Role of membrane charge and semiquinone structure on oxygen consumption rates

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Rates of oxygen consumption of air-saturated aqueous solutions containing either 2,3-dimethoxy-5-methylbenzoquinone (UQ-0), 1,4-naphthoquinone (NQ) or phenanthroquinone (PHQ) and ascorbate were measured in the presence or absence of large unilamellar vesicles (LUVs) composed of either dimyristoylphosphatidylcholine (DMPC) or equimolar mixtures of DMPC with dimyristoylphospahtidic acid (DMPA) or hexadecyltrimethylammonium bromide (CTAB), at physiological pH. Semiquinone hydrophobicities follow the same order as that of the corresponding quinones. Rates increase with positively charged LUV concentration and decrease with either neutral or negatively charged LUV concentration in samples containing the hydrophobic quinones NQ and PHQ. Rates remain essentially unchanged in the presence of the most hydrophilic quinone, UQ-0. An increase in the ionic strength of the solution partially inhibits the observed changes in the rate of oxygen consumption, R_{ox}, caused by the presence of positively charged membranes, thus implying that such changes are of electrostatic nature. Similar trends with membrane charge are observed for the rates of oxygen consumption in the presence of PHQ and dithiothreitol. The observed increase in the ascorbate oxidation rate in the presence of positively charged lipids occurs in systems where a decrease in semiquinone disproportionation is also detected, thus, implying that an increase in the quinone one-electron redox potential, caused by semiquinone–positively-charged-membrane interaction, could contribute to the observed effects.

Introduction

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

The efficiency of these electron-transfer processes should depend on the thermodynamic stability of the semiquinones which is governed by the combined relationship between semiquinone structure and environmental characteristics. Although quinones could also be reduced to the two-electron reduced species, the hydroquinone (QH_2), direct oxidation of hydroquinones by oxygen is a very slow, spin-forbidden, reaction[6–9] and, thus, hydroquinone autoxidation is proposed to proceed *via* the semiquinone reduction of oxygen.[7,10,11]

Two major environments are present in biological systems. These are either hydrophilic or hydrophobic. A combination of these two environments is found in phosphatidylcholine (PC) membranes. Since oxygen is more soluble at the hydrophobic lipid moiety of PC membranes than in water,[12] the semiquinone–oxygen electron transfer process could be more effective in this site than in the aqueous phase if the semiquinone partitions into the membrane. In addition, semiquinone-membrane interaction could alter the rates and equilibrium constant governing the semiquinone disproportionation equilibrium (eqn. (1)).

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

In a previous work, we reported the role of PC membrane charge and semiquinone structure on the semiquinone membrane–buffer distribution coefficients and semiquinone disproportionation equilibrium.[13] It was found that in the presence of neutral or positively charged membranes (much more pronounced in the latter case), the semiquinone disproportionation equilibrium is shifted to the left for hydrophobic semiquinones (juglone-, naphthazarin-, 1,4-naphtho- and 1,2-naphthosemiquinones) and to the right for a hydrophilic semiquinone (1,4-benzosemiquinone) (eqn. (1)). In the presence of negatively charged membranes the opposite is observed for all the semiquinones included in that study. In the present work we report the role of PC membrane electrical charge on the rates of oxygen consumption. A negatively charged (ascorbate) and a neutral (dithiothreitol) reducing agent were used. Two hydrophobic (1,4-naphtho- and phenanthro-) and one hydrophilic (2,3-dimethoxy-5-methyl-1,4-benzo-) quinones are studied here. The semiquinones of these are all anions at neutral pH (pK_a values of $NQH^{\bullet}$, $PHQH^{\bullet}$ and $UQ\text{-}0H^{\bullet}$ are within 3–5[14,15,16]).

