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Electron Paramagnetic Resonance of the Interaction of Semiquinone, Na^+ Ion Pairs with Phosphatidylcholine in Ethereal Solvents

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The solubilization of sodium salts of 1,4-benzosemiquinone ($BQ^{\bullet -}$), 1,4-naphthosemiquinone ($NQ^{\bullet -}$),2,3,5,6-tetramethylbenzosemiquinone ($DQ^{\bullet -}$), 2-methyl-1,4-naphthosemiquinone ($MNQ^{\bullet -}$), 2,6-di-*tert*-butyl-1,4-benzosemiquinone ($DTBQ^{\bullet -}$) and 9,10-anthrasemiquinone ($AQ^{\bullet -}$) in 1,2-dimethoxyethane (DME) and diethyl ether (DEE), in the presence and absence of egg yolk phosphatidylcholine (PC), under very dry conditions, has been studied utilizing electron paramagnetic resonance (EPR) spectroscopy. EPR spectra corresponding to both the ion pair and the 'free' ion species were observed simultaneously for $NQ^{\bullet -}$, $DQ^{\bullet -}$ and $MNQ^{\bullet -}$ in the presence of small quantities of PC in DME. Weighted-average EPR spectra were observed for $DTBQ^{\bullet -}$ and $AQ^{\bullet -}$ in DME, evidenced by the monotonic variation of their corresponding g values or proton coupling constants as a function of added PC. Binding constants of the sodium semiquinone salts to PC were determined for both DME and DEE solutions. Binding constants of the solvated sodium–semiquinone ion pairs to PC were also determined for DME solutions. The roles of the solvent and PC in solvating the sodium–semiquinone ion pairs are strongly interrelated. Addition of methyl or *tert*-butyl groups and extension of the π cloud to both the $BQ^{\bullet -}$ and $NQ^{\bullet -}$ structures decreases the solvated sodium–semiquinone binding to PC in DME implying that solubilization occurs preferentially at the charged sites of the PC molecules.

Semiquinones ($Q^{\bullet -}$) are important intermediates in antitumour drug activity,[1] radical scavenging processes,[2] redox reactions in membranes[3] and photoelectron-transfer mechanisms.[4] Owing to their organic and ionic nature, semiquinones are soluble in organic protic and aqueous media. The thermodynamic stability of semiquinones is known to vary strongly with changes in their solvating environment, as illustrated by the strong dependence of the quinone half-wave redox potential on the type of solvent.[5] Thus, the interaction of the semiquinone with its environment should play a major role in determining its stablity and activity in all the above-mentioned processes.

Semiquinones are anions in organic solvents. Most *p*-benzosemiquinones are also anions in water, at neutral pH, since their aqueous pK_a values are close to 4.[6,7] Therefore, one of their major stabilization processes in non-aqueous solvents is ion-pair formation.[7–11]

Several reports have dealt with the interaction of crown ethers with semiquinone, Na^+ ion pairs[12–16] in ethereal solvents which influences the rates of cation migration in semiquinones and also the equilibrium concentrations of the different ion pairs involved in these equilibria. However, only ion-paired species are observed in these solvents, even in the presence of crown ethers.

In this work we have found that phosphatidylcholine (PC), under very dry conditions, acts as an efficient semiquinone ion-pair dissociator and promotes the semiquinone salt solubility in ethereal solvents. Since semiquinones are known to be involved in redox processes in membranes and since the environment plays a major role in determining the semiquinone stability and activity in these processes, the $Q^{\bullet -}$–PC interactions described in this study may be relevant to the redox processes of semiquinones in membranes.

Experimental

Materials

All of the quinones used, *i.e.* 1,4-benzoquinone (BQ), 1,4-naphthoquinone (NQ), 2,3,5,6-tetramethylbenzoquinone (DQ), 2,6-di-*tert*-butylbenzoquinone (DTBQ), 9,10-anthrasemiquinone (AQ), menadione (MNQ) and the solvents DME and DEE were purchased from Aldrich. BQ, DQ, AQ, MNQ and DTBQ were sublimed prior to use. NQ was recrystallized from ethanol and vacuum dried. All the quinones were stored in a desiccator prior to use. The solvents DME and DEE were dried with Na and benzophenone under N_2 and stored under vacuum over an Na–K alloy. Egg yolk phosphatidylcholine (99%) at a concentration of 100 mg cm^{-3} in $CHCl_3$ was purchased from Sigma and used without further preparation. Galvinoxyl was purchased from Aldrich and its purity was determined by measuring the absorbance of a 10^{-3} mol dm^{-3} benzene solution at 772–773 nm ($\varepsilon = 607$ dm^3 mol^{-1} cm^{-1}).[18] Solutions of galvinoxyl, in the corresponding ethereal solvent, were prepared and stored under vacuum at −20 °C until used as spin standards.

Procedure

The quinones were reduced with sodium in the corresponding ether, under vacuum, utilizing the glass apparatus depicted in Fig. 1. Quinone (10–20 mg) was added into the calibrated tube A. Na (0.2–1.0 g) was placed into tube B and sublimed under vacuum (10^{-6} Torr†) into bulb C. Dry ether (*ca.* 10 cm^3) was distilled from Na–K alloy into bulb A and the quinone solution transferred under vacuum into bulb C for reduction. The semiquinone solutions were prepared after *ca.* 5–10 h of stirring the quinone solution over the Na mirror, as detected by their corresponding EPR spectra and the formation of semiquinone and/or dianion salt precipitate. A maximum EPR signal was obtained after salt precipitation was initiated as expected for a saturated solution in $Q^{\bullet -}$, Na^+. At this point, an EPR sample tube was sealed off from the apparatus and centrifuged at 3000 r.p.m. for 15 min before recording its corresponding spectra. The salt precipitate was excluded from the EPR cavity prior to recording the EPR spectra. Alternatively, the solution was filtered through a glass frit and the filtrate submitted to EPR analysis.

†1 Torr = (101 325/760) Pa.

