemulsion, 0.1 mol dm^{-3} CTAB/CPB was added to cyclohexane. 1-Butanol was added to this solution to achieve a 1-butanol/surfactant ratio of 5:1.[13] Water was added to this system to obtain W_0 values of 10, 20, 30, and 40. The samples were sonicated or vigorously shaken to obtain a homogeneous and transparent solution adequate for optical absorption measurements.

2.2. Methods: Pulse Radiolysis. The pulse radiolysis setup using 7 MeV electron pulses coupled with transient spectroscopy has been described elsewhere.[27] The absorbed dose was measured using an air-saturated solution containing 5×10^{-2} mol dm^{-3} KSCN assuming Gε for $(SCN)_2^{\bullet-} = 2.6 \times 10^{-4}$ m^2 J^{-1} at 475 nm.[28] The kinetic spectrophotometric detection system covered the wavelength range from 250 to 800 nm. The electron pulse used was 500 ns, and the dose per pulse was 30 Gy. Relative absorption of radicals in the UV−vis region has been observed against time and wavelength to get kinetic and absorption characteristics, respectively.

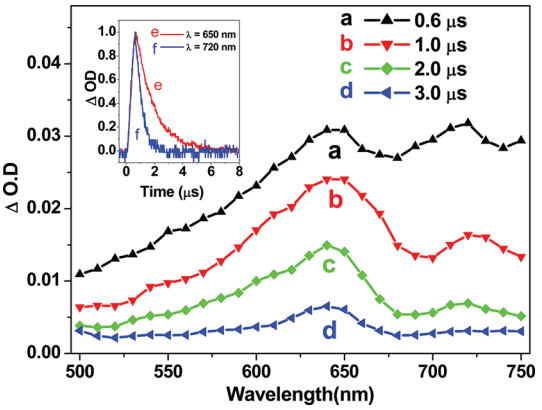

Figure 1. Transient absorption spectrum of hydrated electrons at (a) 0.6, (b) 1, (c) 2, and (d) 3 μs after the electron pulse of N_2 saturated solution of CTAB/cyclohexane/*n*-butanol/water with W_0 = 20. O.D. = absorbance. Inset: (e) Decay of hydrated electron at 650 nm and (f) at 720 nm in microemulsion with W_0 = 20.

3. RESULTS AND DISCUSSION

3.1. Hydrated Electron. Hydrated electrons are formed in a microemulsion from two sources. One is the scavenging of excess electrons produced in the oil phase by water pools. The scavenging process is very fast and completed during the pulse of our experiment.[2,3] The other source of hydrated electrons is the direct radiolysis of water pools. The transient absorption spectra obtained in the pulse radiolysis of a microemulsion containing CTAB/H_2O/*n*-butanol/cyclohexane solutions bubbled with nitrogen are shown in Figure 1. The hydrated electron spectra at different times after the electron pulse was obtained from a solution with W_0 = 20 is represented in this figure where two peaks at 650 and 720 nm are visible. The latter is the typical hydrated electron spectrum one expects in pure aqueous solution. The interesting

observation is another absorption maximum at 650 nm, which, is even more intense than the usual peak at 720 nm at longer times. This absorption peak can be attributed to electrons solvated in the interface of the reverse micelle. By calculating the effective packing parameter of the *n*-pentanol/CTAB/*n*-hexane/water microemulsion system, Giustini et al. have shown that in the dispersed phase not only CTAB and water but pentanol is also present.[14] Besides, it is also reported that solvated electrons in long chain alcohols give rise to an absorption peak at around 630−650 nm.[15] In the present microemulsion system, as 1-butanol is present in the interface, the absorption peak at 650 nm is ascribed to the electrons solvated in this region. Moreover, in another study it had been shown that dual sites of solvation is possible in microemulsion containing NaLS/H_2O/1-pentanol/cyclohexane.[20g] The hydrated electron spectrum obtained from solutions with W_0 = 30 and 40 are not shown here as they are similar but show higher yield of hydrated electrons (Table 1). It must be noted that the yield of solvated electrons at the interface shows a higher value with an increase in water content of the system. However, it has also been observed that, at the end of the electron pulse, the absorption due to the electrons solvated in the interfacial region becomes lower and that for the water core becomes higher as the water content of the system is increased showing bulk-water-like behavior at higher W_0. The electrons at the interface decay slower than the ones in the water pool. Thus, at a particular W_0, due to slower decay, the yield of solvated electrons at the interface might show a higher value at longer times. Further, as the solvated electrons have a broad absorption spectra, the absorption at 650 nm would always have some contribution from that at 720 nm. Consequently, quantitative assessment in the properties of these two types of electrons would always have a contribution from the other.

