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One- and Two-electron Reduction of Quinizarin and 5-Methoxyquinizarin: A Pulse Radiolysis Study

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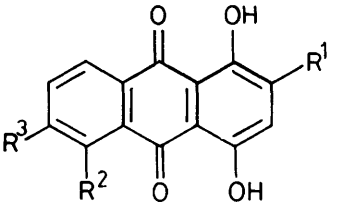
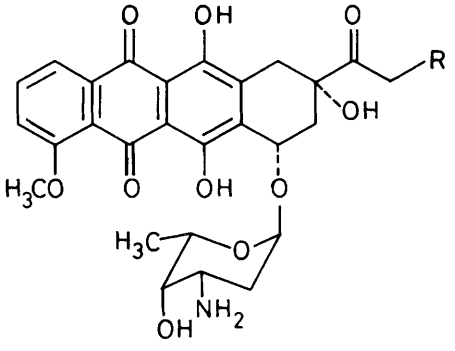
Absorption characteristics of the semiquinone free radicals formed by one-electron reduction of quinizarin (QH_2), 5-methoxyquinizarin (MQH_2) and quinizarin 2-sulphonate (QSH_2) have been studied by pulse radiolysis in a mixed solvent system consisting of water, isopropyl alcohol and acetone. Second-order rate constants have been determined for the reactions of $(CH_3)_2\dot{C}OH$ with the quinones, of the semiquinones with O_2 and of the semiquinones with each other. The one-electron reduction potentials (*vs.* NHE) are $E_7^1 = -269$ mV for QH_2, -333 mV for MQH_2 and -298 mV for QSH_2. They vary with pH in accordance with the pK_a values of the parent quinones and the semiquinones. The radicals are stable within the approximate pH range 5–11. The stability constant is highest at pH 8.5 ($K_s \approx 0.09$) for QH_2, at pH $\approx$ 9.5 for QSH_2 ($K_s \approx 10$) and pH $\approx$ 10.8 for MQH_2 ($K_s \approx 4.8$), respectively. The one-electron reduction potentials of the semiquinones and the two-electron reduction potentials of the quinones are calculated to be $E_7^2 = -188$, -192 and -216 mV, and $E_7^m = -229$, -263 and -257 mV for QH_2, MQH_2 and QSH_2, respectively. The effect of solvent on the properties of the semiquinones is discussed.

The significance of the semiquinones derived from quinizarin (see scheme 1) [**I**(*a*): 1,4-dihydroxy-9,10-anthraquinone; QH_2] and its 2-sulphonate [**I**(*b*); $Q2SH_2$] and 6-sulphonate [**I**(*c*); $Q6SH_2$] derivatives has been highlighted by us earlier.[1] Quinizarin [**I**(*a*); QH_2] and 5-methoxyquinizarin [**I**(*d*); MQH_2] are closely related to adriamycin [**II**(*a*)] and daunomycin [**II**(*b*)], two of the most potent quinone antitumour drugs.[2]



(I)



(II)

Scheme 1. Structure **I**(*a*) $R^1 = R^2 = R^3 = H$, QH_2; (*b*) $R^1 = SO_3^-$, $R^2 = R^3 = H$, $Q2SH_2$; (*c*) $R^1 = R^2 = H$, $R^3 = SO_3^-$, $Q6SH_2$; (*d*) $R^1 = R^3 = H$, $R^2 = OCH_3$, MQH_2. Structure **II**(*a*) R = OH, adriamycin; (*b*) R = H, daunomycin.

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In fact, it has recently been shown[3] that quinizarin has moderate anti-tumour activity with associated dose-related toxic effects.

Both quinizarin and 5-methoxyquinizarin are very sparingly soluble in water. However, the one-electron reduction of quinones can be studied by pulse radiolysis of de-aerated aqueous solutions containing excess of methanol, ethanol, isopropyl alcohol–acetone or formate,[1,4–9] and it has been shown that the same semiquinones are formed in each system. For pulse radiolysis studies of QH_2 and MQH_2 we have used an aqueous solution of 5 mol dm^{-3} isopropyl alcohol and 1 mol dm^{-3} acetone. This mixed solvent system has been used earlier[5] in pulse radiolysis studies of several methyl derivatives of 1,4-benzoquinone and 1,4-naphthoquinone. We have included quinizarin 2-sulphonate [**I**(*b*), hereafter simply QSH_2] so as to compare the aqueous formate[1] and aqueous isopropyl alcohol–acetone systems.

Experimental

5-Methoxyquinizarin was synthesised by refluxing for 3 h a solution containing naphthazarin (5,8-dihydroxy-1,4-naphthoquinone) with a large excess of 1-methoxycyclohexa-1,3-diene dissolved in dichloromethane. The black–brown Diels–Alder adduct (*ca.* 75% yield) thus obtained was purified by column chromatography on silica gel using ethyl acetate–hexane–dichloromethane (1 : 3 : 4) as eluent and recrystallisation from ethanol, to afford yellow crystals, m.pt. 159–161 °C. The adduct was then oxidised to 5,8-ethano-5,8-dihydro-5-methoxyquinizarin in 91% yield with aerated aqueous sodium hydroxide (reaction time *ca.* 2 h). The deep red needles obtained on acidification and crystallisation (hexane) were finally refluxed in *o*-xylene for 30 min to give 5-MQH_2 in *ca.* 92% yield, purified by recrystallisation from petroleum ether. The melting point, 247–248 °C [ref. (10), 247–249 °C] and the NMR spectrum of the red crystals were consistent with the structure [**I**(*d*)]. Quinizarin (Sigma) was purified by recrystallisation from benzene.

Quinizarin 2-sulphonate was synthesised as described earlier.[1] The redox references and all other chemicals were as

described earlier,[1] except for sodium anthraquinone-2-sulphonate, which was repeatedly recrystallised from ethanol.

For preparation of the experimental solutions, solid quinone was first dissolved in isopropyl alcohol. To an aliquot of this solution was added the appropriate volume of acetone, followed by water and buffer solution, to obtain the solution of desired concentration. Pulse radiolysis and all other experiments were conducted essentially as described earlier.[7,8] Solutions were all bubbled for at least 30 min with O_2-free argon (Air Products Ltd) saturated with solvent vapour as a precautionary measure for any solvent loss by evaporation. The temperature of the solutions was maintained at 298 K.

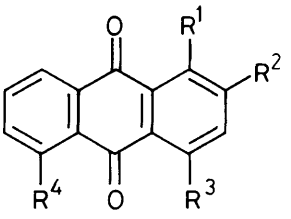
The difficulty in defining the pH of an aqueous organic system has been discussed elsewhere.[11–13] As in previous studies,[4,5] the pH measurements in this work were undertaken using a combination electrode, since our solvent system contains 30.2 mol dm^{-3} water besides 5 mol dm^{-3} isopropyl alcohol and 1 mol dm^{-3} acetone, *i.e.*, the bulk of the medium is still aqueous and the reporting of pH seems to be justifiable. Wherever possible, the data for the system have been compared with data for solutions lacking isopropyl alcohol and acetone.

Results and Discussion

Absorption Spectra and pK_a of Quinizarin Derivatives

In aqueous–organic solutions, QH_2, MQH_2 and QSH_2 can exist in their fully protonated forms **III(*a*)** (yellow orange), mono-anionic forms **III(*b*)** (violet) or di-anionic forms **III(*c*)** (deep blue for MQ^{2-} and QS^{2-} and deep violet for Q^{2-}) depending on the pH, see scheme 2.

The measured spectral data and the acid dissociation constants (K_1 and K_2) are given in table 1.