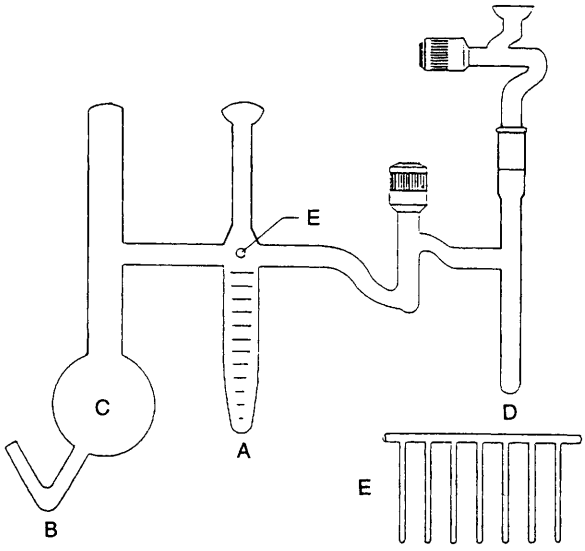

Fig. 1 Vacuum apparatus utilized in the sodium metal reduction of the quinones in DME and DEE and for the gradual addition of PC

Additions of PC to semiquinone salt solutions were performed as follows. Measured volumes of egg yolk PC in chloroform were added into tube D. The sample was submitted to high vacuum for *ca.* 30 min. PC was dissolved vigorously into the semiquinone solution and an EPR sample tube sealed off from the apparatus. An excess of the salt was still available for solubilization after PC addition. The EPR sample tubes were centrifuged and the corresponding EPR spectra recorded as indicated above. Alternatively, the solution was filtered through a glass frit and the filtrate submitted to EPR analysis.

In order to determine the semiquinone concentrations, the double integrated areas of the overmodulated semiquinone spectra were compared with that corresponding to a galvinoxyl standard dissolved in the same solvent.

Spectroscopic Measurements

EPR spectra were recorded on an X-band Bruker ER-200D EPR spectrometer. Double integrations were carried out with the 'EPR Software' version 2.1 from IBM, utilizing and IBM CS-9000 computer coupled to the EPR spectrometer. The g value determinations were performed as previously described utilizing DTBQ in hexamethylphosphoramide as a g standard ($g = 2.004\,184 \pm 0.000\,008$).[19] Coupling constants were corrected by comparison with those corresponding to the cyclooctatetrane radical anion in hexamethylphosphoramide.[20] Critical coupling of the EPR cavity was performed before recording double integrations as recommended by Goldberg.[20] EPR spectra were recorded at 25 °C.

Water Content per PC Molecule

The number of water molecules per PC molecule was determined by Karl Fischer titration[22] using a Mettler DL18 autotitrator. The pyridine-based titrant was standardized against water in methanol. Samples of PC in DME, prepared by using PC submitted to high vacuum for 30 min and DME distilled from Na–K, were titrated. Samples of DME, dried as indicated above, were also titrated and the amount of water determined subtracted from that corresponding to the PC solution. All titrations were also corrected for small leaks of water into the sample per unit time ('drift').

In order to compare this $[H_2O]$:[PC] molar ratio to that in the presence of a solubilized semiquinone salt such as $DQ^{\bullet -}$,Na^+, a Karl Fischer titration was also performed on DME solutions of $DQ^{\bullet -}$,Na^+ in the presence of 10 mg PC cm^{-3}, prepared as indicated above. Again, a solvent blank titration was subtracted from that of the sample.

Results and Discussion

$DQ^{\bullet -}$,Na^+–PC; $NQ^{\bullet -}$,NA^+–PC and $MNQ^{\bullet -}$,Na^+–PC Systems

Sodium reduction of DQ, NQ or MNQ in DME produced the corresponding semiquinone and/or dianion salts and the ion-paired semiquinone species with the EPR spectra depicted in Fig. 2, 3 and 4, respectively. The ion-paired semiquinone spectra corresponding to $DQ^{\bullet -}$,Na^+ and $MNQ^{\bullet -}$,Na^+ are characterized by linewidth alternation effects. These effects have been previously reported for $DQ^{\bullet -}$,Na^+ and $NQ^{\bullet -}$,Na^+ [9,10,23] ion pairs and ascribed to an exchange, at an intermediate rate of modulation, of the sodium cation between the oxygen atoms. Consecutive additions of PC result in an increase in the total EPR signal intensity (Table 1), and the generation of new EPR spectra, Fig. 2–4. These new EPR spectra show neither alkali-metal splitting nor linewidth alternation effects. Thus, abstraction of the sodium metal cation from these semiquinones has occurred. The proton hyperfine splittings of these new EPR spectra are similar to those observed for the free semiquinone species as reported elsewhere, *i.e.* $DQ^{\bullet -}$,Cs^+ in DME [a_H (12 H) = 1.92 G],[24] $MNQ^{\bullet -}$ in DMF [electrolytic reduction, a_H

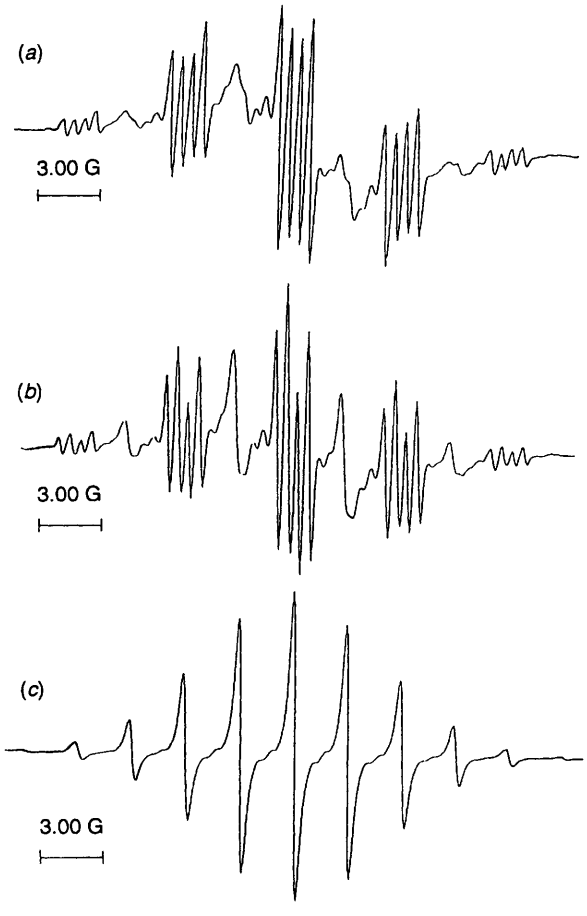

Fig. 2 EPR spectra corresponding to $DQ^{\bullet -}$,Na^+ in DME at 25 °C: (*a*) no PC added (previously reported).[17] [PC]/mg cm^{-3}: (*b*) 0.3, (*c*) 1.88; a_H (12 H) = 1.88 G.