In the inset of Figure 1, e and f represent the decay of solvated electrons at 650 and 720 nm, respectively, in the microemulsions (W_0 = 20). It is seen that the decay of the hydrated electron in these systems is very fast, even faster than that in pure aqueous solution, and the decay at 720 nm is faster than that at 650 nm. The half-lives as calculated from this trace are ∼1 and ∼1.6 μs in the microemulsions with W_0 = 20 as measured at 720 and 650 nm, respectively. On the contrary, the half-life of the electron was reported to be 12 μs as measured at 720 nm in anionic microemulsion with W_0 = 16.[20g] The anionic nature of the charged surfaces in the NALS system makes the electrons more stable. Moreover, the reactivity of hydrated electrons with the CTAB micelle is higher than that with NALS,[29] which makes the half-life of hydrated electrons greater in NALS system.

Table 1. Yields of Hydrated Electrons in CTAB/H_2O/Cyclohexane/*n*-Butanol Microemulsion[a]

W_0 (CTAB system)	$G(e_{aq}^-)$ at 650 nm	$G(e_{aq}^-)$ at 720 nm
10	0.45	0.504
20	0.708	0.731
30	0.738	0.733
40	0.789	0.736

[a] The yields were calculated assuming the extinction coefficient of solvated electrons in the microemulsion system is same as that in pure water.

In microemulsions, the decay of hydrated electrons follows a kinetic equation, and by carrying out the reaction of solutes with hydrated electrons, it is possible to characterize these organized systems.[21] In an early report, we could determine the water pool radius and location of probes in the NaLS/H_2O/1-pentanol/cyclohexane water-in-oil microemulsion.[20g] However, the intrinsic decay of the hydrated electron should be reasonably slow to obtain the parameters with accuracy. Unfortunately, in the present case, it is very difficult to characterize the system by following electron decay because of the short life of the hydrated electron itself. Moreover, it is worth mentioning here that Gebicki and Maciejewska[23] have already shown that it is impossible to get a pure hydrated electron spectrum in the microemulsion system with low water content.

It should be noted in Figure 2 that the decay of solvated electron in the interface is slower than that in the water core. This reveals that the electrons are more stabilized and are well separated with other electrons when they are solvated in the interfacial region. The decay of electrons solvated in the water core is not dependent on W_0, whereas that in the interface varies with W_0. When the electron decay at the interface in microemulsions with different water content is compared, a faster decay is observed with an increase in W_0. It has already been mentioned in the Introduction that with the increase in water content an increase in interfacial fluidity was observed in an earlier study.[19] In the present case, due to the increase in interfacial fluidity with the increase in W_0, the mobility of electrons solvated in this region increases, and so, the escape of these electrons into the water core might also be favored. All of this would lead to an increased interaction between the electrons present in the interface and also with those that exist in the water core. Another interesting point to be noted is that the decay rates of electrons solvated in the water core are not significantly different at various W_0's. This is due to the cationic interface which makes the interaction between e_{aq}^- faster, and also the time window of our measurement may not be appropriate to differentiate the decay patterns of e_{aq}^- in this particular media.

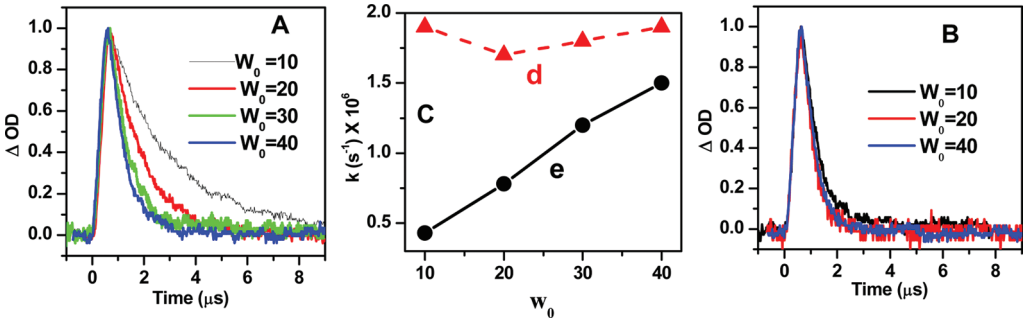

Figure 2. (A and B) Decay pattern of solvated electrons at the interface and in the water core. (C) Plot of decay constants of solvated electrons versus W_0 (d) in the water core and (e) at the interface, respectively. O.D. = absorbance.