(III)

Scheme 2. Structure **III**: $R^2 = R^4 = H$, quinizarin; $R^2 = SO_3^-$, $R^4 = H$, quinizarin 2-sulphonate; $R^2 = H$, $R^4 = OCH_3$,5-methoxyquinizarin; (*a*) $R^1 = R^3 = OH$, $QH_2(QSH_2, MQH_2)$; (*b*) $R^1 = O^-$, $R^3 = OH$, $QH^-(QSH^-, MQH^-)$; (*c*) $R^1 = R^3 = O^-$, $Q^{2-}(QS^{2-}, MQ^{2-})$.

The absorption spectra of QSH_2 measured in the aqueous solution containing formate[1] and the present aqueous organic systems were almost identical, except for a slight broadening of the visible region peak in the latter system. The first pK_a value was *ca.* one pK_a unit higher in the mixed solvent system, *i.e.* the presence of alcohols or acetone leads to decreased ionisation. The pK_a values of MQH_2 are higher than those of QH_2, possibly due to the mesomeric electron-releasing property of the methoxy group which increases the electron density of the system, thus destabilising the anion and, therefore, making ionisation more difficult. Surprisingly, the second pK_a value was very similar in the two systems.

Semiquinone–Quinone Difference Spectra, Acid Dissociation Constants and Absolute Absorption Spectra of the Radicals

When electron pulses are delivered to argon-bubbled dilute aqueous–organic solutions of quinizarin derivatives (conc. $\leqslant 10^{-4}$ mol dm^{-3}), containing isopropyl alcohol (5 mol dm^{-3}) and acetone (1 mol dm^{-3}), the principal action of the radiation is on the major component (water) followed by a series of reactions as follows:

$$H_2O \rightsquigarrow H^\bullet, e_{aq}^-, OH^\bullet \quad (I)$$

$$OH^\bullet(H^\bullet) + (CH_3)_2CHOH \longrightarrow (CH_3)_2\dot{C}OH + H_2O(H_2) \quad (II)$$

$$e_{aq}^- + (CH_3)_2CO \longrightarrow (CH_3)_2CO^{\bullet -} \quad (III)$$

$$(CH_3)_2CO^{\bullet -} + H_2O \longrightarrow (CH_3)_2\dot{C}OH + OH^- \quad (IV)$$

$$(CH_3)_2\dot{C}OH + \text{quinone} \longrightarrow \text{semiquinone} + (CH_3)_2CO + nH^+ \quad (V)$$

with the parent quinone in one of the forms represented by structure **III** and the semiquinone in one of the four different forms in structure **IV(*a*)–(*d*)** (*e.g.* $OH_3^\bullet$, QH_2^-, $QH^{\bullet 2-}$ or $Q^{\bullet 3-}$), depending on the pH of the solution, see scheme 3.

Protonation to $QH_4^{\bullet +}(MQH_4^{\bullet +}$ or $QSH_4^{\bullet +})$ may occur in very strongly acidic media, but such solutions are not considered here. At the high concentration of the organic solutes in this system, direct action of the radiation on the solute must also be considered. This will consist essentially of the formation of further $(CH_3)_2\dot{C}OH$ which will then react according to reaction (V).

The differences between the absorbance of the semiquinone and the parent quinone were measured several microseconds after giving electron pulses to argon-bubbled solutions containing QH_2, MQH_2 and QSH_2 (*ca.* 1×10^{-4} mol dm^{-3}). The difference spectra for QH_2 after 20 μs at pH 1.2 and 5.4,

Table 1. Absorption characteristics and pK_a values of quinizarin and its derivatives in the mixed solvent system of 5 mol dm^{-3} isopropyl alcohol and 1 mol dm^{-3} acetone in water

	λ_{max}/nm ($\varepsilon/10^3$ dm^3 mol^{-1} cm^{-1})				
compound	neutral	anionic	di-anionic	pK_1	pK_2
quinizarin	465 (8.32) 477 (8.43) *ca.* 510 sh (4.74)	547 (9.74) 581 (8.63)	562 (10.43) 600 (10.83)	9.90 ± 0.1	12.75 ± 0.1
5-methoxyquinizarin	470 (8.32) 488 (8.42) *ca.* 524 sh (4.72)	552 (9.74) 584 (8.62)	572 (10.42) 602 (10.82)	10.60 ± 0.1	13.4 ± 0.1
sodium quinizarin 2-sulphonate	468 (8.8)	600 (9.65)	586 (13.68) 618 (14.7)	10.1 ± 0.1	12.6 ± 0.1

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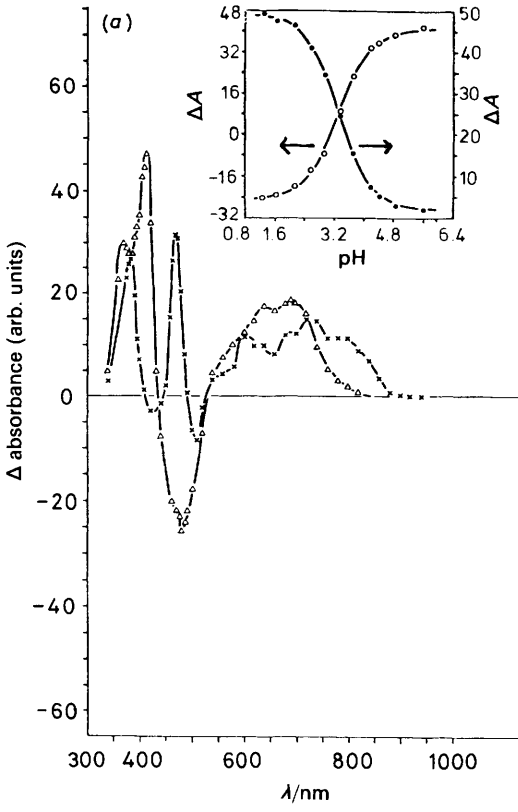


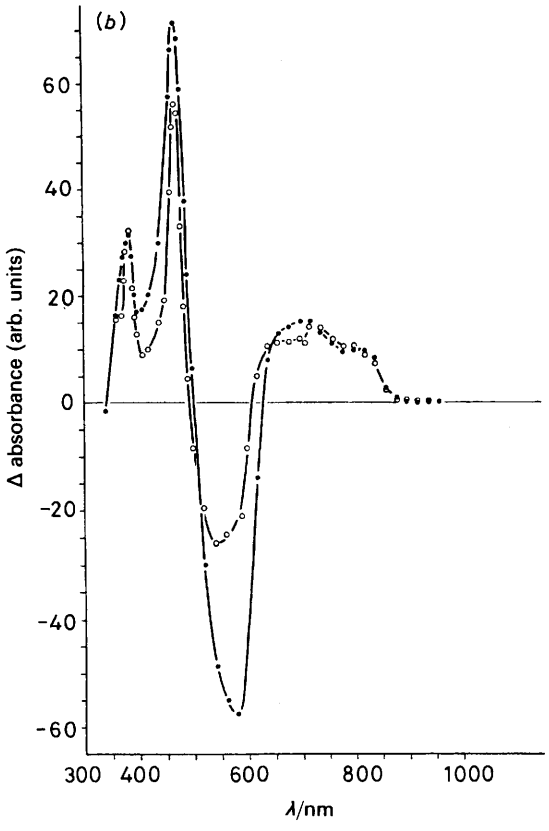
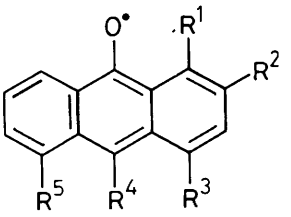


Fig. 1. Change in absorbance caused by reactions in argon-bubbled aqueous solutions of quinizarin containing 5 mol dm^{-3} isopropyl alcohol and 1 mol dm^{-3} acetone. (*a*) pH 1.2 (△), measured 20 μs after the pulse; pH 5.4 (X), measured 20 μs after the pulse. (*b*) pH 10.4 (○), measured 50 μs after the pulse; pH *ca.* 14 (●), measured 80 μs after the pulse. 1 cm pathlength; dose 9 Gy. Inset (*a*): Change in absorbance at 410 nm (●) and 475 nm (○) with pH. (———) Computer best fit [eqn (1)] with $pK_1^R = 3.3$.



