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Thermodynamics of semiquinone disproportionation in aqueous buffer

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The thermodynamic parameters, K_{disp}, ΔH° and ΔS°, controlling the disproportionation of semiquinones derived from 1,4-benzoquinone (BQ), 1,4-naphthoquinone (NQ), 2-methylbenzoquinone (MBQ), menadione (MNQ), naphthazarin (NZQ) and quinizarin (QNZ), have been determined. Smaller disproportionation constants, K_{disp}, are observed upon addition of a fused benzene ring to the semiquinone structure. Negative enthalpies and positive entropies of disproportionation govern the disproportionation equilibria. Addition of OH groups to the 5 and 8 positions in $NQ^{\cdot -}$ displaces the disproportionation equilibrium to the semiquinone probably due to intramolecular hydrogen bonding.

Semiquinones ($Q^{\cdot -}$), the one-electron reduction products of quinones (Q), are important intermediates in biological[1] and photochemical[2] processes. These are postulated as cytotoxic intermediates in quinone-containing antitumour drug activity.[3,4] Semiquinones are readily reoxidized under aerobic conditions and, in biological systems, can enter a redox cycle with molecular oxygen forming superoxide ions, hence, producing damaging hydroxyl radicals *via* the iron-catalysed Haber–Weiss reaction.[5]

The efficiency of these electron-transfer processes should depend on the thermodynamic stability of the semiquinones in the given environment in which they are immersed. Thus, the thermodynamic parameters controlling the relevant equilibria in which these species are involved should be determined and understood.

One of these equilibria is the semiquinone disproportionation in water at physiological or near-neutral pH, eqn. (1), where QH_2 is the corresponding hydroquinone.

$$2Q^{\cdot -} + 2H^+ \rightleftarrows Q + QH_2 \qquad (1)$$

Few studies are encountered in the literature regarding the determination of semiquinone disproportionation equilibrium constants in water and all have used mixtures of water and alcohols, or relatively high pH values, to obtain relatively strong semiquinone absorption spectra.[6–8] Here, we have taken advantage of the fact that aqueous semiquinone solutions are stable at room temperature for several hours at neutral pH[9,10] and the intrinsic sensitivity of electron paramagnetic spectroscopy (detection at $\geqslant 10^{-8}$ M) to determine the thermodynamic parameters controlling the disproportionation equilibria of several *para*-semiquinones in neutral aqueous buffer and look for possible trends in these parameters with structural modifications.

Experimental

Materials

The compounds, 1,4-benzoquinone (BQ), hydroquinone (BQH_2), 1,4-naphthoquinone (NQ) and menadione (MNQ), Fig. 1, were purchased from Aldrich. Naphthazarin (NZQ), 1,4-dihydroxy-9,10-anthraquinone (quinizarin, QNZ) were purchased from Fluka and 2-methyl-1,4-benzoquinone (MBQ) and its hydroquinone ($MBQH_2$) were purchased from Pfaltz and Bauer. Quinones and hydroquinones were purified by single or double sublimation. Fresh stock solutions of these compounds were prepared in water and used the same day. Samples were prepared in phosphate buffer at pH 7.4 using distilled, deionized water.

Equipment

First-derivative EPR spectra were recorded on an X-band Bruker ER-200D EPR spectrometer and coupled to a PC/AT computer for data acquisition and analysis using the software developed by Morse.[11] Spectral simulations were performed utilizing WINSIM.[12] Semiquinone concentrations were obtained by comparing the semiquinone over modulated (10 G modulation amplitude) EPR double-integrated spectral area with that corresponding to a 3-carbamoyl-2,2,5,5-tetramethyl-3-pyrrolin-1-oxide spin standard using the same spectrometer settings for both sample and spin standard with the exception of the receiver gain value in some cases. All sample and spin standard areas were normalized to those corresponding to the same receiver gain value. The relative error obtained in these areas and, thus, in the semiquinone concentrations is estimated as 20%. The latter was obtained from the repetitive double integration of the spin standard, where the EPR instrument was tuned after positioning the sample in the EPR cavity, before each area of determination.