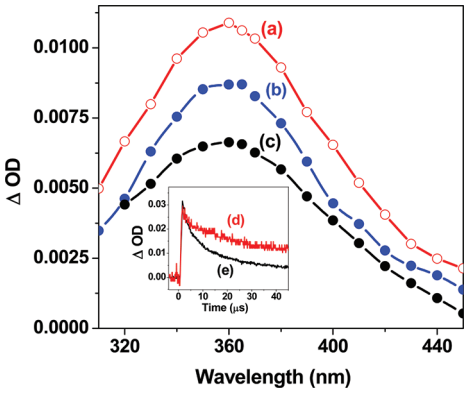

Figure 3. Transient absorption spectrum of dibromide anion radical of the CTAB/cyclohexane/*n*-butanol/water solution after electron pulse (a) N_2O saturated (W_0 = 40), (b) N_2 purged (W_0 = 40), and (c) N_2 purged (W_0 = 30). Inset: Decay of dibromide anion radical at 360 nm at a dose of (d) 34 Gy and (e) 62 Gy in the N_2-bubbled CTAB/*n*-butanol/cyclohexane/water microemulsion.

Solvated electrons could not be observed in the CPB microemulsion in the present experiments. Gratzel et al.[30] had reported the trapping of hydrated electrons by a cetyl pyridinium chloride (CPCl) micelle. According to these authors, the reaction proceeds with a bimolecular rate constant of 7×10^9 dm^3 mol^{-1} s^{-1}. The electron adduct of the pyridinium cation was seen to have a shoulder at around 355 nm in its broad absorption spectrum. When an aggregation number of 95 is assumed, it had also been found that the second order rate constant for the reaction of e_{aq}^- with the CPCl micelle to be more than 1×10^{12} dm^3 mol^{-1} s^{-1}. These authors rationalized the extraordinarily high rate constant for this reaction by the electrical properties of the micellar double layer. In comparison to this rate constant, CTAB micelles react with hydrated electrons with a bimolecular rate constant of 9.2×10^5 dm^3 mol^{-1} s^{-1}.[30] Thus, from the data available in the literature and arguments provided therein, it is not surprising that we could not observe the hydrated electron in the CPB microemulsion.

3.2. Counterion Radical: Dibromide Radical Anion in CTAB and CPB Microemulsions. The absorption spectrum in the lower wavelength region is quite different than that observed and reported earlier in reverse micellar systems. We have observed another species absorbing in the 300−430 nm region with a maximum around 365 nm (Figure 3). The absorption at this wavelength decays much slower than that both at 720 and 650 nm. Other than an electron, the primary radicals that can be formed in the water pool are the $^{\bullet}$OH radical and $^{\bullet}$H atom. Both the radicals absorb in the far UV and have very low extinction coefficients. The possibility of the absorption maximum at 360 nm cannot be accounted for by either of these radicals or radicals formed from cyclohexane. Thus, it can be assumed that a secondary radical is generated in the water pool.

The bromide ion distribution in CTAB water-in-oil microemulsion has been demonstrated earlier by chemical trapping technique.[31,32] The bromide ion concentrations at the interface and water pool were shown to be as high as 7.0 and 6.0 mol dm^{-3}, respectively, at W_0 = 10 in the CTAB/*n*-dodecane/$CHCl_3$/water microemulsion, and with an increase in W_0 from 10 to 40, the bromide ion concentrations decrease to 3.0 and 0.19 mol dm^{-3} at the interface and at the water pool, respectively.[32] In the CTAB/isooctane/*n*-hexanol/water reversed micellar system, the bromide ion concentration in the water pool was calculated to be 1.91 and 0.29 mol dm^{-3} at W_0 = 12 and 44, respectively.[31] The latter system is closer to that used in the present study, and therefore, it is reasonable to assume a bromide ion concentration of about 2 mol dm^{-3} in the water pools at W_0 = 10. Direct radiolysis of water in the core of these reverse micelles produces hydroxyl radicals.[24] Molar concentration of bromide ions in the water pool are expected to scavenge these hydroxyl radicals and produce dibromide radical anion ($Br_2^{\bullet-}$). In a N_2O-saturated aqueous solution containing bromide ion, the following well established reactions occur after the electron pulse:[33,34]