(IV)

Scheme 3. Structure **IV**: $R^2 = R^5 = H$, quinizarin; $R^2 = SO_3^-$, $R^5 = H$, quinizarin 2-sulphonate; $R^2 = H$, $R^5 = OCH_3$, 5-methoxyquinizarin; (*a*) $R^1 = R^3 = R^4 = OH$, $QH_3^{\bullet}$($QSH_3^{\bullet}$, $MQH_3^{\bullet}$); (*b*) $R^1 = R^3 = OH$, $R^4 = O^-$, $QH_2^{\bullet -}$(QSH_2^-, $MQH_2^{\bullet -}$); (*c*) $R^1 = R^4 = O^-$, $R^3 = OH$, $QH^{\bullet 2-}$($QSH^{\bullet 2-}$, $MQH^{\bullet 2-}$); (*d*) $R^1 = R^3 = R^4 = O^-$, $Q^{\bullet 3-}$($QS^{\bullet 3-}$, $MQ^{\bullet 3-}$).

are shown in fig. 1(*a*) and after 50 μs at pH 10.4 and 80 μs at pH *ca.* 14 in fig. 1(*b*).

Examination of the absorbances above 650 nm, where the parent quinones absorb little, shows that at least two of the four forms of the semiquinone can be distinguished spectroscopically in this wavelength region.

A plot of difference in absorbance at 410 and 475 nm [inset of fig. 1(*a*)] as a function of pH over the pH range 1–6, and a best fit with the theoretical curve computed from the equation

$$\Delta A_{obs} = \frac{\Delta A_1}{1 + 10^{pH - pK_a}} + \frac{\Delta A_2}{1 + 10^{pK_a - pH}} \qquad (1)$$

where ΔA_1 and ΔA_2 are the changes in absorbance at pH well beyond the pK_a, were obtained with a pK_a of 3.3 ± 0.1 for the quinizarin semiquinone. No values for the second and third pK_a could be determined spectroscopically owing to insufficient differences in the absorption spectra recorded for the semiquinone in the pH range 6–14.

The semiquinone–quinone difference spectra for 5-methoxyquinizarin at pH 1.2 and 5.5 were recorded and were very similar to those obtained from QH_2 under identical conditions. A pK_a of 3.65 ± 0.1 was obtained from the dependence of the absorbance at 420 and 480 nm on pH in the range 1–8. The result for 420 nm is shown in fig. 2.

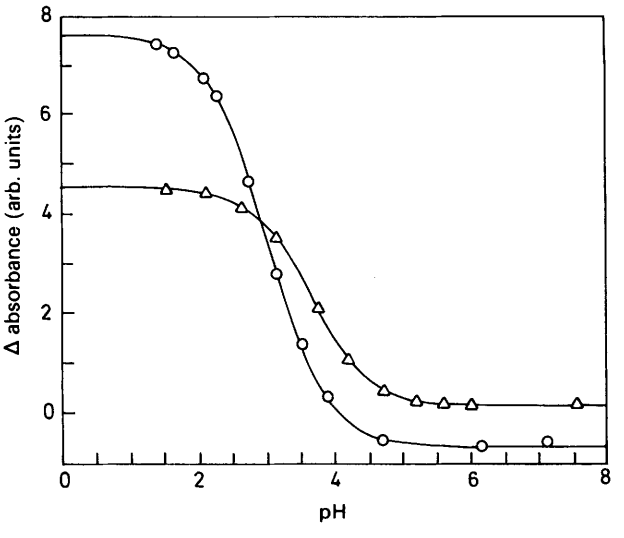


Fig. 2. Change in absorbance caused by reactions I–V with pH for aqueous solutions of 5-methoxyquinizarin (△) at 420 nm and sodium quinizarin 2-sulphonate (○) at 425 nm, containing isopropyl alcohol (5 mol dm^{-3}) and acetone (1 mol dm^{-3}). (———) Computer best fit [eqn (1)] with $pK_1^R = 3.65$ (△) and $pK_1^R = 3.0$ (○).

By analogy with our results on quinizarin sulphonates[1] and our ESR studies[14] in aqueous organic solutions containing isopropyl alcohol, the semiquinone form existing between pH 6 and 9 is attributed to $QH_2^{\bullet-}$ (or, $MQH_2^{\bullet-}$). The pK_1 as determined above is thus attributed to the dissociation

$$QH_3^{\bullet}(\text{or } MQH_3^{\bullet}) \longrightarrow QH_2^{\bullet-}(\text{or } MQH_2^{\bullet-}) + H^+ \quad \text{(VI)}$$

and the semiquinone form present at pH *ca.* 1.2 has been assigned to $QH_3^{\bullet}$ (or $MQH_3^{\bullet}$).

Similarly, the difference between the absorbance of the semiquinone and QSH_2 was measured immediately after essential completion of reactions (I) to (V), following the delivery of a pulse to argon-bubbled aqueous solutions of QSH_2 (*ca.* 1×10^{-4} mol dm^{-3}) containing 5 mol dm^{-3} isopropyl alcohol and 1 mol dm^{-3} acetone. The spectra at pH 1.4 and 7 had λ_{max} at 420 nm and 480 nm, respectively, comparable to those obtained in aqueous formate.[1] This provides further evidence that the semiquinones produced in the formate and in the isopropyl alcohol–acetone–water systems are identical, $QSH_3^{\bullet}$ at pH 1.4 and $QSH_2^{\bullet-}$ at pH 5 and 7.

A pK_a of 3.0 ± 0.1 was obtained from plots of the change in absorbance at 420 and 480 nm as a function of pH over the range 1–7 (fig. 2). This pK_a, attributable to the dissociation of $QSH_3^{\bullet}$, is *ca.* 0.8 pH units higher than in the aqueous system. This difference may offer a basis for comparison between the two solvent systems for other quinones.

An estimated G value of 6.2 molecules $(100\ eV)^{-1}$† [*i.e.* 6.42×10^{-7} mol J^{-1}; 1 mol $J^{-1} = 9.65 \times 10^6$ molecules $(100\ eV)^{-1}$] for the reducing radicals in the mixed solvent systems was assumed, leaving provision for the acetonyl radical, compared to 6.5 molecules $(100\ eV)^{-1}$ in aqueous formate solution, further confirmed by comparing the transient absorption spectrum obtained from methyl-1,4-benzoquinone in aqueous formate and aqueous isopropyl alcohol–acetone systems (pH *ca.* 6.9).