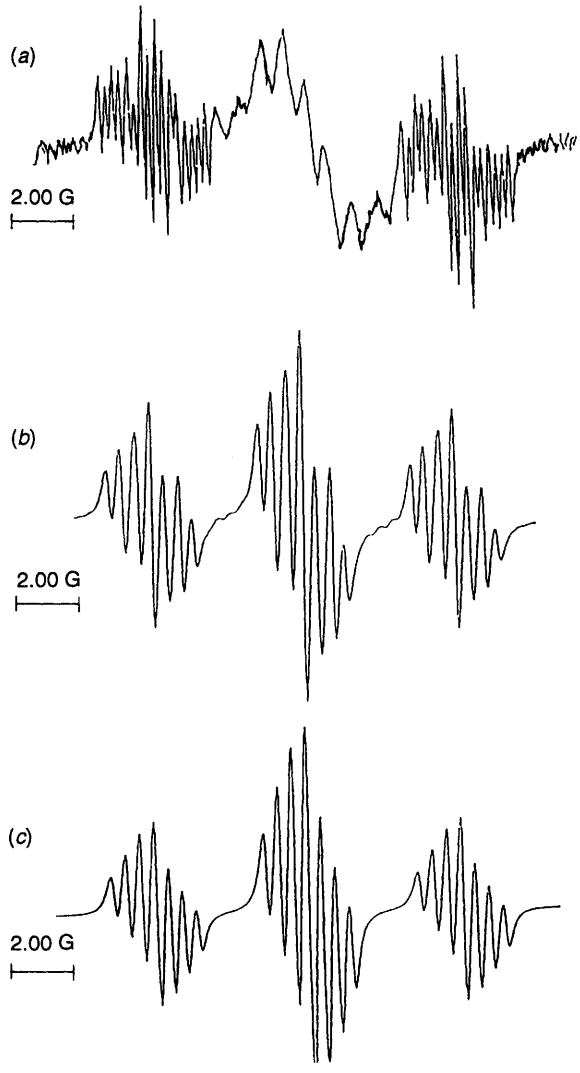

Fig. 3 EPR spectra corresponding to $NQ^{\bullet -}$,Na^+, in DME at 25 °C. (*a*) No PC added (previously reported).[10] (*b*) [PC] = 10 mg cm^{-3}. (*c*) Computer simulation of (*b*) using a_H (2 H) = 0.30 G.

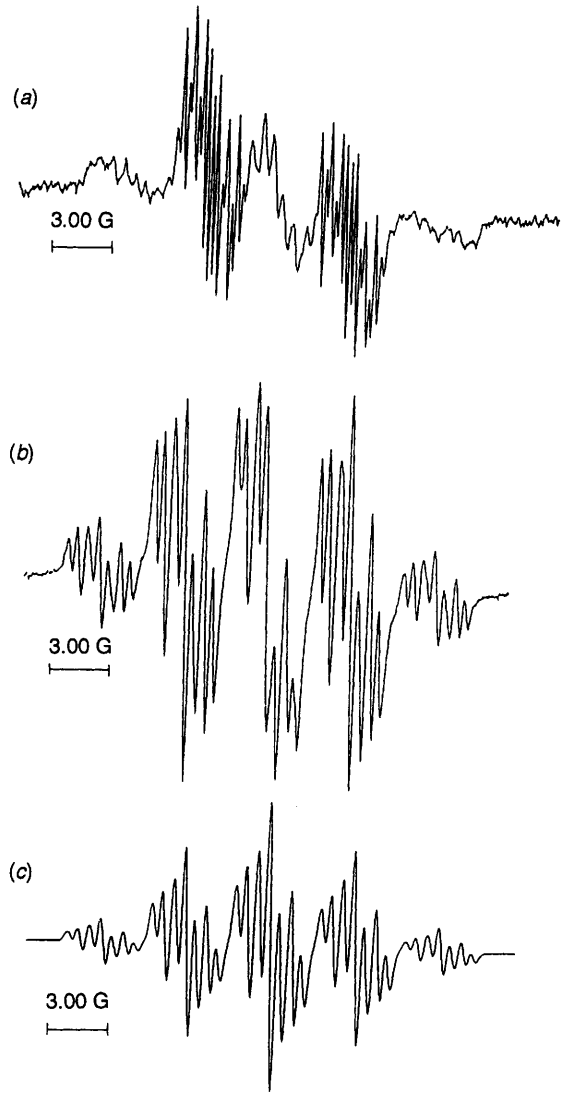

Fig. 4 EPR spectra corresponding to $MNQ^{\bullet -}$,Na^+ in DME at 25 °C. (*a*) No PC added. (*b*) [PC] = 10 mg cm^{-3}. (*c*) Computer simulation of (*b*) using a_H (4 H) = 2.72 G, a_H (2 H) = 0.722 G, a_H (2 H) = 0.298 G.

(4 H) = 2.69 G, a_H (1 H) = 0.22 G, a_H (1 H) = 0.36 G, a_H (1 H) = 0.78 G, (1 H) = 0.61 G][13] and $NQ^{\bullet -}$,K^+ in HMPA [a_H (2 H) = 3.28 G, a_H (2 H) = 0.64 G and a_H (2 H) = 0.23 G].[19] This indicates that these new species are unassociated ions when solubilized by PC.

