# One-Electron Reduction Potential of *m*-AMSA$^+$ [9-(2-Methoxy-4-methylsulphonylaminoanilino)acridinium] as Measured by Pulse Radiolysis

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The absorption spectrum of one-electron reduced *m*-AMSA$^+$ has been measured by pulse radiolysis. The radical species formed, *m*-AMSA$^\cdot$, is readily oxidized by oxygen, flavin mononucleotide, and triquat; *m*-AMSA$^+$ itself acts as an oxidant of the one-electron reduced species of nicotinamide adenine dinucleotide. The one-electron reduction potential of the *m*-AMSA$^+$/*m*-AMSA$^\cdot$ couple at pH 7, $E^1_7$, has been determined to be $-803 \pm 10$ mV (*vs.* NHE) by studying the equilibrium established between *m*-AMSA$^+$/m-AMSA$^\cdot$ and a low-potential bipyridinium compound used as a redox indicator.

*m*-AMSA$^+$ [9-(2-methoxy-4-methylsulphonylaminoanilino)-acridinium] [1] is a promising cytotoxic drug against leukaemia which has successfully completed Phase II clinical studies [2,3] and is now undergoing randomized trials. Its mechanism of action is thought to involve binding to DNA by intercalation.[4] Interest in a possible additional role for *m*-AMSA$^+$ (and other intercalators) as a modifier of radiation damage in cells has stemmed from work *in vitro* with *E. coli* B/r [5] and the mammalian cell lines CHO [6] and V79.[7] At subtoxic concentrations *m*-AMSA$^+$ appears to reduce the width of the shoulder of the *X*-ray survival curve when present during the irradiation. Similar effects have been observed for adriamycin which is also a cytotoxic intercalating agent.[8,9]

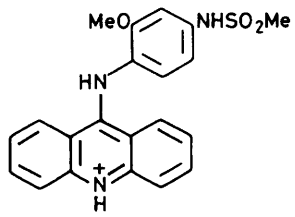
Although the cytotoxic efficacy of intercalating agents is thought to be related to how strongly they can bind to DNA, this factor alone is not predictive.[4] Recently the one-electron reduction potential at pH 7 ($E^1_7$) has been determined for adriamycin as $-328$ mV (*vs.* NHE).[10] This electronic parameter has been shown to be the dominant term in structure–activity studies on the radiosensitization of mammalian [11] and bacterial [12] cells by a series of nitroimidazole compounds. It has been proposed [13] that radiation-induced reactions with DNA result largely from direct ionizations which form positive and negative radical sites throughout the polymer; the action of electron-affinic sensitizers prevents charge recombination by scavenging mobile electrons.

A pulse radiolysis study on acridine [14] has estimated that its one-electron reduction potential at pH 2 is more negative than $-0.48$ V. It was also found that reduction by $(CH_3)_2\dot{C}OH$ and $CO_2^-$ species is rapid and efficient only when the acridine is protonated. In another pulse radiolysis study proflavin bound to DNA was found not to influence the possible electron migration in irradiated DNA.[15]

In the present work we report on aspects of the radiation chemistry of *m*-AMSA$^+$ including electron-transfer reactions to *m*-AMSA$^+$ and subsequent reoxidation reactions involving various oxidizing agents. *m*-AMSA$^+$ has p$K_a$ 8.8,[1] and as work described in this paper was done at pH 7 or less, the reacting substrate is the protonated form of the acridine.

## Experimental

*m*-AMSA$^+$ (1) was prepared and supplied as the sulphonate salt by the Cancer Research Laboratory Auckland Medical School, New Zealand. Dipyrido[1,2-*a*;2′,1′-*c*][1,4]diazepinediium dibromide ($TQ^{++}$) and its 2,3,11,12-tetramethyl derivative ($V^{++}$) were prepared by a method similar to that described in the literature.[16] Flavin mononucleotide (FMN) (99%) and nicotinamide adenine dinucleotide ($NAD^+$) were obtained from Sigma Chemical Co.