The absorption spectra of the semiquinones were obtained from the difference spectra by allowing for depletion of the parent quinone, using the expression

$$\varepsilon_R = \varepsilon_P + \frac{\Delta A \times 2.9 \times 7.1 \times 10^3}{A_{(SCN)_2^{\bullet-}} \times 6.2} \quad (2)$$

† 1 eV ≈ $1.602\,18 \times 10^{-19}$ J.

where, the value[15] of G for $(SCN)_2^{\bullet-}$ is 2.9 molecules $(100\ eV)^{-1}$ (*i.e.* 3 mol J^{-1}) and the extinction coefficient of $(SCN)_2^{\bullet-}$ at 500 nm is 7.1×10^3 dm^3 mol^{-1} cm^{-1}. The absolute spectra of the semiquinones are, however, sensitive to the radical yields assumed and dosimetry calibration factors. The absorption spectra of the radicals obtained from QH_2 at pH 1.2 and 5.5 (10.4) are shown in fig. 3. The spectra obtained from $MQH_2^{\bullet-}$ were similar. The spectra in fig. 3 correspond to $QH_3^{\bullet}$ and $QH_2^{\bullet-}$, respectively. Comparing these systems with other quinones, it is safe to assume that any further pK_a of the semiquinone radicals are > 14.

Kinetic Studies

The rate constants for the formation of the semiquinone by reaction of $(CH_3)_2\dot{C}OH$ with quinizarin were measured by monitoring the first-order build-up of $QH_3^{\bullet}$(or $QH_2^{\bullet-}$) absorption in solutions of pH 1.2, 5.4, 10.4 and *ca.* 14. Values are shown in table 2. The corresponding values for $MQH_2^{\bullet-}$ were similar.

The semiquinone decay rate constants ($2k$) as given by the reaction

$$2\ \text{semiquinone} \xrightarrow{2k} \text{products} \quad \text{(VII)}$$

were measured at pH 1.2 and 5.4, and are shown in table 2. The decay rate constants at higher pH values were difficult to measure because the semiquinones were too stable.

The reactivities of the semiquinone anions towards oxygen were estimated by comparing the decay of the semiquinone at 480 nm in the absence and in the presence of oxygen at pH 6.7 and 11.1. The decay in the absence of O_2 was negligible so that a rate constant for the reaction

$$QH_2^{\bullet-} + O_2 \longrightarrow QH_2 + O_2^{\bullet-} \quad \text{(VIII)}$$

could be easily determined (table 2).

One-electron Reduction Potential of the Quinones

On delivery of an electron pulse of low dose (typically 1–1.5 Gy, to minimise radical–radical reactions) to an argon-bubbled solution containing QH_2, MQH_2 or QSH_2 and an appropriate redox reference (R), the quinone and the refer-

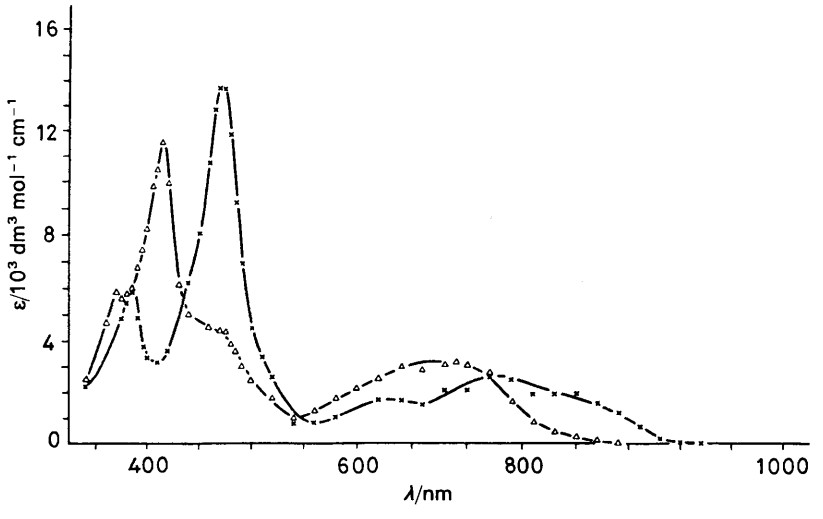


Fig. 3. Absolute absorption spectra of quinizarin semiquinone in aqueous isopropyl alcohol (5 mol dm^{-3})–acetone (1 mol dm^{-3}) system. (△) pH 1.2; (X) pH 5.4 (and pH 10.4).

Table 2. Kinetic parameters for quinizarin and its semiquinone radicals in 5 mol dm^{-3} isopropyl alcohol–1 mol dm^{-3} acetone–water system

reactions	pH	2nd order rate constant/dm^3 mol^{-1} s^{-1}
$QH_2 + (CH_3)_2\dot{C}OH$	1.2	$(3.3 \pm 0.3) \times 10^9$
$QH_2 + (CH_3)_2\dot{C}OH$	5.45	$(3.2 \pm 0.3) \times 10^9$
$QH^- + (CH_3)_2\dot{C}OH$	10.45	$(1.5 \pm 0.2) \times 10^9$
$Q^{2-} + (CH_3)_2CO^{\bullet -}$	*ca.* 14	$(1.8 \pm 0.2) \times 10^9$
$2QH_3^{\bullet} \rightarrow$ products	1.2	$2k = (5.1 \pm 0.4) \times 10^8$
$2QH_2^{\bullet -} \rightarrow$ products	5.45	$2k = (3.8 \pm 0.3) \times 10^8$
$QH_2^{\bullet -} + O_2 \rightarrow QH_2 + O_2^{\bullet -}$	6.7[a]	$(4.4 \pm 0.8) \times 10^7$
$QH_2^{\bullet -} + O_2 + H_2O \rightarrow QH^- + O_2^{\bullet -} + 2OH^-$	11.1[a]	$(3.7 \pm 0.6) \times 10^7$

[a] Solubility of oxygen has been taken as 5×10^{-4} mol dm^{-3} based on molarity of the components and oxygen solubility in air-saturated individual components. This value is in excellent agreement with the value calculated[16] from the measured Ostwald coefficient at the given mole fraction, assuming acetone as equivalent to isopropyl alcohol for oxygen solubility.

ence become reduced by the radiolytically produced $(CH_3)_2\dot{C}OH$ radicals [reactions (V) and (IX)],

$$(CH_3)_2\dot{C}OH + R \rightarrow R^{\bullet -} + (CH_3)_2CO + H^+. \quad \text{(IX)}$$

Under appropriate experimental conditions, an equilibrium (X) is established within a few hundred microseconds after the pulse

$$\text{semiquinone} + R \rightleftharpoons \text{quinone} + R^{\bullet -}. \quad \text{(X)}$$

If the equilibrium constant K and $E^1(R/R^{\bullet -})$ are known, $E^1(Q/Q^{\bullet -})$ can be calculated from the expression

$$E^1(Q/Q^{\bullet -}) = E^1(R/R^{\bullet -}) - 59 \log K \quad (3)$$

where the E^1 values are in mV and the solution temperature is 298 K. The equilibrium constant is obtained by measurement of the absorbance at equilibrium, from which the individual equilibrium radical concentrations can be calculated

$$K = \frac{[\text{quinone}]_{eq}[R^{\bullet -}]_{eq}}{[\text{semiquinone}]_{eq}[R]_{eq}}. \quad (4)$$

In aqueous formate solutions (pH range 9–11), 1,1′-di-β-hydroxyethyl-4,4′-bipyridylium dichloride ($HEBP^{2+}$, $E_7^1 = -410$ mV[17]) came into equilibrium[1] with the other species in reaction (X) when used as a redox reference for QSH_2

$$HEBP^{\bullet +} + QSH^- + H_2O \rightleftharpoons HEBP^{2+} + QSH_2^{\bullet -} + OH^-. \quad \text{(XI)}$$