As shown in Fig. 2(*c*), the ion-paired species has disappeared at 1.88 mg PC cm^{-3}, *i.e.* 2×10^{-3} mol dm^{-3} PC (assuming a PC molecular weight of 800). At this same concentration of 18-crown-6 $DQ^{\bullet -}$,Na^+ is not dissociated at all in THF and it is necessary to lower the temperature to −50 °C to dissociate completely this ion pair.[16] Therefore, PC is more efficient than crown ethers in sequestering the Na^+ cation from the semiquinone and probably from other anionic species in these ethereal solvents. This behaviour of PC as an efficient ion-pair dissociator should be relevant to chemical[25] and photochemical[26] reactions in which ion pairing affects the outcome of these processes in organic solvents.

Sodium reduction of MNQ, DQ or NQ in DEE generates the corresponding semiquinone and/or dianion salt, although

Table 1 Molar solubility of sodium salts of semiquinones in DME and DEE at 25 °C[a]

	DME		DEE	
$Q^{\bullet -}$	0 mg PC cm^{-3}	10 mg PC cm^{-3}	0 mg PC cm^{-3}	10 mg PC cm^{-3}
$DQ^{\bullet -}$	$(2.8 \pm 1.5) \times 10^{-3}$	$(4.9 \pm 2.0) \times 10^{-3}$	$< 10^{-7}$ [b]	$(8.4 \pm 5) \times 10^{-5}$
$NQ^{\bullet -}$	$(6.6 \pm 1.4) \times 10^{-5}$	$(2 \pm 1) \times 10^{-3}$	$< 10^{-7}$ [b]	$(1.2 \pm 1.0) \times 10^{-6}$
$BQ^{\bullet -}$	$< 10^{-7}$ [b]	$(1.2 \pm 1.0) \times 10^{-5}$	$< 10^{-7}$ [b]	$< 10^{-7}$ [b]
$AQ^{\bullet -}$	$(3.4 \pm 0.5) \times 10^{-4}$	$(4.30 \pm 0.2) \times 10^{-3}$	$< 10^{-7}$ [b]	$(4.3 \pm 4) \times 10^{-5}$
$DTBQ^{\bullet -}$	$(3.5 \pm 3.6) \times 10^{-4}$	$(1.6 \pm 1.2) \times 10^{-3}$	$< 10^{-7}$ [b]	$(1.2 \pm 0.1) \times 10^{-3}$
$MNQ^{\bullet -}$	$(1.6 \pm 0.3) \times 10^{-4}$	$(6.7 \pm 1.9) \times 10^{-4}$	$< 10^{-7}$ [b]	$(7.0 \pm 4.9) \times 10^{-5}$

[a] Average and standard deviations of at least three determinations. [b] EPR spectrum not observed. This is our instrument sensitivity limit as determined by successive dilutions of galvinoxyl solutions.

no detectable EPR signal is observed up to a PC concentration of *ca.* 0.5 mg cm^{-3}. Above this PC concentration, EPR spectra similar to those of the 'free' ions detected in DME, in the presence of PC, are observed (spectra not shown). An increase in the corresponding EPR signal intensity, upon addition of PC, is also detected in DEE, Table 1.

The fact that no detectable EPR signal is observed for these semiquinones in DEE, although detected in DME in the absence of PC, should be related to the more exergonic solvation of the sodium cation by DME than by DEE.[27] However, a Gibbs energy difference in the solvation of the $Q^{\bullet -}$,Na^+ ion pair, as a whole, by DEE and DME should also be involved.

Since water always adheres to PC molecules,[28,29] a reasonable conjecture for these observations could be that the changes detected in the semiquinone EPR spectra described above, upon addition of PC, are due to water solvation of semiquinones. Thus, experiments involving the addition of water, in the absence of PC, to $Q^{\bullet -}$,Na^+ solutions in DME were performed (see below).

The amount of water determined by Karl Fischer titration per PC molecule (assuming a PC molecular weight of 800) is 2 ± 1 water molecules per PC molecule in the absence of semiquinone salt. However, in the presence of $DQ^{\bullet -}$,Na^+ a $[H_2O]$: [PC] molar ratio of 0.2 ± 0.2 was determined indicating that further drying of the PC molecules occurs in the presence of the semiquinone salts. Possible causes for the further drying of the PC molecules are: (1) PC dehydration occurring upon quinone dianion protonation, owing to the large pK_a values of the corresponding hydroquinones ($pK_a \approx 8$–10);[30] (2) reaction of water molecules with sodium metal particles which are transferred with the salts into tube A (Fig. 1).

Addition of 0 to 15 times the amount of water present in $DQ^{\bullet -}$,Na^+–PC solutions, in the absence of PC, did not have the same effect as that provoked by PC addition on the previously described spectra, Fig. 5. Therefore, the observed changes in the $DQ^{\bullet -}$, $MNQ^{\bullet -}$ and $NQ^{\bullet -}$ spectra upon addition of PC should not be ascribed to water solvation. The common spectroscopic feature induced by addition of water in the absence of PC is an increase in the spectral broadening. An NMR study suggests that small amounts of water in DEE form ice-like globules or clusters, with small amounts of free inter-cluster water molecules.[31] The adsorption of semiquinone ions onto the surface of these highly structured water clusters should decrease the semiquinone tumbling rate and, thus, increase its EPR line broadening in a similar fashion to that occurring with semiquinones adsorbed on water-containing reversed micelles of cetyltrimethylammonium bromide (CTAB).[32]

Furthermore, it is well known that PC forms reverse micelles in diethyl ether with an average aggregation number of 40 under conditions where no free water has been added or where no dry conditions have been imposed. Under such conditions an $[H_2O]$/[PC] molar ratio of 6–7 has been found.[33,34] Therefore, at the low levels of hydration used in the present work, *i.e.* $[H_2O]/[PC] = 0.2$, not enough water molecules are available for sodium cation solvation (six water molecules per Na^+) or inducing PC reverse micellization.