(1)

Details of the Brunel [17] and the new Gray Laboratory [18] pulse radiolysis and radical detection systems (used for spectral and electron transfer studies respectively) have been published. Dosimetry was carried out using aerated KSCN solution, assuming the $(SCN)_2^-$ radical produced had a yield ($G$) of 0.29 μmol J$^{-1}$ with a molar absorptivity, ε, of 7 600 l mol$^{-1}$ cm$^{-1}$ at 480 nm.[19]

## Results and Discussion

On irradiation water breaks down to yield (among other intermediates) ·OH radicals and aquated electrons, $e^-_{aq}$, the yields ($G$) of these two species being *ca.* 0.28 μmol J$^{-1}$. In studies of the reactions of $e^-_{aq}$ with solutes, 2-methylpropan-2-ol is commonly used to scavenge the highly reactive and oxidizing ·OH radicals [reaction (1)], the β-alcohol

$$\cdot OH + (CH_3)_3COH \longrightarrow H_2O + \cdot CH_2C(CH_3)_2OH \quad (1)$$

radical formed being relatively unreactive. In studies of ·OH radical reactions it is common practice to use solutions saturated with nitrous oxide to convert the reducing radical into ·OH [equation (2)].

$$e^-_{aq} + N_2O \longrightarrow \cdot OH + N_2 + H_2O \quad (2)$$

In solutions containing propan-2-ol, reaction with ·OH results in the formation of the strongly reducing α-alcohol radical [equation (3)].