$$^{\bullet}OH + Br^- \rightarrow [OHBr]^{\bullet-} \rightarrow OH^- + Br^{\bullet} \quad (1)$$

$$Br^{\bullet} + Br^- \rightarrow Br_2^{\bullet-} \quad (2)$$

The rate constant for the formation of $Br_2^{\bullet-}$ radical is 1.2×10^9 dm^3 mol^{-1} s^{-1}. In our experiments the radical formation was complete just at the end of the pulse, and therefore, the rate constant could not be determined at such a high concentration of bromide ion. The dibromide radical anion thus produced has a strong absorption in the wavelength range of 300−500 nm (λ_{max} = 360 nm).[33] In the present case the absorption spectrum matches well with that reported earlier for dibromide radical anion in aqueous solution.

The $Br_2^{\bullet-}$ radical in pure aqueous solution decays by a second order process with a rate constant of 3.3×10^9 dm^3 mol^{-1} s^{-1} and so a first half-life of ca. 0.2 ms.[34] We have also observed a second order decay of the radical as is evident from inset of Figure 3. The decay at 360 nm becomes faster with an increase in dose from 34 to 62 Gy because of an increase in the concentration of the radical at higher dose. The calculated first half-life is ~40 μs which is faster compared to bulk aqueous solution (200 μs).

The faster decay of $Br_2^{\bullet-}$ radical in the microemulsion can be attributed due to the close proximity of the interacting radicals in a small water pool. Another important point is that the yield of $Br_2^{\bullet-}$ radical is increased when the solution is saturated with N_2O (Figure 3 and Table 2). The yield of the $^{\bullet}$OH radical increases in the presence of N_2O by a scavenging process of hydrated electron by the gas through the following reaction:

$$e_{aq}^- + N_2O \rightarrow {}^{\bullet}OH + N_2 + OH^- \quad (3)$$

In this context it is worth mentioning that $Br_2^{\bullet-}$ radical formation and its dismutation reaction was observed earlier in the CTAB micelle. However, the observations were made in an aqueous solution of 0.02 mol dm^{-3} NaBr and 0.005 mol dm^{-3} CTAB.[35] Consequently the radicals were generated from the added bromide ion of NaBr and not from the counterion of CTAB. In the present case, the $G(Br_2^{\bullet-})$ as calculated assuming the extinction coefficient of $Br_2^{\bullet-}$ to be same as that in bulk water is 0.27 per 100 eV at W_0 = 40, N_2 bubbled. We have also observed that that the yield of $Br_2^{\bullet-}$ radicals does not change with added bromide ion (0.005 mol dm^{-3}) in the solution. Therefore, the yield of the hydroxyl radical in the water pool corresponds to that of the observed $Br_2^{\bullet-}$ radicals. Another way to measure the hydroxyl radical yield experimentally is to use KSCN at a concentration of 0.05 mol dm^{-3} in the water pool and measuring absorption at 475 nm due to the $(SCN)_2^{\bullet-}$ radical. The rate constant for the reaction of the $^{\bullet}$OH radical with SCN^- is 1.4×10^{10} dm^3 mol^{-1} s^{-1} [36]

Table 2. Yields of Dibromide Radical Anion ($Br_2^{\bullet-}$) in the CTAB(or CPB)/H_2O/Cyclohexane/*n*-Butanol Microemulsion (W_0 = 40)[a]