Therefore, as suggested by one of the reviewers, ion-pair dissociation should be mediated mostly by an interaction of the sodium cation with the PC phosphate group and of the semiquinone with the tetramethylammonium group, leaving the semiquinone free enough to tumble rapidly and, consequently, producing a well resolved spectrum.

Another piece of evidence that further suggests that each semiquinone interacts with a single PC molecule is the low [PC] : $[Q^{\bullet -}]$ molar ratio. For example, at 10 mg PC cm^{-3},

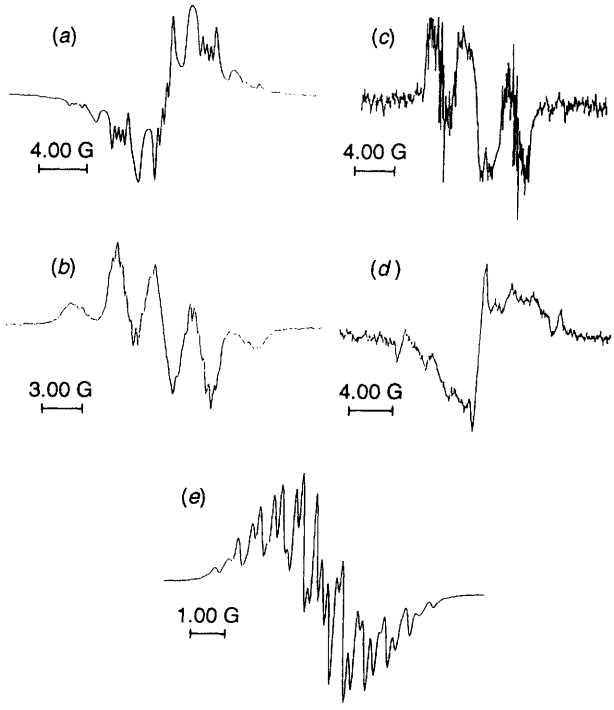

Fig. 5 EPR spectra corresponding to (*a*) $DQ^{\bullet -}$, (*b*) $MNQ^{\bullet -}$, (*c*) $NQ^{\bullet -}$, (*d*) $BQ^{\bullet -}$ and (*e*) $AQ^{\bullet -}$ in DME containing a water concentration *ca.* 15 times larger than that determined for $DQ^{\bullet -}$,NA^+ solutions containing 10 mg PC cm^{-3}, *i.e.* $[H_2O] = 4.0 \times 10^{-2}$ mol dm^{-3}. No PC is present.

the $DQ^{\bullet -}$,Na^+ ion pair concentration in DME is $(4.9 \pm 2.0) \times 10^{-3}$ mol dm^{-3} (see Table 1) and all of the $DQ^{\bullet -}$,Na^+ ion pairs interact with PC, Fig. 1(*c*). The PC concentration is 1.3×10^{-2} mol dm^{-3}, assuming a PC molecular weight of 800. At this PC concentration, under such dry conditions, no PC aggregation is expected since no driving force for reverse micelle formation is present. Furthermore, the [PC] : $[Q^{\bullet -}]$ molar ratio is close to 3. Therefore, at most, one out of three PC molecules should be interacting with $DQ^{\bullet -}$,Na^+. The probability of finding two semiquinone ion pairs per PC molecule should be very low at the semiquinone concentrations used here. Thus, only a 1 : 1 molecular complex for the interaction between $Q^{\bullet -}$,Na^+ and PC is expected. The well resolved EPR spectra observed in this work for all the semiquinones under study here also supports the idea that one PC molecule is interacting with $Q^{\bullet -}$,Na^+ and that no more than one $Q^{\bullet -}$,Na^+ per $Q^{\bullet -}$,Na^+–PC complex should exist, since loss of spectral resolution is expected with an increase in the size of the $Q^{\bullet -}$,Na^+–PC complex (by decreasing the $Q^{\bullet -}$ tumbling rate) and/or by a Heisemberg exchange process if more than one semiquinone ion pair is encountered per complex.[35]

$BQ^{\bullet -}$,Na^+–PC

Reduction of BQ in DME with a sodium mirror and either centrifugation or filtration did not yield a detectable EPR spectrum at room temperature. Thus, the crystal lattice Gibbs free energy of $BQ^{\bullet -}$,Na^+ surpasses the Gibbs free energy change in the solvation of this salt by DME (or by DEE, see below). Addition of a small amount of PC (*ca.* 0.1 mg cm^{-3}) produced a detectable five-line EPR spectrum corresponding to $BQ^{\bullet -}$ with a_H (4 H) = 2.44 G, similar to that reported previously.[36] The intensity of this spectrum also increases upon increasing the PC concentration. No sodium splitting is detected. No EPR signal is observed after sodium

mirror reduction in DEE. Addition of water without added PC to DME solutions produces a large broadening increase in the EPR spectra accompanied by a small contribution of a linewidth-alternated $BQ^{\bullet -},Na^+$ spectrum which could also be attributed to an exchange at an intermediate rate of modulation of the sodium cation between the oxygen atoms, Fig. 5(*d*). The same explanation as presented above for similar samples of semiquinones is adopted also for $BQ^{\bullet -},Na^+$ in DME upon addition of water.