$$\cdot OH + (CH_3)_2CHOH \longrightarrow H_2O + (CH_3)_2\dot{C}OH \quad (3)$$

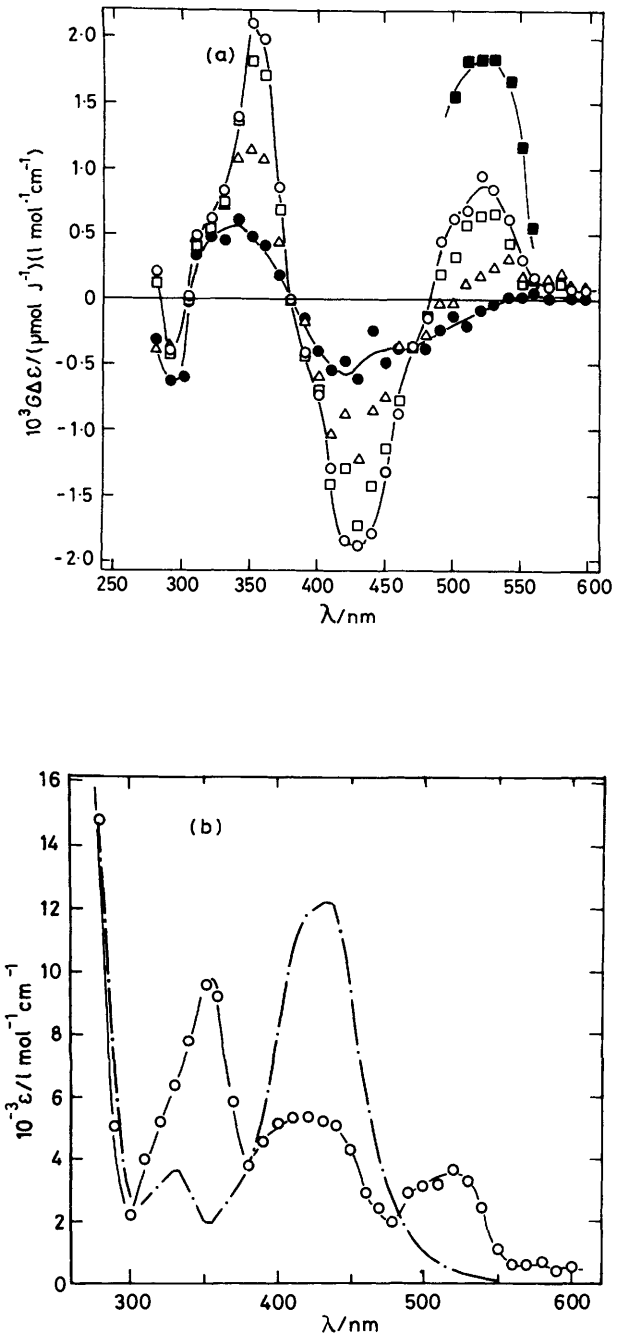



**Figure 1.** (a) Changes in absorption [presented as the product of the yield of radicals ($G$) and the change in molar absorptivity ($\Delta\varepsilon$)] following pulse radiolysis (5 Gy in 0.2 μs) of (i) a $N_2$-saturated solution containing *m*-AMSA$^+$ (50 μmol l$^{-1}$) and 2-methylpropan-2-ol (0.2 mol l$^{-1}$) at pH 5.9: spectra measured at ○ 10 μs, □ 50 μs, △ 200 μs, and ● 1 ms following the pulse, and (ii) a $N_2O$-saturated solution containing *m*-AMSA$^+$ (200 μmol l$^{-1}$) and propan-2-ol (0.2 mol l$^{-1}$) at pH 7 ■ measured 15 μs after the pulse. (b) The absorption spectrum of *m*-AMSA· measured 10 μs after the pulse from Figure 1a, ○; corrected for the bleaching of *m*-AMSA$^+$ absorption — · — · —

*Spectra.*—Figure 1a shows spectra measured at four different times following a 5 Gy pulse delivered to a $N_2$-saturated aqueous solution of *m*-AMSA$^+$ (50 μmol l$^{-1}$) containing 2-methylpropan-2-ol (0.2 mol l$^{-1}$) at pH 5.9. The ordinate is expressed as $G\Delta\varepsilon$, where $G$ is the yield of radicals (μmol J$^{-1}$) and $\varepsilon$ is the molar absorptivity (l mol$^{-1}$ cm$^{-1}$) derived from the expression (4) where $A$ is the absorbance,

$$G = A/td \qquad (4)$$

$t$ the pathlength of the cell (cm), and $d$ the dose per pulse (Gy). The Figure also shows the spectrum over a limited wavelength region for an $N_2O$-saturated solution of *m*-AMSA$^+$ (0.2 mmol l$^{-1}$) containing propan-2-ol (0.2 mol l$^{-1}$) at pH 7.

The full spectrum, due to the reaction (5), shows bleaching

$$e^-_{aq} + m\text{-AMSA}^+ \longrightarrow m\text{-AMSA}\cdot \qquad (5)$$

between 380 and 480 nm, due to removal of *m*-AMSA$^+$, which must absorb more strongly in this region than the electron adduct. Figure 1b shows the spectrum of *m*-AMSA$^+$, and the absorption spectrum of the *m*-AMSA· radical, calculated assuming that the $e^-_{aq}$ is reacting quantitatively. It has three bands with $\varepsilon$ 9 900, 5 400, and 3 475 mol$^{-1}$ cm$^{-1}$ at 320, 420, and 520 nm respectively. Neta [14] has measured the spectrum of the electron adduct of acridine and finds a broad band between 400 and 500 nm, possibly showing more than one transition and with $\varepsilon_{max.}$ 4 000 l mol$^{-1}$ cm$^{-1}$.

The relatively slow decay of the *m*-AMSA· radical shows two well defined isosbestic points, implying a clean reaction. The decrease in bleaching possibly implies re-formation of the substrate, and the reaction may involve disproportionation to *m*-AMSA$^+$ and its two-electron reduced product.

The fact that over the wavelengths measured for the propan-2-ol–$N_2O$ system the spectrum is similar to but the yield double that for the 2-methylpropan-2-ol–$N_2$ system is consistent with the $(CH_3)_2\dot{C}OH$ radical reducing *m*-AMSA$^+$ to the same transient product as the aquated electron [equation (6)]. The molar absorptivity of *m*-AMSA$^+$ is almost zero in this range of wavelengths.

$$(CH_3)_2\dot{C}OH + m\text{-AMSA}^+ \longrightarrow (CH_3)_2CO + H^+ + m\text{-AMSA}\cdot \qquad (6)$$

*Rates of Electron Transfer.*—The rate of reaction (5) was determined by following the decay of $e^-_{aq}$ at 680 nm in 2-methylpropan-2-ol (0.2 mol l$^{-1}$) at *m*-AMSA$^+$ concentrations of 40, 60, and 200 μmol l$^{-1}$. The decays were exponential and the observed first-order rate constants proportional to *m*-AMSA$^+$ concentration, with the bimolecular rate constant $k_5 = 3.95 \pm 0.4 \times 10^{10}$ l mol$^{-1}$ s$^{-1}$. The high value is consistent with a diffusion-controlled reaction between reactants of opposite charge. From the exponential grow-ins at 540 and 575 nm for $N_2O$-saturated solutions containing *m*-AMSA$^+$ (200 μmol l$^{-1}$) and propan-2-ol (0.2 mol l$^{-1}$) we obtained a value for $k_6$ of $1.2 \pm 0.2 \times 10^9$ l mol$^{-1}$ s$^{-1}$.