The above was studied at 395 nm, corresponding to the absorption maximum[18] of $HEBP^{\bullet +}$. However, in the present mixed solvent system the initial absorption remained constant even after several hundred microseconds and no equilibrium could be detected even when the ratio $HEBP^{2+}$: QSH_2 (pH 9) was varied between 50 : 1 and 1 : 10. This observation could, in principle, be explained if (*a*) E_9^1 of QSH_2 is equal to E_9^1 of $HEBP^{2+}$ in this solvent system, (*b*) the equilibration is extremely slow or (*c*) the $(CH_3)_2\dot{C}OH$ is not acting exclusively as an electron donor, but is also forming an adduct with the quinone.[19–21]

The redox equilibrium (XI) was studied in solutions buffered to pH 9 (i) in argon-bubbled aqueous formate[1] (10^{-1} mol dm^{-3}) and (ii) in N_2O-saturated solutions containing isopropanol (10^{-1} mol dm^{-3}). In the latter case, e_{aq}^- is converted to $OH^{\bullet}$ by N_2O; the $OH^{\bullet}$, along with $H^{\bullet}$, then react with isopropyl alcohol to give $(CH_3)_2\dot{C}OH$. Good equilibria were obtained in both systems. The E_9^1 values in the two solutions were calculated as (i) −286 mV and (ii) −283 mV, respectively, mean −285 mV. This closeness in E^1 value in the two systems strongly suggests that the principal product for the reaction of $(CH_3)_2\dot{C}OH$ with QSH_2 is the semiquinone $QSH_2^{\bullet -}$. This implies that the $(CH_3)_2\dot{C}OH$ is acting as a reducing radical by electron transfer, and not forming an adduct with the quinone. Further support was obtained by UV spectroscopy and TLC analysis of the solutions (i) and (ii) after giving a dose of *ca.* 10 Gy and then exposing to air, when the resultant spectra were the same and only a single TLC spot was obtained.

It has been reported[22] that changing the solvent from pure water to aqueous organic mixtures containing alcohol and acetone has the effect of making E^1 less negative for both paraquat (1,1-dimethyl-4,4′-bipyridylium dichloride) and diquat (DQT^{2+}). The cationic $HEBP^{2+}$ will be much more favourably solvated by water, isopropyl alcohol and acetone, than its mono-cation reduction product, $HEBP^{\bullet +}$. However, the difference in solvation energy between $HEBP^{2+}$ and $HEBP^{\bullet +}$ will be greater in water than in an aqueous organic medium, resulting in a decrease in the free energy difference between $HEBP^{2+}$ and $HEBP^{\bullet +}$ on changing to a less aqueous environment and will thus result in a net increase of the E^1 value. From the data available from studies of aqueous ethanol and aqueous acetone solutions,[22] it is possible to estimate that the E^1 value for $HEBP^{2+}$ in the present mixed solvent system would be *ca.* −310 mV (compared with −410 mV in water[17]). The experiments described above show that E^1 for QSH_2 at pH 9 is −285 mV in the system containing little organic solvent. This value is expected to vary slightly in the present aqueous organic mixed solvent owing to small variation in the pK_a values. It is possible, therefore, that E^1 ($HEBP^{2+}$) and E^1 (QSH_2) are more or less equal in the mixed solvent, thus explaining the absence of the equilibrium reaction (X).

Sodium 9,10-anthraquinone-2-sulphonate (AQS) was chosen as a redox reference, since its E_7^1 value in water (−380 mV[23]) is the same in aqueous and in aqueous organic solutions[21] and its semiquinone has no pK_a in the pH range used for the redox study.[24] A good equilibration was obtained within a few hundred microseconds after the pulse:

$$AQS^{\bullet -} + QSH_2 \rightleftharpoons AQS + QSH_2^{\bullet -} \quad \text{(XII)}$$

$$AQS^{\bullet -} + QSH^- + H_2O \rightleftharpoons AQS + QSH_2^{\bullet -} + OH^- \quad \text{(XIII)}$$

The equilibrium constants, for solutions in the pH range 8–10.5, were obtained in the usual way[1] (measurements at 500 nm, which corresponds to the absorption maximum[24] of $AQS^{\bullet -}$). The E^1 values calculated by using eqn (3) are shown in fig. 4.

Similarly, the E^1 values of MQH_2 within the pH range 8–11 were determined using AQS as a redox standard, from the equilibrium

$$AQS^{\bullet -} + MQH_2 \rightleftharpoons AQS + MQH_2^{\bullet -} \quad \text{(XIV)}$$

$$AQS^{\bullet -} + MQH^- + H_2O \rightleftharpoons AQS + MQH_2^{\bullet -} + OH^- \quad \text{(XV)}$$

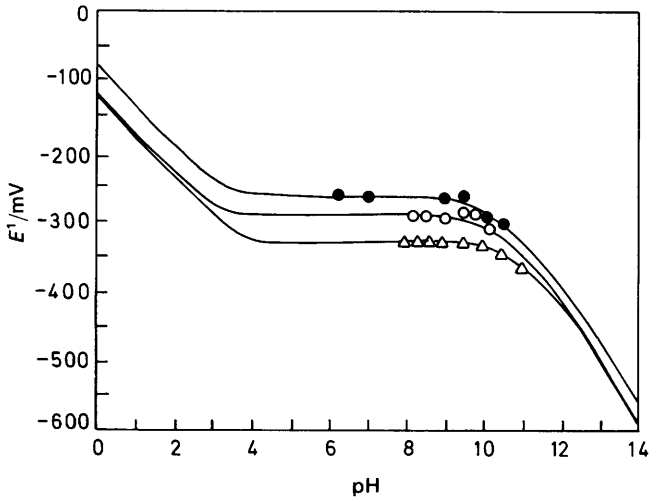


Fig. 4. pH dependence of one-electron reduction potential in aqueous isopropyl alcohol (5 mol dm^{-3}) and acetone (1 mol dm^{-3}), of (○) sodium quinizarin 2-sulphonate, (●) quinizarin, (△) 5-methoxyquinizarin. (———) Computed best fit [eqn (5)–(9)].

as studied at 400 and 500 nm, corresponding to the absorption maximum of $AQS^{\bullet -}$. The experimental values obtained are shown in fig. 4.

The E^1 values of quinizarin were determined over the pH range 6–10.5 using AQS as well as duroquinone (DQ) as redox standards.[25] The E^1 of DQ, as studied with AQS as a redox standard, did not change significantly from the aqueous formate to the present solvent system and was independent of pH within the above range. The wavelength chosen was 445 nm corresponding to the absorption maximum[5] of the durosemiquinone radical $DQ^{\bullet -}$. The calculated E^1 values from the equilibrium

$$QH_2^{\bullet -} + DQ \rightleftharpoons QH_2 + DQ^{\bullet -} \qquad \text{(XVI)}$$

and

$$QH_2^{\bullet -} + DQ + OH^- \rightleftharpoons OH^- + DQ^{\bullet -} + H_2O \qquad \text{(XVII)}$$

are shown in fig. 4.

It can be seen that the E^1 values of QH_2, QSH_2 and MQH_2 are independent of pH in the region 6–8. This shows that no proton is involved in the reduction process, thus providing further evidence that the radical exists as $QH_2^{\bullet -}$ ($QSH_2^{\bullet -}$ or $MQH_2^{\bullet -}$) in this region. Since the parent is present as the neutral molecule throughout this range, an expression can readily be derived relating E_a^1 at pH a to E_b^1 at pH b, as follows:

$$E_a^1 = E_b^1 + 59 \log\left(\frac{AD}{BC}\right) \qquad (5)$$

where

$$A = 10^{-3a} + K_1^R 10^{-2a} + K_1^R K_2^R 10^{-a} + K_1^R K_2^R K_3^R \qquad (6)$$

$$B = 10^{-3b} + K_1^R 10^{-2b} + K_1^R K_2^R 10^{-b} + K_1^R K_2^R K_3^R \qquad (7)$$

$$C = 10^{-2a} + K_1 10^{-a} + K_1 K_2 \qquad (8)$$

$$D = 10^{-2b} + K_2 10^{-b} + K_1 K_2. \qquad (9)$$

In eqn (6) to (9) K_1, K_2, K_1^R, K_2^R and K_3^R correspond to the various ionisation constants of the parent and the semiquinone, respectively.