Semiquinone generation

For hydrophobic quinones such as NQ, QNZ, NZQ and MNQ, semiquinone solutions were generated as described previously under an N_2 atmosphere, *i.e.* the corresponding

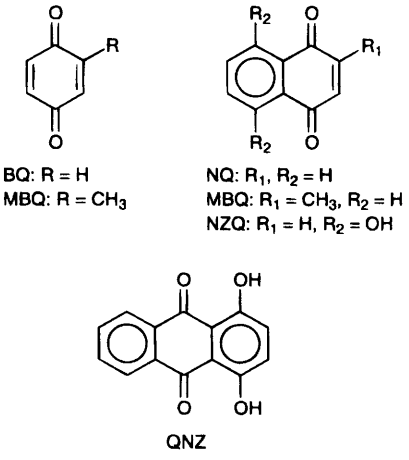

Fig. 1 Quinones under study in this work

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quinone was dissolved in methanol in a septum-stoppered test-tube and reduced with the minimum amount of $NaBH_4$ which produced the semiquinone EPR spectra in the aqueous buffer (see below);[9] the solvent was then removed using an N_2 stream and vacuum, followed by addition of a 50 mM N_2-saturated phosphate buffer solution. The sample was then equilibrated at pH 7.4 and centrifuged for 10 to 15 min at 4500 rpm. The supernatant was then extracted for EPR analysis. A septum-stoppered EPR quartz flat cell (60 × 10 × 0.25 mm) was used. In some cases the centrifugation process was repeated twice to ensure that the supernatant is free of dispersed particles. The presence of the parent quinone in the precipitated insoluble solid was confirmed by HPLC analysis for all the hydrophobic quinones under study here (see below).

Determination of quinone and hydroquinone equilibrium concentrations for hydrophobic quinones

UV spectral overlap of Q, $Q^{\cdot -}$, QH_2 and buffer for most of the quinones under study here introduced irreproducibilities in quinone and hydroquinone concentrations which were circumvented by the chemical or air-oxidation of the reduced species ($Q^{\cdot -}$ and QH_2) to the corresponding quinone followed by HPLC determination of the total quinone concentration in the aqueous sample at equilibrium, *i.e.* $[Q]_{tot,\,eq} = [Q]_{eq} + [Q^{\cdot -}]_{eq} + [QH_2]_{eq}$. This was done by first extracting a 250 μl sample of the aqueous solution containing the semiquinone of either MNQ, QNZ, NZQ or NQ with several portions of HPLC-grade dichloromethane until the aqueous phase was colourless. After evaporating the solvent, the remaining solid was redissolved in 5.00 ml of dry benzene and oxidized, following the reported method of Ansell *et al.*[13] by addition of 0.3 g of AgO and 0.1 g of dry $MgSO_4$. The benzene used in this reaction was then evaporated and replaced by 1.00 ml of CH_2Cl_2 to avoid overlap with the quinone chromatographic peak during HPLC analysis (see next section). The quinone concentration in this CH_2Cl_2 solution, produced by the oxidation reaction, was then determined by HPLC analysis by interpolation in a calibration curve of [Q] *vs.* chromatographic peak area. It was found that <30 min of duration of this reaction were needed until a maximum concentration of quinone was detected. QNZ buffered semiquinone solutions were oxidized by exposing the sample to air as detected by HPLC analysis. $[Q]_{tot,\,eq}$ was determined from the quinone concentration obtained in the HPLC analysis, the CH_2Cl_2 volume and the volume of aqueous sample analysed.

The quinone concentration at equilibrium, $[Q]_{eq}$, was assumed as the solubility of each quinone in phosphate buffer, pH 7.4, since undissolved solid quinone was always present in the precipitated solid after buffer addition to the solid mixture left in the test tube after methanol evaporation. The latter was determined by washing this solid precipitate several times with buffer followed by dissolving this sample in the N_2-saturated MeOH–H_2O solvent mixture used for HPLC analysis and detecting the quinone peak by HPLC. In this case a helium-purged mobile phase was used to avoid air-oxidation of reduced species. No ESR signal was detected from the precipitated solids after submitting an N_2-saturated buffer dispersion of the water-washed solid to EPR analysis. Thus, the quinone peak detected is not produced by semiquinone salt disproportionation or hydroquinone oxidation upon addition of the HPLC eluent solvent mixture to this solid.