$AQ^{\bullet -},Na^+$–PC and $DTBQ^{\bullet -},Na^+$–PC

Sodium reduction of AQ and DTBQ in DME generates the corresponding semiquinone EPR spectrum, where no sodium splitting or linewidth alternation is observed, Fig. 6(*a*). However, this observation does not mean that the 'free' ion is obtained, since ion pairs have been previously observed where no alkali-metal splitting is present.[19] Even though no metal splitting is detected in the case of $AQ^{\bullet -},Na^+$ without PC, the EPR spectrum under this condition (four sets of different proton coupling constants) is very different from that obtained in the presence of 10 mg PC mm^{-3} (two sets of different proton coupling constants), Fig. 6(*c*). In fact, one type of $AQ^{\bullet -}$ EPR spectrum gradually changes to the other type upon addition of PC, owing to a gradual change in the proton coupling constants and *g* value, Fig. 7. An increase in a_H corresponding to $DTBQ^{\bullet -}$ and in the $AQ^{\bullet -}$ *g* value is observed upon increasing PC concentration. This means that weighted-average species are observed for $AQ^{\bullet -}$ and $DTBQ^{\bullet -}$ upon addition of PC. The initial species (no lecithin added) should be a solvent-separated ion pair, as defined by Szwarc,[8] since no metal splitting is detected. This species enters into an equilibrium with $Q^{\bullet -},Na^+$–PC (complexes) with increasing PC concentration. A final constant *g* value or proton-coupling constant indicates that a constant microenvironment for $Q^{\bullet -}$ has been achieved. An increase in a_H for $DTBQ^{\bullet -}$ and in the *g* value of $AQ^{\bullet -}$ has also been detected previously for these species with a decrease in KI concentration in hexamethylphosphoramide (HMPA).[19] Therefore, as occurs in the case of $DQ^{\bullet -},Na^+$; $NQ^{\bullet -},Na^+$; $BQ^{\bullet -},Na^+$ and $MNQ^{\bullet -},Na^+$, ion-pair dissociation with an increase in PC concentration is implied by these changes in the spectral parameters corresponding to $DTBQ^{\bullet -},Na^+$ and $AQ^{\bullet -},Na^+$.

Again, no EPR signal was detected upon sodium reduction of AQ or DTBQ in DEE. EPR spectra, which are similar to those observed in the presence of PC in DME, were also detected in DEE (spectra not shown). Therefore, only the

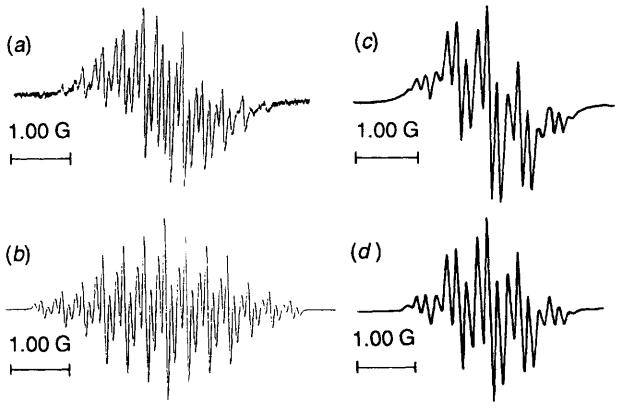

Fig. 6 EPR spectra corresponding to (*a*) $AQ^{\bullet -},Na^+$ in DME, no PC added. (*b*) Computer simulation of (*a*) assuming a_H (2 H) = 1.90 G, a_H (2 H) = 1.26 G, a_H (2 H) = 0.63 G and a_H (2 H) = 0.19 G. (*c*) $AQ^{\bullet -},Na^+$ in DME; [PC] = 10 mg cm^{-3}. (*d*) Computer simulation of (*c*) using a_H (4 H) = 1.02 G and a_H (4 H) = 0.29 G.

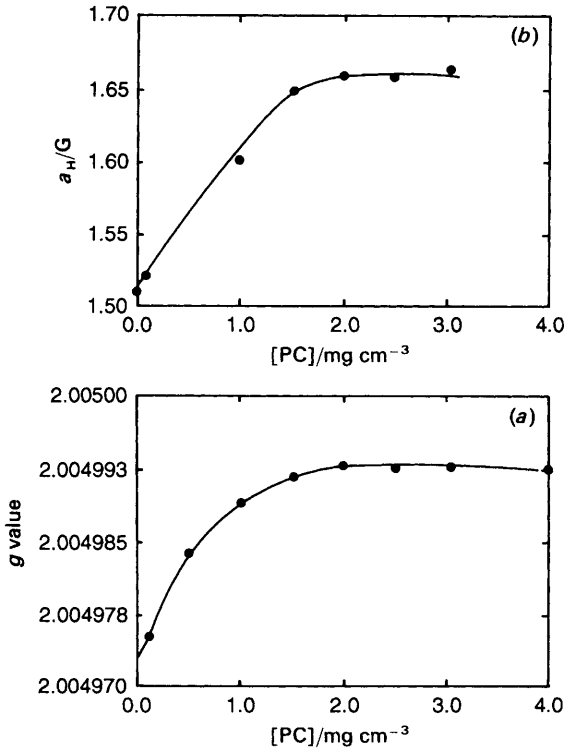

Fig. 7 (*a*) Variation in the *g* value of $AQ^{\bullet -},Na^+$ as a function of PC concentration in DME. (*b*) $DTBQ^{\bullet -},Na^+$ proton coupling constant dependence on PC concentration in DME.

$AQ^{\bullet -}$ PC and $DTBQ^{\bullet -}$–PC species are observed in DEE, which is the same situation as detected for the other semiquinone,Na^+ systems mentioned above. An increase in the semiquinone EPR signal is also observed upon increasing the PC concentration in both solvents, Table 1.

A spectral broadening increase was also detected for $DTBQ^{\bullet -},Na^+$ and $AQ^{\bullet -},Na^+$ upon addition of water, in the absence of PC, up to 0.040 mol dm^{-3}, Fig. 5. Such a broadening increase may be explained as above for other semiquinones.

$Q^{\bullet -},Na^+$–PC Binding Constants

Constant *g* values and proton coupling constants are observed for all the semiquinones under study, after *ca.* 2 mg PC cm^{-3} in DME. This is evidence that the microenvironment of the semiquinone does not change after this PC concentration. Thus, all of the $Q^{\bullet -},Na^+$ pairs should be interacting with PC molecules at 10 mg PC mm^{-3} in both DME and DEE. Since only lecithin-solubilized semiquinone,Na^+ species are observed in DEE, all the $Q^{\bullet -},Na^+$ ion pairs should also be interacting with PC, almost exclusively, at 10 mg PC cm^{-3}.