Experimental

Materials

The quinones (Fig. 1), 1,4-naphthoquinone (NQ), phenanthroquinone (PHQ) and 2,3-dimethoxy-5-methyl-1,4-benzoquinone (UQ-0) were purchased from Aldrich. Egg-yolk phosphatidylcholine (PC), dimyristoylphosphatidic acid (DMPA) and dimyristoylphosphatidylcholine (DMPC) were obtained from Avanti Polar Lipids. Quinones were purified by double sublimation. Fresh stock solutions of quinones were prepared in water and used the same day. Ascorbic acid (99+%), sodium

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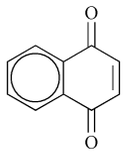
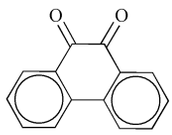
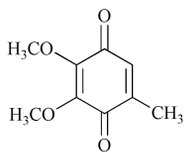


1,4-naphthoquinone (NQ)



phenanthroquinone (PHQ)



2,3-dimethoxy-5-methyl-1,4-benzoquinone (UQ-0)

Fig. 1 Quinones under study here.

phosphates, Na_2HPO_4 and NaH_2PO_4, of highest grade, dithiothreitol (DTT) and Chelex 100 were purchased from Alfa-Aesar. Stock solutions of reagents were prepared in deionized distilled water. Anaerobic solutions were prepared by bubbling nitrogen through septa-stoppered test tubes.

Semiquinone generation for EPR analyses

Semiquinones were produced as previously described.[17,18] Since two of the quinones under study here are slightly soluble in the buffer phase, semiquinones were first generated in absolute methanol by reacting from 10 to 20 mg of quinone with $NaBH_4$ in a convenient molar ratio such that semiquinone spectral intensity is maximized when transferred to aqueous buffer. The solvent was then evaporated to dryness using a dry nitrogen flow. The sample was submitted to high vacuum for at least 30 min. A nitrogen-purged aqueous sodium phosphate buffer solution was then added to the dry semiquinone sample and the resulting solution equilibrated to pH 7.4 by addition of small aliquots of HCl or NaOH. This aqueous solution was diluted to 1.50 ml and centrifuged at 3500 rpm for 5 min. The supernatant was then submitted to EPR analysis or used in the multilamellar vesicles (MLVs) preparation.

Liposome preparation for EPR analyses

Multilamellar vesicles (MLVs) were prepared as described previously.[13] A thin film of PC (1.0–100.0 mg), with or without CTAB or DMPA in a 1:1 ratio with PC, was prepared in a septum-stoppered test tube, by evaporating the solvent from its chloroform solution, using first a nitrogen gas flow followed by high-vacuum application for 30 min. A sample (400 μl) of an aqueous semiquinone solution, prepared as described above, was then added to this lecithin film and strongly vortexed under nitrogen until a stable suspension was obtained (*ca.* 3–4 min). This suspension was submitted for EPR analysis.

Liposome preparations for oxygen consumption measurements

MLVs and large unilamellar vesicles (LUVs) were prepared in distilled, deionized, chelex-treated water. In order to avoid lipid oxidation DMPC was used instead of egg yolk PC. DMPC with or without an equimolar amount of DMPA or CTAB was dissolved in chloroform followed by stripping-off this solvent with a flow of nitrogen. The lipid sample was then exposed to high vacuum for at least 30 min. Water was then added and the suspension strongly vortexed. This suspension was then submitted to five freeze–thaw cycles, as recommended by Hope and coworkers,[19] followed by ten filtration cycles through a polycarbonate filter of 100 nm pore diameter using a high pressure extruder.