The hydroquinone concentration at equilibrium, $[QH_2]_{eq}$, was, therefore, obtained by subtracting $[Q]_{eq}$, and the semiquinone concentration determined by EPR analysis, $[Q^{\cdot -}]_{eq}$, from $[Q]_{tot,\,eq}$. The semiquinone disproportionation constants were obtained from eqn. (2). At least three K_{disp} determinations were made for each semiquinone at each temperature using different samples for each determination.

$$K_{disp} = [Q]_{eq}[QH_2]_{eq}/[Q^{\cdot -}]^2_{eq}[H^+]^2_{eq} \quad (2)$$

HPLC analyses

A Waters analytical HPLC system equipped with a 600 solvent delivery pump, UV–VIS tunable detector and a μBondapack C_{18} (3.9 × 300 mm) column was used. A mobile phase of MeOH–H_2O (85 : 15 v/v) was used at a flow rate of 1 ml min^{-1}.

Disproportionation equilibrium constant determination for $BQ^{\cdot -}$ and $MBQ^{\cdot -}$

Since BQ, BQH_2, MBQ and $MBQH_2$ are all water-soluble and commercially available, the semiquinones were produced by the comproportionation reaction of the corresponding quinone and hydroquinones, opposite of reaction (1). Again, equilibrium concentrations of semiquinones were obtained by EPR analysis and those corresponding to quinone and hydroquinone were obtained by subtracting half the semiquinone concentration from the initial quinone and hydroquinone concentrations, respectively; at least three determinations of K_{disp} were made for each semiquinone.

Semiquinone disproportionation enthalpies

These were obtained from Van't Hoff plots, *i.e.* from the slope of $\ln K_{disp}$ *vs.* $10^3/RT$ plots. The temperature was controlled by placing the sample inside a quartz dewar located at the EPR instrument cavity. A Bruker ER 4111 VT temperature controller was used to stabilize the cavity temperature. A temperature range of 25–50 °C was investigated.

The quinone and hydroquinone concentrations at equilibrium were obtained by subtracting half of the semiquinone equilibrium concentration at each temperature from the initial quinone and hydroquinone concentrations determined at 25 °C.

Results and Discussion

The EPR spectra corresponding to the semiquinones prepared as described in the Experimental section are similar to those previously reported in water-containing samples, Table 1. Only the anionic semiquinone and the diprotonated hydroquinone are considered in eqn. (1) due to the low pK_a values of the protonated semiquinones, the large pK_a values of the hydroquinones ($pK_a > 9$) and the pH values used in this work, Table 1.

The semiquinone disproportionation constants are listed in Table 2. Those corresponding to $BQ^{\cdot -}$ and $MBQ^{\cdot -}$ are near those which can be calculated from the data reported by Ilan *et al.* extrapolated to pH 7.4.[6] It can be observed from Table 2 that the disproportionation equilibrium constant decreases upon addition of a fused benzene ring to the quinone *i.e.* $BQ^{\cdot -}$ *vs.* $NQ^{\cdot -}$, $MBQ^{\cdot -}$ *vs.* $MNQ^{\cdot -}$ and $NZQ^{\cdot -}$ *vs.* $QNZ^{\cdot -}$. Thus, the semiquinone stability increases at the expense of the corresponding quinone and hydroquinone stabilities upon increasing the electron delocalization through addition of a fused benzene ring, probably due to a decrease in the electron–electron repulsion at the semiquinone. Addition of a methyl group to $BQ^{\cdot -}$ results in an increase in K_{disp}, implying that addition of an electron-releasing group to the semiquinone ring destabilizes the semiquinone relative to the non-charged quinone and hydroquinone species. However, no change in K_{disp} was detected upon addition of a methyl group to $NQ^{\cdot -}$ in an analogous position as that in $MBQ^{\cdot -}$, *i.e.* at the carbon position which is ortho to the carbonyl group. Thus, the larger electron delocalization occurring in $MNQ^{\cdot -}$ as compared to that in $MBQ^{\cdot -}$ serves as a buffer in avoiding changes in K_{disp}.