		$G(Br_2^{\bullet-})$, N_2 bubbled		$G(Br_2^{\bullet-})$, N_2O saturated	
sr. no.	cosurfactant and other alcohol	CTAB ME	CPB ME	CTAB ME	CPB ME
1	1-butanol	0.27	0.56	0.29	0.48
2	1-pentanol	0.25	0.53	0.30	0.53
3	1-hexanol	0.30	0.59	0.36	0.52
4	1-butanol and 0.07 mol dm^{-3} methanol	0.29	0.58	0.25	0.57
5	1-butanol and 0.5 mol dm^{-3} methanol	0.30	0.59	0.31	0.54
6	1-butanol and 1.0 mol dm^{-3} methanol	0.31	0.67	0.24	0.61
7	1-butanol and 0.07 mol dm^{-3} ethanol	0.25	0.59	0.29	0.49
8	1-butanol and 0.5 mol dm^{-3} ethanol	0.31	0.59	0.25	0.55
9	1-butanol and 1.0 mol dm^{-3} ethanol	0.20	0.66	0.24	0.55

[a] The yields were calculated assuming that the extinction coefficient of the $Br_2^{\bullet-}$ radical in the microemulsion system is the same as that in pure water.

and that with Br^- is 1.2×10^9 dm^3 mol^{-1} s^{-1}. At W_0 = 10, if the concentration of Br^- is assumed to be 1.9 mol dm^{-3} in the water pool, about 30% of the $^{\bullet}OH$ radicals are expected to react with SCN^-. It is not feasible to scavenge all of the $^{\bullet}OH$ radicals in the water pool unless the concentration of SCN^- reaches a value of 16.28 mol dm^{-3} in the water pool. Indeed, we could not succeed in observing measurable absorption due to $(SCN)_2^{\bullet-}$ radicals with a KSCN concentration of 0.05 mol dm^{-3} in the water pool. From the aforesaid results it is now clear that the free bromide ions present in the system as dissociated counterions are capable of scavenging all of hydroxyl radicals produced in the water pool after irradiation. The yield of the $Br_2^{\bullet-}$ radical in CTAB and CPB microemulsions using different cosurfactants and also in the presence of other alcohols are shown in Table 2. It is evident that the cosurfactant does not have any significant role in the yield of the counterion radical. Methanol and ethanol are known scavengers of hydroxyl radicals and are expected to be present in the water core in addition to the dispersed phase. However, it is clear from the table that these alcohols are not able to scavenge the hydroxyl radicals, and thus, the yield of the $Br_2^{\bullet-}$ radical is unchanged up to a concentration of 1 mol dm^{-3} of the alcohols in the microemulsions. The yield of $Br_2^{\bullet-}$ radical in CPB microemulsion is always higher as compared to that in CTAB systems. This anomaly is due to the overlapping absorption of the electron adduct of the pyridinium cation of CPB and the $Br_2^{\bullet-}$ radical.[29]

It may be recalled from the last section that the observed half-life of hydrated electron in CTAB microemulsion is much lower compared to that in SDS system and also in pure water. It is known that the bimolecular rate constant of e_{aq}^- with $Br_2^{\bullet-}$ is 1.3×10^{10} dm^3 mol^{-1} s^{-1}.[34] Therefore the interaction with the $Br_2^{\bullet-}$ radical should also be accounted for besides the presence of charged interface, for the faster decay of the hydrated electron in the CTAB microemulsion system.

3.2. Reaction of the $Br_2^{\bullet-}$ Radical with ABTS: A Protocol for Antioxidant Assay for Substrates Insoluble in Water. The ABTS radical assay employing the technique of kinetic spectroscopy is a standard method to determine antioxidant activity of different samples of unknown composition and contents.[37] These samples are generally herbal extracts obtained in different organic solvent systems. The ABTS radical is also known as a reference system for electron transfer kinetic studies where the free radical is considered as the one electron oxidant.[38] Thus, the

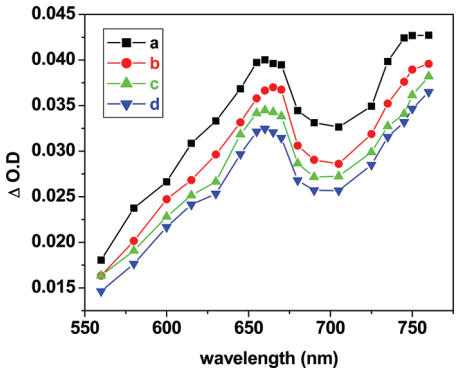

Figure 4. Transient absorption spectrum of the ABTS radical in N_2O-saturated the CTAB/*n*-butanol/cyclohexane microemulsion (W_0 = 40) containing ABTS (1×10^{-3} mol dm^{-3}) at (a) 5, (b) 20, (c), 30, and (d) 40 μs after the electron pulse. O.D. = absorbance.