Eqn (5) to (9) have been used in conjunction with the measured $E_{8.5}^1$ of −298 mV for QSH_2 to calculate E^1 for QSH_2 within the pH range 0–14, using our experimental values of $K_1 = 10^{-9.1}$, $K_2 = 10^{-12.6}$, $K_1^R = 10^{-3.0}$, $K_2^R = 10^{-14}$ and $K_3^R \ll 10^{-14}$. The agreement of the calculated E^1 values, represented by the solid line in fig. 4, with the experimental values is very good.

Similarly, for MQH_2, a plot of E^1 *vs.* pH, with the measured $E_8^1 = -333.5$ mV, together with our experimental values of $K_1 = 10^{-10.6}$, $K_2 = 10^{-13.4}$, $K_1^R = 10^{-3.65}$, $K_2^R < 10^{-14}$ and $K_3^R \ll 10^{-14}$, gave the solid line in fig. 4. The calculated values were in excellent agreement with the experimental values. For QH_2, a plot of E^1 *vs.* pH, with $E^1 = -272$ mV at pH 9, with $K_1 = 10^{-9.9}$, $K_2 = 10^{-12.75}$, $K_1^R = 10^{-3.3}$, $K_2^R < 10^{-14}$ and $K_3^R \ll 10^{-14}$ gave an excellent fit with the experimental points.

At pH 7, in the mixed solvent system, the best values for the one-electron reduction potentials at pH 7 (E_7^1) of QH_2, MQH_2 and QSH_2 are found by extrapolation of fig. 4 to be −269 mV, −333 mV and −298 mV, respectively (all values *vs.* NHE). The introduction of the electron-donating methoxy group evidently causes the E^1 value to become distinctly more negative.

Disproportionation of the Semiquinones: Equilibrium Constants

The disproportionation of the semiquinones of quinizarin, methoxyquinizarin and quinizarin 2-sulphonate was treated in terms of the simple equation:

$$2\ \text{semiquinone} \rightleftharpoons \text{quinone} + \text{hydroquinone}. \qquad \text{(XVIII)}$$

It was studied by making observations at a number of wavelengths over the pH range 1–14 using de-oxygenated solutions of the quinone in the mixed solvent. Buffers were used for the pH range 5–11. For quantitative measurements the chosen wavelength was one at which neither the quinone nor the hydroquinone absorb, and the semiquinone has an absorbance maximum. This was 720 nm for $QH_2^{\bullet -}$ and 780 nm for $MQH_2^{\bullet -}$ and $QSH_2^{\bullet -}$.

At pH values in the range 1–5 and >12 the equilibrium was almost completely over to the right, so that the disappearance of the semiquinone was almost quantitative. However, within the pH range of approximately 5–12, the absorption due to the radicals did not disappear completely, but relaxed to a value which remained constant for hundreds of microseconds at least. Studies at several wavelengths and doses established the equilibrium reaction (XVIII).

In the case of MQH_2, it was found that within the pH range 10–11.5, the absorption at 780 nm produced by the pulse remained constant for several tenths of a second at least. Thus in that case the equilibrium (XVIII) appears to be almost wholly over to the left.

The concentration of the semiquinone present at equilibrium, $[R]_{eq}$, can be calculated from the equilibrium absorbance attained and known ε of the semiquinone, on the assumptions that the equilibrium is governed solely by reaction (IX) and that only the semiquinone absorbs at the wavelength of observation. The equilibrium constant K_{eq} for reaction (XVIII) is then given by

$$K_{eq} = \left(A_0 - \frac{[R]_0 + [R]_{eq}}{2}\right)\left(\frac{[R]_0 - [R]_{eq}}{2}\right)\Big/[R]_{eq}^2 \qquad (10)$$

where, A_0 = initial quinone concentration and $[R]_0$ = concentration of semiquinone immediately after the pulse. Plots of log K_{eq} *vs.* pH for QH_2, MQH_2 and QSH_2 are shown in fig. 5. On extrapolation to the minimum log K_{eq}, the maximum stability seems to be attained at around pH 9.5 for QSH_2, pH 8.5 for QH_2 and pH 10.8 for MQH_2, with the corresponding stability constant K_S [inverse of K_{eq} in eqn (10)] values of *ca.* 10 for QSH_2, *ca.* 0.09 for QH_2 and *ca.* 4.8

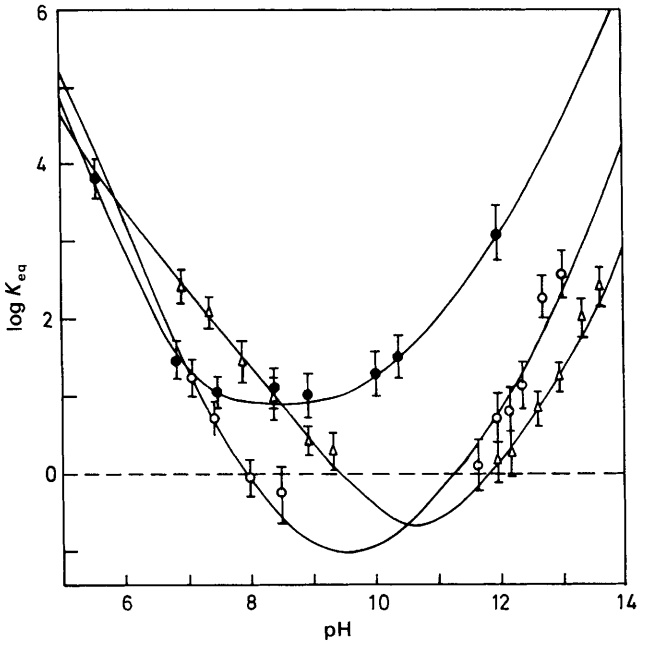


Fig. 5. Variation of log K_{eq} with pH for (○) sodium quinizarin 2-sulphonate, (●) quinizarin and (△) 5-methoxyquinizarin. Solvent: 5 mol dm^{-3} isopropyl alcohol and 1 mol dm^{-3} acetone in water. Theoretical best fit based on experimental parent and semiquinone pK_a and assumed hydroquinone pK_a values [eqn (6)–(9) and (14)–(16)].

for MQH_2, respectively. The maximum K_S of *ca.* 10 around pH *ca.* 9–10 for QSH_2 compared to *ca.* 1.45 around pH 7.5–8.8 in the aqueous formate system[1] shows greater stability of the semiquinone in the aqueous organic solvent. This variation may be attributed to the difficulty in defining the pH of an aqueous organic system.[11–13] It may also be a function of the ionic forms of the quinone and the hydroquinone, since the pK_a values of the quinone and the hydroquinone are dependent upon the solvent system employed.