Table 1. Hyperfine coupling constants for semiquinones under study here in nitrogen-saturated phosphate buffer (pH 7.4) and the corresponding pK_a values of the protonated semiquinones

semiquinone	a_H[a]/G	a_H[b]/G	pK_a
$BQ^{\cdot -}$	2.38 (2H)	2.35 (4H)	4.1[c]
$MBQ^{\cdot -}$	2.09 (3H), 1.67 (1H), 2.39 (1H), 2.55 (1H)	2.10 (3H), 1.70 (1H), 2.55 (1H), 2.37 (1H)	4.45[c]
$MNQ^{\cdot -}$	2.93 (3H), 2.29 (2H), 0.64 (4H)	3.01 (3H), 2.34 (1H), 0.59 (1H), 0.73 (1H), 0.66 (2H)	4.7[c]
$NQ^{\cdot -}$	3.18 (2H), 0.63 (4H)	3.11 (2H), 0.63 (2H), 0.55 (2H)	4.1[d]
$NZQ^{\cdot -}$	2.34 (4H), 0.59 (2H)	2.356 (4H), 0.587 (2H)	2.7[e]
$QNZ^{\cdot -}$	2.25 (2H), 1.00 (2H), 0.61 (4H)	2.084 (2H), 0.905 (2H), 0.554 (2H), 0.520 (2H)	3.3[f]

[a] This work. [b] Ref. 17 (in alkaline aqueous solution). [c] Ref. 6. [d] Ref. 15. [e] Ref. 13. [f] Ref. 16.

Semiquinone disproportionation equilibria have been previously studied, and the corresponding disproportionation constants determined at high pH values by Bishop and Tong.[7] Data regarding the semiquinone disproportionation of several substituted *p*-benzosemiquinones at high pH values (pH 9–13) have also been compiled by these authors in that study. However, since the hydroquinone species can be in different ionization states depending on the solution pH, a semiquinone formation constant is defined as $K = [Q^{\cdot -}]^2/[Q][R]$, where [R] is the sum of all the hydroquinone species concentration at each pH value, *i.e.* QH_2, QH^- and Q^{2-}. It was found that, for all of the quinones under study in Bishop and Tong's work, K increases with an increase in pH, which means that the semiquinone stability increases with a rise in pH at the expense of the corresponding quinone and R species stabilities, most probably due to an increase in the concentration of Q^{2-} and the consequent augmentation of the electron–electron repulsion within R. From the values of K corresponding to $R = Q^{2-}$ (K' values) and the corresponding acid ionization constants of BQH_2 and $MBQH_2$, also determined in the cited work, K_{disp} values of 4.2×10^{20} and 1.8×10^{21} can be calculated, respectively, which are in good agreement with those obtained in our work. It is also shown in ref. 7 that addition of an electron-donating group, such as a methyl substituent, or an electron-withdrawing group, such as a sulfonate substituent decreases and increases, respectively, the semiquinone stability relative to those of Q and Q^{2-}. Interestingly, analogous trends are observed in our work even though QH_2, a neutral species, where electron–electron repulsion is not as large as in Q^{2-}, is the only R species. Thus, it seems that the combined increase or decrease in the free energies of two semiquinones is more important in governing the disproportionation equilibrium position than the changes in free energies of Q and R and that this is also independent of the ionic state of R.

The enthalpies of disproportionation are negative, Fig. 2, Table 2. Thus, more heat is evolved upon forming the hydroquinone and quinone than the heat needed to break ion–solvent interactions. A possible cause for this heat evolution is the production of an aromatic ring and O—H bonds at the hydroquinone, as well as the elimination of the electron–electron repulsion occurring at the semiquinone.

The positive entropy changes should be due to solvent disordering taking place upon releasing the reactant ions to solvate non-ionic products.