reactivity of unknown or speculated radicals can be determined from the formation of the strongly absorbing ABTS radical. As an example peroxyl radicals of glycine and vaniline radicals have been known to be reactive toward the ABTS dianion to form the $ABTS^{\bullet-}$ radical.[39] In pulse radiolysis study, generation of the ABTS radical in aqueous solution involves scavenging of primary radicals ($^{\bullet}OH$) by azide ion (N_3^-) producing azidyl radical ($N_3^{\bullet}$), which in turn generates ABTS radical if present in the solution. This radical shows its absorption maxima at 415 nm, 645 and 734 nm.[40] By following the decay of this radical at any of these wavelengths in presence of known antioxidant such as vitamin C one can form a standard curve. Looking at the change in the decay pattern in presence of an extract and determining the decay constant of ABTS radical, it is trivial to fit the value in the standard curve and determine the antioxidant efficiency in terms of ascorbate equivalent. However, problems arise when the substrate is not water-soluble. Mixing of a very low amount of these nonpolar solvent extracts with aqueous solution obviously makes the whole system optically nontransparent and inappropriate for kinetic spectrophotometric analysis. Even for alcohol soluble substrates, it becomes difficult to test the radical scavenging efficacy because the alcohols scavenge the primary radical ($^{\bullet}OH$). In the present investigation we intended to observe ABTS radical via the reaction of ABTS with $Br_2^{\bullet-}$ radical in the first step. The ABTS radical formation and its transient