In strongly acid solutions, the equilibrium reaction can be written as:

$$2QH_3^{\bullet} \rightleftharpoons QH_2 + QH_4 \qquad \text{(XIX)}$$

where

$$K_{11} = \frac{[QH_2][QH_4]}{[QH_3^{\bullet}]^2}. \qquad (11)$$

The value of K_{11} is independent of pH, but at other pH, the position of the equilibrium does depend on pH, as H^+ enters the equilibrium equation. At pH *ca.* 5,

$$2QH_2^{\bullet -} + 2H^+ \rightleftharpoons QH_2 + QH_4 \qquad \text{(XX)}$$

$$K_{12} = \frac{[QH_2][QH_4]}{[QH_2^{\bullet -}]^2[H^+]^2}. \qquad (12)$$

Again, the value of K_{12} does not depend on pH, but the concentrations of the three other species are pH dependent. The equilibrium constant we calculate is defined as:

$$K_{eq} = \frac{[QH_2]_T[QH_4]_T}{[QH_3^{\bullet}]_T^2} \qquad (13)$$

where T denotes the total concentration of the species involved.

Expressing the total concentrations in the usual way, and considering two pH values, *a* and *b*, one can obtain,

$$\log(K_{eq})_a = \log(K_{eq})_b + \log\left(\frac{CEB^2}{DFA^2}\right) \qquad (14)$$

where *A*, *B*, *C*, *D* are given by eqn (6), (7), (8) and (9), respectively, and *E* and *F* are given by:

$$E = 10^{-4a} + K_1^{HQ}10^{-3a} + K_1^{HQ}K_2^{HQ}10^{-2a} + K_1^{HQ}K_2^{HQ}K_3^{HQ}10^{-a} + K_1^{HQ}K_2^{HQ}K_3^{HQ}K_4^{HQ} \qquad (15)$$

and

$$F = 10^{-4b} + K_1^{HQ}10^{-3b} + K_1^{HQ}K_2^{HQ}10^{-2b} + K_1^{HQ}K_2^{HQ}K_3^{HQ}10^{-b} + K_1^{HQ}K_2^{HQ}K_3^{HQ}K_4^{HQ}. \qquad (16)$$

K_1^{HQ}, K_2^{HQ}, K_3^{HQ} and K_4^{HQ} in eqn (15) and (16) are the pK_a of the hydroquinone. Following eqn (14), a theoretical plot of log K_{eq} *vs.* pH could be drawn (solid line of fig. 5), based on best fit pK^{HQ} values of 6.9 and 7.1 for QH_2, 5.0 and 10.75 for MQH_2 and 7.5 and 9.0 for QSH_2, the other pK^{HQ} being $\gg 14$ for all three compounds.

Second One-electron Reduction Potential of the Quinones

At 298 K, K_{eq} [eqn (10)] and E^1 (quinone/semiquinone) are related to the second one-electron reduction potential E^2 by:

$$E^1 - E^2 = -59 \log K_{eq} \qquad (17)$$

where the potentials are expressed in mV. E^2 in eqn (17) is also the one-electron reduction potential of the semiquinones.

The experimental values of log K_{eq} and the E^1 values at the corresponding pH values, have been obtained to yield the E^2 values within the pH range 5–14 for QH_2, MQH_2 and QSH_2. The values of E^2 as a function of pH are shown in fig. 6 for all the three quinones.

E^2, however, is a function of the pK_a of the semiquinone as well as of the hydroquinone. As in the case of the variation of E^1 with pH, the variation of E^2 with pH can be expressed as:

$$E_a^2 = E_b^2 + 59 \log\left(\frac{EB}{FA}\right) \qquad (18)$$

where *A*, *B*, *E* and *F* are given by eqn (6), (7), (15) and (16), respectively.

As would be expected, the best fit to points derived from the experiments summarised in fig. 4 and 5 were obtained for QH_2 with $pK_1^{HQ} = 6.9$, $pK_2^{HQ} = 7.1$; for MQH_2, $pK_1^{HQ} = 5.0$

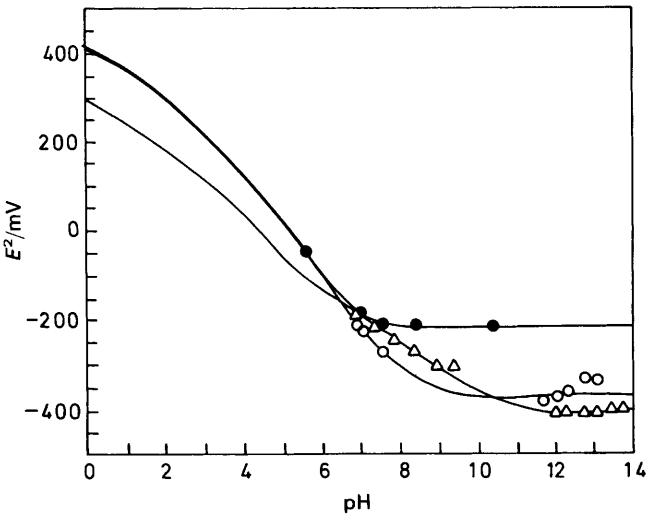


Fig. 6. Variation of the second one-electron reduction potential of (○) sodium quinizarin 2-sulphonate, (●) quinizarin and (△) 5-methoxyquinizarin, with pH. The points are based on the measured log K_{eq} (fig. 5) and the calculated E^1 values (fig. 4). A single best-fit line is calculated on the basis of assumed pK_a (HQ) values and eqn (18).

and $pK_2^{HQ} = 10.75$ and for QSH_2, $pK_1^{HQ} = 7.5$ and $pK_2^{HQ} = 9.0$; pK_3 and $pK_4 \gg 14$ were used for each quinone. The lines calculated on the basis of these values are shown in fig. 6.

The best values for the second one-electron reduction potential (E_7^2) of the quinones at pH 7, were found to be -188, -192 and -216 mV for QH_2, MQH_2 and QSH_2, respectively.

Two-electron Reduction Potential of the Quinones

The two successive one-electron reduction potentials of a quinone are related to the two-electron potential E^m (quinone/hydroquinone) by the expression:

$$E(\text{quinone/semiquinone}) + E(\text{semiquinone/hydroquinone}) = 2E(\text{quinone/hydroquinone})$$

or

$$E^1 + E^2 = 2E^m. \quad (19)$$

E^m must be a function of the pK_a of the parent quinone and the hydroquinone. On this basis, the expression relating E^m at pH a to that at pH b, E_b^m becomes

$$E_a^m = E_b^m + 29.5 \log\left(\frac{ED}{FC}\right) \quad (20)$$

where C, D, E and F are defined by eqn (8), (9), (15) and (16), respectively.

Fig. 7 shows the curves obtained using eqn (20) with the pK_a^{HQ} values employed in the treatment of K_{eq} *vs.* pH and E^2 *vs.* pH. At pH 7, the best E^m values in the mixed solvent for QH_2, MQH_2 and QSH_2 become -229 mV, -263 mV and -257 mV, respectively. The corresponding value for QSH_2 in the aqueous formate system[1] is -258 mV. It can be seen that change of solvent from the aqueous formate to the aqueous isopropanol–acetone system, has virtually no effect on the two-electron reduction potential of the hydroxyquinone at pH 7.

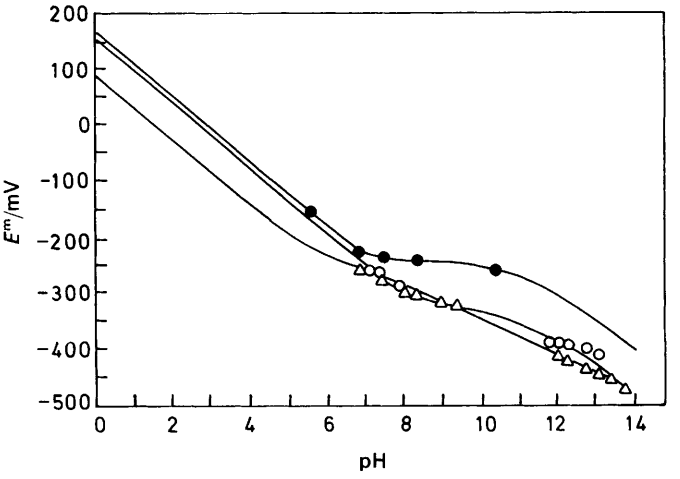


Fig. 7. Variation of the two-electron reduction potential of (○) sodium quinizarin 2-sulphonate, (●) quinizarin and (△) 5-methoxyquinizarin, with pH. The points are based on theoretical values from fig. 4 and experimental points from fig. 5. The line is calculated, based on constants used for fig. (4)–(6) and eqn (20).