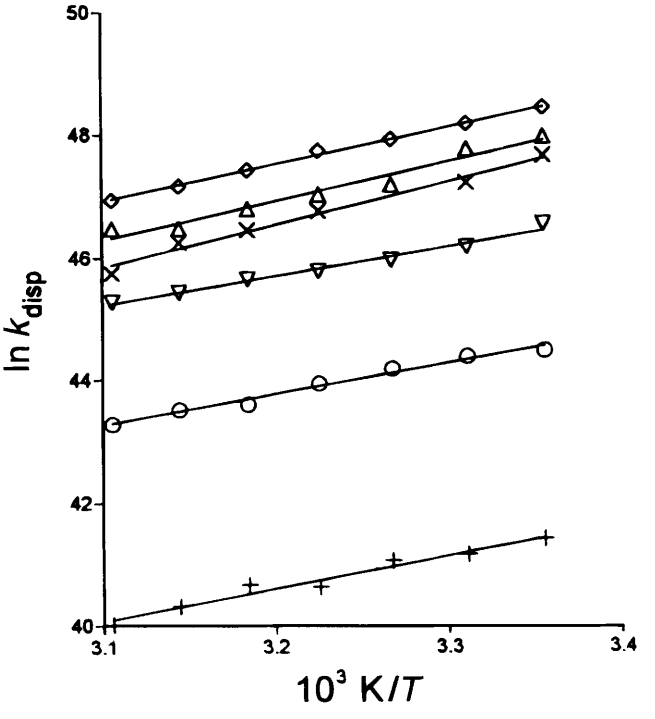
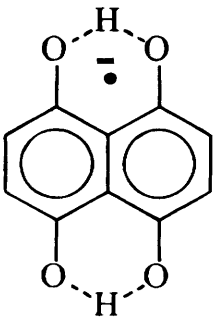


Fig. 2 Average Van't Hoff plots used in the determination of the semiquinone disproportionation enthalpies in phosphate buffer, pH 7.4. (△) $BQ^{\cdot -}$; (▽) $MNQ^{\cdot -}$; (◇) $MBQ^{\cdot -}$; (○) $NZQ^{\cdot -}$; (×) $NQ^{\cdot -}$; (+) $QNZ^{\cdot -}$.

Table 2 Thermodynamic parameters controlling semiquinone disproportionation in phosphate buffer (pH 7.4)

semiquinone	K_{disp}[a]	$\Delta H°$/kJ mol^{-1} [b]	$\Delta S°$/J K^{-1} mol^{-1} [c]
$BQ^{\cdot -}$	$(6.0 \pm 1.0) \times 10^{20}$	-53 ± 5	220 ± 8
$MBQ^{\cdot -}$	$(1.2 \pm 0.3) \times 10^{21}$	-49 ± 3	239 ± 12
$NQ^{\cdot -}$	$(1.5 \pm 0.3) \times 10^{20}$	-57 ± 10	195 ± 10
$MNQ^{\cdot -}$	$(1.6 \pm 0.2) \times 10^{20}$	-42 ± 4	246 ± 6
$NZQ^{\cdot -}$	$(2.1 \pm 0.8) \times 10^{19}$	-42 ± 5	229 ± 20
$QNZ^{\cdot -}$	$(9.6 \pm 2.0) \times 10^{17}$	-44 ± 8	197 ± 10

[a] Errors are standard deviations for at least three independent determinations. [b] Errors are propagated from the Van't Hoff plots slope errors. [c] Errors are propagated from those in K_{disp} and $\Delta H°$.



$NZQ^{\bullet -}$

Addition of OH groups to the 5 and 8 positions in $NQ^{\bullet -}$ strongly decreases the disproportionation constant ($NQ^{\bullet -}$ *vs.* $NZQ^{\bullet -}$). An increase in semiquinone stability, upon addition of these hydroxy substituents, should occur due to intramolecular hydrogen bonding in $NZQ^{\bullet -}$. Intramolecular, hydrogen bonding in $NZQ^{\bullet -}$ has been postulated to explain the low pK_a value of NZQH (2.7 for NZQH *vs.* 4.1 for NQH) and large pK_a values for the OH groups in NZQH (pK_a 13.8) as compared to that corresponding to NZQ (pK_a 10.7).[14]

In summary, the thermodynamic parameters, K_{disp}, $\Delta H°$ and $\Delta S°$, controlling the semiquinone disproportionation equilibrium of several *p*-semiquinones, have been determined. Smaller disproportionation constants are observed upon addition of a fused benzene ring to the semiquinone structure. Negative enthalpies and positive entropies of disproportionation govern the disproportionation equilibria. Addition of OH groups to the 5 and 8 positions in $NQ^{\bullet -}$ displaces the disproportionation equilibrium to the semiquinone probably due to intramolecular hydrogen bonding.

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