Assuming that one $Q^{\bullet -},Na^+$ ion pair is interacting with a single PC molecule (see above), then we can define K_I as the equilibrium constant for reaction (I).

$$Q^{\bullet -},Na_s^+ + PC_{sol} \rightleftharpoons Q^{\bullet -},Na^+\text{–}PC_{sol} \qquad \text{(I)}$$

K_I is, of course, a measure of the semiquinone ion-pair affinity for PC and can be calculated using eqn. (1),

$$K_I = [Q^{\bullet -},Na^+\text{–}PC_{sol}]/[PC_{sol}] \qquad (1)$$

where $[PC_{sol}] = [PC]_{tot} - [Q^{\bullet -},Na^+\text{–}PC]_{sol}$ and $[PC]_{tot}$ is the total concentration of PC, free plus interacting, in solution. K_I values evaluated at 10 mg PC cm^{-3} in both DME and DEE are shown in Table 2.

Table 2 Binding constants K_I and K_{IV} obtained for sodium-semiquinone solutions in DME and DEE at 25 °C

	DME[a]		DEE[a]
$Q^{\bullet -}$	K_I[b]	K_{IV}[c]	K_I[b]
$DQ^{\bullet -}$	0.61 ± 0.19	$(2.2 \pm 0.7) \times 10^3$	$(6.5 \pm 4.6) \times 10^{-3}$
$NQ^{\bullet -}$	0.18 ± 0.04	$(2.7 \pm 0.6) \times 10^3$	$(9.2 \pm 7.0) \times 10^{-5}$
$BQ^{\bullet -}$	$(9.2 \pm 3.0) \times 10^{-4}$	$>(9.2 \pm 4.0) \times 10^3$	$<8 \times 10^{-8}$
$AQ^{\bullet -}$	0.49 ± 0.13	$(1.4 \pm 0.3) \times 10^3$	$(3.3 \pm 3.2) \times 10^{-3}$
$DTBQ^{\bullet -}$	0.028 ± 0.0018	$(8.0 \pm 4.1) \times 10^1$	0.10 ± 0.05
$MNQ^{\bullet -}$	0.054 ± 0.021	$(3.3 \pm 1.3) \times 10^2$	$(5.4 \pm 4.2) \times 10^{-3}$

[a] Solution contains 10 mg PC cm^{-3}. [b] Errors are propagated from those corresponding to $Q^{\bullet -},Na^+$ salt solubilities. [c] Errors are propagated from those corresponding to K_I and the $Q^{\bullet -},Na^+$ salt solubilities without PC.

Semiquinones are know to be involved in a disproportionation equilibrium with their corresponding quinone and dianion, eqn. (II).[37,38]

$$2Q^{\bullet -},Na^+_{sol} \rightleftharpoons Q_{sol} + Q^{2-},2Na^+_{sol} \quad \text{(II)}$$

The three species depicted in eqn. II may all interact with PC molecules. These interactions should affect the equilibrium position of eqn. (II). However, this shift in eqn. (II) cannot affect the solubility of $Q^{\bullet -},Na^+$, depicted by eqn. (I), since no degrees of freedom are present in eqn. (I) at a given PC concentration, *i.e.* the solubility equilibrium [eqn. (I)] will shift to the products or reactants in order to maintain a constant semiquinone concentration at a given PC concentration and in the presence of semiquinone salt.

It is evident, upon inspection of Table 1, that solubilities are larger in DME than in DEE with and without added PC. This is consistent with the more negative Gibbs free energy of solvation of Na^+ in DME than in DEE.[27] Therefore, the roles of PC and solvent in determining the extent of solubilization of $Q^{\bullet -},Na^+$ are strongly interrelated. It is also observed in Table 2 that a different relative order in K_I is obtained in DME than in DEE and that larger relative differences in K_I values ($K_I = 10^{-6}$–10^{-1}, five orders of magnitude) are found in DEE as compared to those in DME ($K_I = 10^{-4}$–10^{-1}, three orders of magnitude). Therefore, Table 2 also provides evidence for the strong interrelated roles of solvent and PC in the solvation of $Q^{\bullet -},Na^+$.

The binding constants, K_I, are dependent on the relative crystal lattice Gibbs free energies of the semiquinone salts. However, if the salt solubility reaction in the absence of PC (only obtained in DME) is subtracted from reaction (1), then the reaction in which $Q^{\bullet -},Na^+$ is exchanged between the solvent and PC is obtained, Scheme 1, eqn. (IV)

The equilibrium constant governing eqn. (IV) is another binding constant, K_{IV}, which measures the relative affinities of the solvent and PC for $Q^{\bullet -},Na^+$. It is seen in Scheme 1 that K_{IV} can be obtained from the ratio of K_I and the corresponding $Q^{\bullet -}$, Na^+ solubility in the absence of PC, $[Q^{\bullet -},Na^+_{sol}]$, eqn. (2).

$$K_{IV} = K_I/[Q^{\bullet -},Na^+_{sol}] \quad (2)$$

Values of K_{IV} are also shown in Table 2.

Differences of at least two orders of magnitude in K_{IV} values are detected in Table 2, *i.e.* from 80 ($DTBQ^{\bullet -}$) to 9200 ($BQ^{\bullet -}$). Although, $BQ^{\bullet -},Na^+$ is the least soluble salt, its relative change in solubility upon addition of PC is larger than for any other semiquinone,Na^+ ion pair in this study. Addition of alkyl groups to $BQ^{\bullet -}$ (as in the case of $DQ^{\bullet -}$ and $DTBQ^{\bullet -}$) and $NQ^{\bullet -}$ (as in the case of $MNQ^{\bullet -}$) decreases the semiquinone,Na^+ affinity for PC relative to that for the solvent. Interestingly, increasing the π cloud extension, as from $BQ^{\bullet -}$ to $AQ^{\bullet -}$ *via* $NQ^{\bullet -}$, also decreases the semiquinone,Na^+ affinity for PC relative to that for the solvent. These observations suggest that the more hydrophobic the semiquinone is made, by the addition of methyl, *tert*-butyl or aromatic groups, the less strongly it is associated to PC molecules. This implies that solubilization of $Q^{\bullet -},Na^+$ occurs preferentially at the charged sites of the PC molecule which is consistent with the sequestering of the sodium cation by PC. A similar conclusion was reached by Kurreck and co-workers regarding the localization of substituted *p*-benzosemiquinone radical anions in CTAB reverse micelles. However, this system is appreciably different to that discussed in the present work owing to the large amount of water used to promote CTAB reverse micelle formation.[32]

In summary, well resolved EPR spectra are observed for a series of *p*-semiquinones in both DME and DEE, upon addition of PC, which can be ascribed to semiquinones that are not associated with their Na^+ counterion. The magnitude of the binding constants of the semiquinone—PC complexes implies that solubilization of the semiquinone,Na^+ ion pair occurs preferentially at the charged sites of the PC molecules. The latter is consistent with the efficient sequestering of the sodium cation by PC.