A major aim of this work was to determine the one-electron reduction potential, $E^1_7$, of *m*-AMSA$^+$. It has been shown that pulse radiolysis provides a powerful technique for making such measurements. The method involves establishing an observable thermodynamic equilibrium of the type (7), and

$$A^{-\cdot} + B \rightleftharpoons A + B^{-\cdot} \qquad (7)$$

determining the equilibrium concentrations of $A^{-\cdot}$ and $B^{-\cdot}$ at various known concentration ratios of A and B.[20,21] From this the equilibrium constant can be determined, and if $E^1_7$ for one of the compounds is known then the other can be calculated.

In the case of *m*-AMSA$^+$ it is necessary to establish the equilibrium (8), where S is a substrate of known one-electron

$$m\text{-AMSA}\cdot + S \rightleftharpoons m\text{-AMSA}^+ + S^{-\cdot} \qquad (8)$$

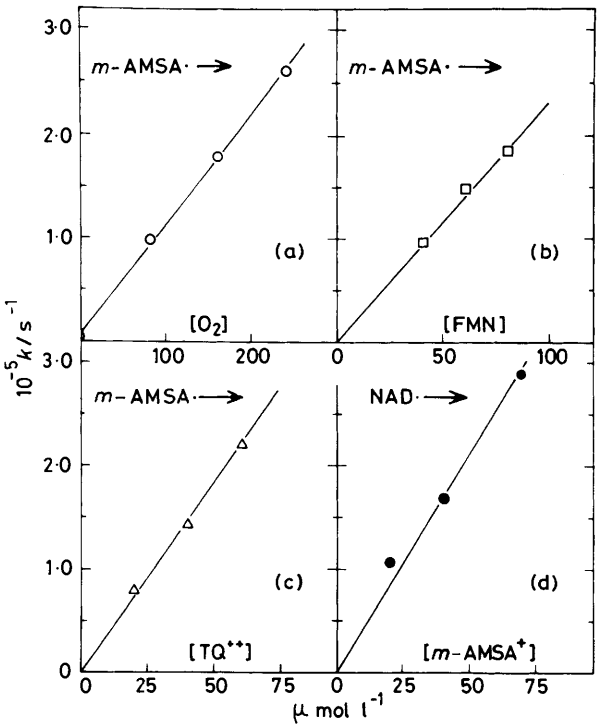



**Figure 2.** First-order oxidation rates of (a) *m*-AMSA· by $O_2$, (b) *m*-AMSA· by FMN, (c) *m*-AMSA· by $TQ^{++}$, and (d) NAD· by *m*-$AMSA^+$. Radical species were produced by pulse radiolysis (2.5 Gy in 0.2 μs) of $N_2$-saturated solutions containing 2-methylpropan-2-ol (0.2 mol $l^{-1}$) at pH 7.0

reduction potential sufficiently close to the required $E^1_7$(*m*-$AMSA^+$/*m*-AMSA·) for an observable equilibrium. If $E^1_7$(S/$S^{-\cdot}$) is not sufficiently close to $E^1_7$(*m*-$AMSA^+$/*m*-AMSA·) reaction (8) will proceed to completion in either the forward or the reverse direction. We therefore initially studied reaction (8) with a number of substrates, S, of known $E^1_7$ values, to find limits of $E^1_7$(*m*-$AMSA^+$/*m*-AMSA·). All solutions contained 2-methylpropan-2-ol (0.2 mol $l^{-1}$) and were buffered to pH 7 with a low concentration of phosphate. The results are presented in Figure 2.