Comparative Data for Anthrasemiquinones

A comparison of the spectroscopic and redox properties of different anthraquinones is summarised in table 3. The following points appear to be of interest.

(1) The main absorption band of non-hydroxy neutral anthrasemiquinones is around 385 ± 10 nm while that of the hydroxy ones is around 420 ± 10 nm, with almost equal extinction coefficients. On the other hand, the semiquinone anions of the hydroxyquinones have their main absorption bands in the 390 and 480 nm regions, the same as 9,10-anthrasemiquinone, although the extinction coefficient for the hydroxy derivatives is nearly twice that of the non-hydroxy ones. The non-hydroxy anthrasemiquinones do not have any

Table 3. Comparison of spectroscopic and redox properties of several anthrasemiquinone derivatives

quinone (Q)	λ_{max}/nm ($\varepsilon_{max}/10^3$ dm^3 mol^{-1} cm^{-1}) QH$^\bullet$	λ_{max}/nm ($\varepsilon_{max}/10^3$ dm^3 mol^{-1} cm^{-1}) Q$^{\bullet -}$	pK_a of QH$^\bullet$	E_7^1	E_7^2	E_7^m	ref.
9,10-anthraquinone	375 (11.0)	395 (7.8) 480 (7.3)	5.3	—	—	—	6
9,10-anthraquinone-1-sulphonate	385 (12.0)[a]	400 (8.0)[a] 500 (8.0)[a]	5.4	−415	—	—	24, 26
9,10-anthraquinone-2-sulphonate	390 (12.5)[a]	400 (8.2)[a] 500 (8.2)[a]	3.25	−380	−76	−228	23, 24, 26
9,10-anthraquinone-1,5-disulphonate	385 (8.4)	390 (5.6) 500 (5.7)	6.1	−418	−283	−350	26
9,10-anthraquinone-2,6-disulphonate	388 (9.5)	396 (6.4) 515 (8.3)	3.0	−255	−146	−201	6, 26
quinizarin[b]	410 (11.6) 680 (3.0)	388 (5.8) 475 (13.7) 720 (2.7)	3.3	−269	−188	−229	this work
quinizarin 2-sulphonate	4.25 (12.4) 680 (3.0)	390 (6.4) 475 (17.2) 700 (2.6) 780 (2.3)	2.2	−270	−246	−258	1
	420[b] (12.4)	480[b] (17.2)	3.0[b]	−298[b]	−216[b]	−257[b]	this work
quinizarin 6-sulphonate	425 (12.4) 680 (3.0)	390 (6.4) 475 (17.2) 700 (2.6) 780 (2.3)	2.2	−249	−213	−231	1
5-methoxyquinizarin[b]	370 (6.6) 420 (12.1)	375 (6.5) 480 (16.9)	3.65	−333	−192	−263	this work

[a] should be multiplied by 5.8/6.5 for corrected ε; see ref. (26) for details. [b] 5 mol dm^{-3} isopropyl alcohol, 1 mol dm^{-3} acetone in water.

absorption beyond 700 nm, which is common for all hydroxy derivatives.

(2) The effect of the 2-SO_3^- or 2,6-$(SO_3^-)_2$ groups and of the hydroxy groups is to bring down the pK_a of the semiquinone, but the presence of two hydroxy groups makes the —OH protons more labile.

(3) The groups —OH and —SO_3^- increase the reduction potential substantially, *i.e.* make the semiquinone molecule a weaker reducing agent, while the group —OCH_3 decreases the reduction potential, *i.e.* makes the semiquinone molecule a stronger reducing agent.

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References

1 T. Mukherjee, E. J. Land, A. J. Swallow, P. M. Guyan and J. M. Bruce, *J. Chem. Soc., Faraday Trans. 1*, 1988, **84**, 2855.
2 T. Mukherjee and N. J. F. Dodd, *Can. J. Chem.*, 1985, **62**, 1297.
3 D. A. Van Echo, M. Y. Whitaere, J. Aisner and P. H. Wiernik, *Cancer Treat. Rep.*, 1981, **65**, 831.
4 R. L. Willson, *Trans. Faraday Soc.*, 1971, **67**, 3020.
5 K. B. Patel and R. L. Willson, *J. Chem. Soc., Faraday Trans. 1*, 1973, **69**, 814.
6 A. J. Swallow, in *Function of Quinones in Energy Conserving Systems*, ed. B. L. Trumpower (Academic Press, New York, 1982), p. 59.
7 E. J. Land, T. Mukherjee, A. J. Swallow and J. M. Bruce, *Br. J. Cancer*, 1985, **51**, 515.
8 E. J. Land, T. Mukherjee, A. J. Swallow and J. M. Bruce, *J. Chem. Soc., Faraday Trans. 1*, 1983, **79**, 391.
9 H. Pal, D. K. Palit, T. Mukherjee and J. P. Mittal, to be published.
10 K. Krohn and K. Tolkiehn, *Chem. Ber.*, 1979, **112**, 3453.
11 G. Jackson and G. Porter, *Proc. R. Soc. London, Ser. A*, 1961, **260**, 13.
12 C. C. Westcott, in *pH Measurements* (Academic Press, New York, 1978).
13 R. G. Bates, M. Paabo and R. A. Robinson, *J. Phys. Chem.*, 1963, **67**, 1833.
14 N. J. F. Dodd and T. Mukherjee, *Biochem. Pharmacol.*, 1984, **33**, 379.
15 G. E. Adams, J. W. Boag, J. Currant and B. D. Michael, in *Pulse Radiolysis*, ed. M. Ebert, J. P. Keene, A. J. Swallow and J. H. Baxendale (Academic Press, London, 1965), p. 117.
16 K. Tokunaga, *J. Chem. Eng. Data*, 1975, **20**, 41.
17 R. F. Homer, G. C. Mees and T. E. Tomlinson, *J. Sci. Food Agric.*, 1960, **11**, 309.
18 J. A. Farrington, M. Ebert and E. J. Land, *J. Chem. Soc., Faraday Trans. 1*, 1978, **84**, 665.
19 A. J. Swallow, *Prog. React. Kinet.*, 1978, **9**, 195.
20 V. Jagannadham and S. Steenken, *J. Am. Chem. Soc.*, 1984, **106**, 6542.
21 P. Wardman and E. D. Clarke, *Br. J. Cancer*, 1987, **55** (suppl. VIII), 172.
22 A. Ledwith, in *Biochemical Mechanisms of Paraquat Toxicity*, ed. A. P. Autor (Academic Press, New York, 1977), p. 19–37.
23 D. Meisel and P. Neta, *J. Am. Chem. Soc.*, 1975, **97**, 5198.
24 B. E. Hulme, E. J. Land and G. O. Phillips, *J. Chem. Soc., Faraday Trans. 1*, 1972, **68**, 1992.
25 P. Wardman and E. D. Clarke, *J. Chem. Soc., Faraday Trans. 1*, 1976, **72**, 1377.
26 H. Pal, D. K. Palit, T. Mukherjee and J. P. Mittal, submitted for publication.

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