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$$Q^{\bullet -},Na^+_s + PC_{sol} \rightleftharpoons Q^{\bullet -},Na^+\text{–}PC_{sol} \quad \text{(I)}$$

$$Q^{\bullet -},Na^+_{sol} \rightleftharpoons Q^{\bullet -},Na^+_s \quad \text{(III)}$$

$$Q^{\bullet -},Na^+_{sol} + PC_{sol} \rightleftharpoons Q^{\bullet -},Na^+\text{–}PC_{sol} \quad \text{(IV)}$$

Scheme 1

References

1 G. Powis, *Free Rad. Biol. Med.*, 1989, **6**, 63.
2 M. Almgrem, F. Fireser and J. K. Thomas, *J. Phys. Chem.*, 1979, **83**, 3232.
3 A Brunmark and E. Cadenas, *Free Rad. Biol. Med.*, 1989, **7**, 435.
4 V. G. Chamupathi and G. Tollin, *Photochem. Photobiol.*, 1990, **51**, 611.
5 J. S. Jaworski, E. Lesniewski and M. K. Kalinowski, *J. Electroanal. Chem. Interfacial Electrochem.*, 1979, **105**, 329.
6 R. L. Willson, *Trans. Faraday Soc.*, 1971, **67**, 3020.
7 R. Bensasson and E. J. Land, *Biochim. Biophys. Acta*, 1973, **325**, 175.
8 M. Szwarc, *Ions and Ion Pairs in Organic Reactions*, Wiley, New York, 1974, vol. 2.
9 J. Oakes and M. C. R. Symons, *Trans. Faraday Soc.*, 1970, **66**, 10.
10 N. J. Flint and B. J. Tabner, *J. Chem. Soc., Faraday Trans. 1*, 1988, **84**, 3917.
11 L. Echegoyen, I. Nieves and G. R. Stevenson, *J. Phys. Chem.*, 1982, **86**, 1611.
12 H. B. Stegman, D. B. Hoang, M. Maurer, K. Scheffler, H. Buchner and E. Hartmann, *Magn. Reson. Chem.*, 1988, **26**, 547.
13 J. M. Fritsch, S. V. Tatwawadi and R. N. Adams, *J. Phys. Chem.*, 1967, **71**, 338.

14 M. P. Eastman, G. V. Bruno, C. A. McGuyer, A. R. Gutierrez and J. M. Shannon, *J. Phys. Chem.*, 1979, **83**, 5223.
15 G. F. Pedulli and A. Alberti, *Chem. Phys. Lett.*, 1977, **48**, 72.
16 S. Konishi, S. Niizuma and H. Kokubun, *Chem. Phys. Lett.*, 1980, **71**, 164.
17 J. Oakes and M. C. R. Symons, *Trans. Faraday Soc.*, 1970, **66**, 10.
18 P. D. Bartlett and T. J. Funahashi, *J. Am. Chem. Soc.*, 1962, **84**, 2596.
19 G. R. Stevenson, A. McB Block and A. E. Alegria, *J. Am. Chem. Soc.*, 1975, **97**, 4859
20 G. R. Stevenson and J. G. Concepcion, *J. Phys. Chem.*, 1972, **76**, 2176.
21 I. B. Goldberg, *J. Magn. Reson.*, 1978, **32**, 233.
22 E. Scholz, *Karl Fischer Titration—Determination of Water—Chemical Laboratory Practice*, Springer-Verlag, New York, 1984.
23 N. J. Flint and B. J. Tabner, *J. Chem. Soc., Faraday Trans. 1*, 1987, **83**, 167.
24 R. D. Allendoerfer and J. R. Papez, *J. Phys. Chem.*, 1972, **76**, 1012.
25 K. Okamoto, *Adv. Carbocation Chem.*, 1989, **1**, 171.
26 R. Billing, D. Rehorek and H. Hennig, *Top. Curr. Chem.*, 1990, **158**, 151.
27 M. H. Abraham and J. Liszi, *J. Chem. Soc., Faraday Trans. 1*, 1978, **74**, 1604.
28 H. F. Eicke and H. Christen, *Helv. Chem. Acta*, 1978, **61**, 2258.
29 U. Herman and Z. A. Schelly, *J. Am. Chem. Soc.*, 1979, **101**, 2665.
30 S. I. Bailey and I. M. Ritchie, *Electrochim. Acta*, 1985, **30**, 3.
31 Sh. A. Markaryan, D. A. Petrosyan and N. M. Beileryan, *Zh. Strukt. Khim.*, 1978, **3**, 460.
32 D. Niethammer, B. Kirste and H. Kurreck, *J. Chem. Soc., Faraday Trans.*, 1990, **86**, 3191.
33 P. H. Poon and M. A. Wells, *Biochemistry*, 1974, **13**, 4928.
34 A. M. Giuliani, C. Biocelli, C. Andrea and P. L. Luisi, *Chem. Phys. Lipids*, 1990, **53**, 265.
35 G. I. Lichtenstein, *Spin Labelling Methods in Molecular Biology*, Wiley-Interscience, New York, 1976.
36 J. A. Pedersen, *Handbook of EPR Spectra from Quinones and Quinols*, CRC Press, Boca Raton, FL, 1985, p. 67.
37 Y. A. Ilan, G. Czapski and D. Meisel, *Biochim. Biophys. Acta*, 1976, **430**, 209.
38 A. E. Alegría, C. Muñoz and S. Rodríguez, *J. Phys. Chem.*, 1990, **94**, 930.

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