When a $N_2$-saturated solution of *m*-$AMSA^+$ (250 μmol $l^{-1}$) was pulsed in the presence of air at various partial pressures, the absorption at 535 nm due to *m*-AMSA· decayed completely in an exponential fashion at a rate dependent on the oxygen concentration. From the slope of Figure 2(a), the value of $1.2 \pm 0.2 \times 10^9$ l $mol^{-1}$ $s^{-1}$ for $k_9$ was obtained. The value of $E^1_7(O_2/O_2^{-\cdot})$ is −155 mV,[22,23] so we next studied electron

$$m\text{-AMSA}\cdot + O_2 \longrightarrow m\text{-AMSA}^+ + O_2^{-\cdot} \quad (9)$$

transfer to FMN [$E^1_7$(FMN/FMNH·) = −314 mV].[24] In $N_2$-saturated solution of *m*-$AMSA^+$ (200 μmol $l^{-1}$) and FMN (40—160 μmol $l^{-1}$) a grow-in at 580 nm due to the flavosemiquinone was observed. This was exponential and proportional to FMN concentration, and from the slope of Figure 2(b) $k_{10}$ was determined as $2.2 \pm 0.2 \times 10^9$ l $mol^{-1}$ $s^{-1}$.

$$m\text{-AMSA}\cdot + \text{FMN} \longrightarrow m\text{-AMSA}^+ + \text{FMNH}\cdot \quad (10)$$

Next we used triquat, $TQ^{++}$ [$E^1_7(TQ^{++}/TQ^{+\cdot})$ = −548 mV][25] detecting the $TQ^{+\cdot}$ species in the infra-red at 900 nm.[26] Again *m*-AMSA· reduced the substrate, a grow-in at 900 nm being detected on pulsing solutions containing an excess of *m*-$AMSA^+$ (200 μmol $l^{-1}$) and various amounts of $TQ^{++}$ (20—60 μmol $l^{-1}$). From the slope of Figure 2(c), $k_{11} = 3.6 \pm 0.3 \times 10^9$ l $mol^{-1}$ $s^{-1}$, the reaction again going to completion. However, on going to $NAD^+$ [$E^1_7$($NAD^+$/

$$m\text{-AMSA}\cdot + TQ^{++} \longrightarrow m\text{-AMSA}^+ + TQ^{+\cdot} \quad (11)$$

NAD·) = −918 mV],[27,28] it was found that a grow-in occurred at 530 nm, this showing *m*-AMSA· was being formed and that reaction (8) was now proceeding in the reverse direction. Figure 2(d) shows the pseudo-first-order rate constants for solutions containing an excess of $NAD^+$ (1 mmol $l^{-1}$) and various amounts of *m*-$AMSA^+$ (20—80 μmol $l^{-1}$). The yield of *m*-AMSA· slightly decreased with decreasing *m*-$AMSA^+$ concentration, implying that reaction (12) was not going fully to completion, and this reverse action is reflected in the high pseudo-first-order rate constant at the lowest *m*-$AMSA^+$ concentration. From the higher concentrations, $k_{12} \simeq 4 \times 10^9$ l $mol^{-1}$ $s^{-1}$.

$$\text{NAD}\cdot + m\text{-AMSA}^+ \longrightarrow \text{NAD}^+ + m\text{-AMSA}\cdot \quad (12)$$

Having established −918 mV < $E^1_7$(*m*-$AMSA^+$/*m*-AMSA·) < −548 mV, a low-potential bipyridinium compound in this region was used. The value of $E^1_7(V^{++}/V^{+\cdot})$ has been established as $-775 \pm 9$ mV for $V^{++}$ (Anderson, unpublished data) using reference compounds employed previously to determine $E^1_7$ for another low-potential bipyridinium compound.[27] $V^{++}$ scavenges $e^-_{aq}$ ($k = 5 \times 10^{10}$ l $mol^{-1}$ $s^{-1}$) and oxidizes propan-2-oxyl radicals ($k = 8 \times 10^8$ l $mol^{-1}$ $s^{-1}$) to form $V^{+\cdot}$, which absorbs in the i.r. (Anderson, unpublished data) and was detected at 900 nm. Reaction (13) was found to produce a measurable equilibrium. A typical

$$m\text{-AMSA}\cdot + V^{++} \rightleftharpoons m\text{-AMSA}^+ + V^{+\cdot} \quad (13)$$

oscillogram is presented in Figure 3, which shows the rapid scavenging of $e^-_{aq}$ to form an initial amount of absorbing $V^{+\cdot}$ which increases as equilibrium (13) is established. This final absorbance is considerably less than that found following pulse radiolysis of $V^{++}$ alone, and as *m*-AMSA· absorbs negligibly at 900 nm, the relative concentrations of $V^{+\cdot}$ and *m*-AMSA· at equilibrium can be found and hence $K_{13}$.