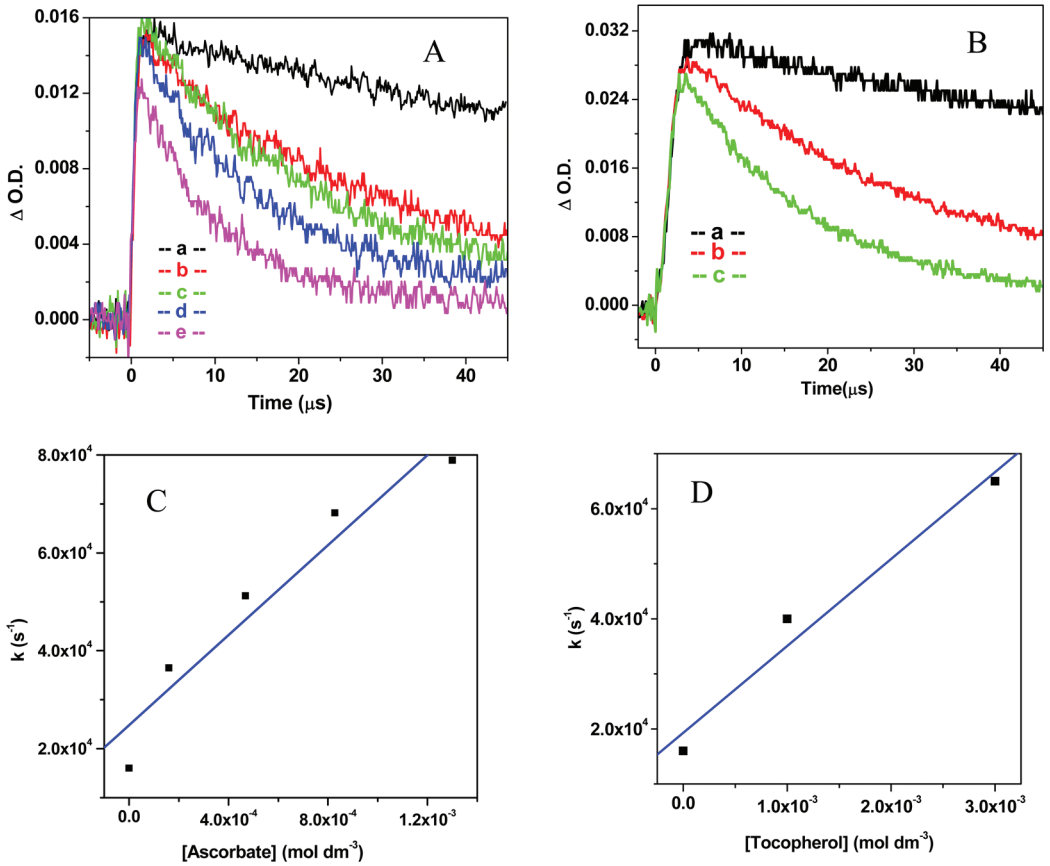

Figure 5. (A) Decay curve of ABTS radical in presence and absence of ascorbate. (B) Decay curve of ABTS radical in presence and absence of tocopherol. (C and D) Plots of rate constant versus concentration of ascorbate and tocopherol, respectively, for scavenging the ABTS radical. O.D. = absorbance.

absorption spectrum are shown in figure 4. Similar to that in aqueous solution, it shows absorption maxima at 650 and 745 nm within 5 μs after the electron pulse. The radical is formed with a bimolecular rate constant of 1.18×10^9 dm^3 mol^{-1} s^{-1}.

Under such experimental conditions, it should be possible to study the ABTS radical scavenging process with the molecules soluble in solvents with a wide polarity assortment. For instance, we have considered tocopherol (water insoluble) and ascorbate (water-soluble) as scavengers of this radical. Figure 5, panels A and B, show the decay of the ABTS radical at 645 nm in the presence and absence of different concentrations of ascorbate and tocopherol, respectively.

It is clearly seen that with an increase in the scavenger concentrations the decay of the ABTS radical becomes faster. By plotting the decay constant versus concentration of ascorbate and tocopherol, we have determined the bimolecular rate constant for the reactions and also standard curves (Figure 5, panels C and D) have been constructed. The bimolecular rate constants for the reaction of the $ABTS^{\bullet-}$ radical with ascorbate and tocopherol are 1.6×10^7 and 4.6×10^7 dm^3 mol^{-1} s^{-1}, respectively. The obvious reason for the higher reactivity of ascorbate compared to tocopherol is the location of these molecules in the microemulsion. Ascorbate being water-soluble, it resides in the water core where the reacting radical is produced, whereas tocopherol may reside both in the oil phase as well as in the interface, the reaction of which involves diffusion of $ABTS^{\bullet-}$ radical to or through the interface. Thus, it is demonstrated in this study that a two phase reaction can occur in the microemulsion without the use of a phase transfer catalyst. It must be pointed out that the initial yield of the ABTS radical is dependent on the concentrations of ascorbate or tocopherol. With the increase in concentration of ascorbate or tocopherol, the probability of scavenging the primary and/secondary radicals ($^{\bullet}OH$ and $Br_2^{\bullet-}$) progressively increases leading to a lower yield of ABTS radical.

In the next step by following the decay of the ABTS radical in the presence of different concentrations (mg/mL) of extracts, it would not be difficult to obtain the vitamin E or C equivalent antioxidant capacity of any water-soluble/insoluble extracts or mixtures of compounds.

4. CONCLUSION

In summary, the hydrated electrons could be observed in a water-in-oil CTAB/H_2O/*n*-butanol/cyclohexane microemulsion. The lifetime is shorter than that compared to aqueous solution as well as in anionic microemulsion. The electrons are more stabilized in the interface as compared to that in water core. The decay rate constant of the electrons solvated in the interface can provide information about the rigidity in that region. This paper contains the first report on the generation of the counter-ion radical ($Br_2^{\bullet-}$) in both of the CTAB and CPB microemulsions after irradiation with an electron beam. In addition, it has been demonstrated that a two phase reaction occurring between radicals produced in the aqueous core and a molecule that is completely insoluble in water was possible. We were able to

generate the ABTS radical in the water pool and observe its reaction with molecules soluble in either phase in CTAB microemulsion. The spinoff of this result is a significant improvement in the established kinetic protocol involving the ABTS radical for free radical scavenging and antioxidant activity assay, which is now applicable to molecules soluble in solvents with a wide assortment of polarities.

AUTHOR INFORMATION

Corresponding Author

*E-mail: asoumya@barc.gov.in. Phone: +91-22-25590301. Fax: +91-22-25505151.

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