From four $N_2$-saturated solutions containing *m*-$AMSA^+$ (0.04—0.2 mmol $l^{-1}$), $V^{++}$ (50 μmol $l^{-1}$), and propan-2-ol (0.2 mol $l^{-1}$) at pH 7, we measured $K_{13}$ (corrected for ionic strength effects[26,29]) as $2.87 \pm 0.45$. Also from four $N_2$-saturated solutions containing *m*-$AMSA^+$ (0.2 mol $l^{-1}$), $V^{++}$ (0.02—0.12 mmol $l^{-1}$), and 2-methylpropan-2-ol (0.1 mol $l^{-1}$) at pH 7 we again measured $K_{13}$ as $2.99 \pm 0.12$. Combining the data, $K_{13} = 2.93 \pm 0.24$. The observed rate of approach to equilibrium, $k_{obs}$, also provides a qualitative method for determing $K_{13}$ as $k_{obs} = k_{13}[V^{++}] + k_{-13}$[*m*-$AMSA^+$], where $k_{13}$ and $k_{-13}$ are the rate constants for the forward and reverse reactions. A plot of $k_{obs}$/[*m*-$AMSA^+$] *vs.* [$V^{++}$]/[*m*-$AMSA^+$] yields $k_{-13}$ from the intercept, $k_{13}$ from the slope and hence $K_{13} = k_{13}/k_{-13}$. The kinetic data of the approach to equilibrium obtained using 2-methylpropan-2-ol were used for analysis (Figure 3) as the initial formation of *m*-AMSA· and $V^{+\cdot}$ upon scavenging of $e^-_{aq}$ is faster than their formation by electron transfer from the propan-2-oxyl radical which results in $k_{obs}$ being complex. The plot yields $K_{13} = 3.80 \pm 1.53$, which agrees with the more accurately determined value.

$$E^1_7(m\text{-AMSA}^+/m\text{-AMSA}\cdot) = E^1_7(V^{++}/V^{+}) - 59 \log K_{13}$$
$$= (-775 \pm 9) - (28 \pm 2) = -803 \pm 10 \text{ mV}$$

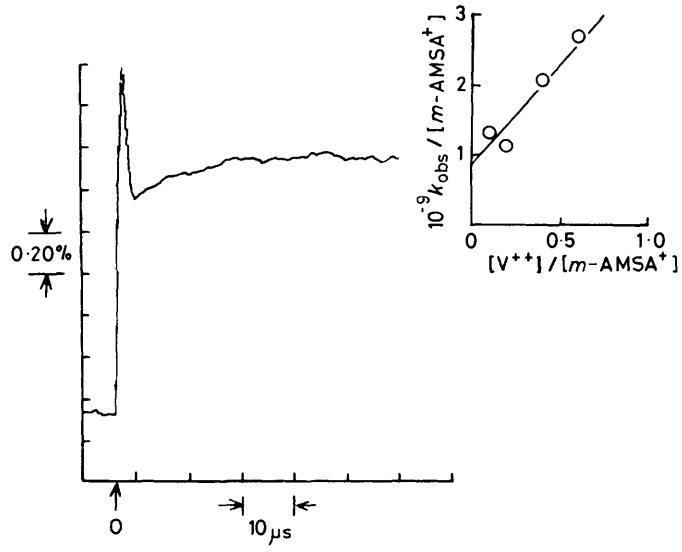



**Figure 3.** Oscilloscope trace showing the change in percentage absorption at 900 nm (ordinate) against time (abscissa) following pulse radiolysis (6.0 Gy in 0.3 μs) at time 0 of a $N_2$-saturated solution containing *m*-AMSA$^+$ (200 mol $l^{-1}$), $V^{++}$ (80 μμmol $l^{-1}$), and 2-methylpropan-2-ol at pH 7; *insert:* kinetic plot based on the rate of approach to equilibrium as described in the text

The low measured potential weighs against a possible direct involvement of the *m*-AMSA· species in its cytotoxic action as we have demonstrated it to be readily oxidized by acceptors such as flavins which possess moderate $E^1_7$ values. This is in contrast to the quinone-containing anticancer drugs such as adriamycin (−328 mV)[10] and mitomycin C (−270 mV);[30] the relative stability of their semiquinones has been suggested to assist in their binding to nucleic acids or subsequent activation.[31,32]

The possibility of *m*-AMSA$^+$, intercalated in DNA, acting as an electron trap following irradiation has yet to be investigated.

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