Oxygen consumption rate measurements

These were performed at 37.0 ± 0.1 °C in 50 mM phosphate buffer, pH 7.4, which was prepared by mixing solutions of Na_2HPO_4 and NaH_2PO_4 without adding any acid or base. Stock and sample solutions were prepared with double distilled deionized water and decontaminated from traces of transition metals by exposure to Chelex 100 resin using the batch method.[20] Air-saturated sample solutions were used containing 6 μM quinone, 1.0 mM or 200 μM ascorbate and 50 mM phosphate buffer (pH 7.4) in the presence or absence of the LUVs indicated above. Oxygen consumption rates were measured with a 5300 Oxygen Biological Monitor (Yellow Springs Instruments Co., USA) using a Clark electrode as a sensor in a YSI 5301 constant temperature bath. Rates were calculated from the initial constant slopes of $[O_2]$ traces. Runs were started in the absence of quinone followed by quinone addition without interrupting oxygen consumption measurements.

EPR measurements

First-derivative EPR spectra were acquired and analyzed using an EMX X-band Bruker EPR spectrometer coupled to a computer. Well-resolved semiquinone first-derivative EPR spectra in the absence of lipids were simulated and optimized (best-fitted to the experimental spectra) using WINSIM,[21] starting with hyperfine coupling constants available in the literature corresponding to aqueous or water-containing samples.[22] First-derivative EPR spectra corresponding to lipid-containing samples, in which both the membrane-bound and the free semiquinone spectra were observed superimposed (hereby known as the composite spectra), were simulated and optimized in the same fashion starting with two superimposed different spectra: the well-resolved spectrum corresponding to the semiquinone in the aqueous phase and a broad (membrane-bound) semiquinone spectrum. A single broad line with peak-to-peak width of 2 Gauss with identical *g*-value to that of the aqueous phase species was used as the starting broad spectral component in the optimization process. Linewidths, relative *g*-values, lineshapes and relative intensities were optimized in the composite spectra. The optimization process produced the EPR spectral intensity ratios corresponding to membrane-bound semiquinones to those of semiquinones in the aqueous phase (I_m/I_{aq}) at appropriate aqueous to lipid volume proportions. Semiquinone membrane-buffer partition coefficients $P_{Q^{\cdot-}}$ were determined using eqn. (2),

$$P_{Q^{\cdot-}} = \frac{I_m V_{aq}}{I_{aq} V_m} \qquad (2)$$

where V_{aq} and V_m are the volumes of aqueous and lipid phases, respectively. The lipid volume was determined from the lipid mass used and its density, assuming that the lipid phase density is equal to the aqueous phase density.[23]

Results and discussion

Upon addition of 6.0 μM of either UQ-0, NQ or PHQ to an air-saturated solution containing 1.0 mM ascorbate and 50 mM phosphate buffer, an initial constant rate of oxygen consumption, R_{ox}, was observed, as reported previously (Fig. 2).[24,25] Addition of NQ to LUV dispersions of DMPC, DMPC/DMPA or DMPC/CTAB produced significant changes in R_{ox} (Fig. 3(a)) characterized by a large increase in R_{ox} with an increase in positively charged lipid concentration and a decrease in R_{ox} with an increase of either neutral or negatively charged lipid concentration, Table 1. A similar trend of R_{ox} with lipid concentration is also observed for PHQ (Fig. 3(b), Table 1). In contrast, no effect of membrane charge on R_{ox} was observed for UQ-0 (Fig. 3(c)). However, since too large rates were measured in the case of UQ-0 when using 1.0 mM ascorbate, these determinations were repeated with a smaller concentration of ascorbate, *i.e.* 200 μM (Fig. 3(d)). Again, the

Table 1 Initial oxygen consumption rates and partition coefficients corresponding to the quinones and semiquinones under study

Quinone	R_{ox}/nM s^{-1}					
	No lipid[a]	DMPC[b]	DMPC/CTAB[b]	DMPC/DMPA[b]	P_Q[c]	$P_{Q^{\bullet-}}$[d]
UQ-0	834 ± 87	783 ± 100	814 ± 112	840 ± 93	1.7 ± 0.1	1.0[f]
NQ	635 ± 10	423 ± 34	1800 ± 200	390 ± 106	16 ± 3	300 ± 70[g]
PHQ	397 ± 27	96 ± 12	2700 ± 400	95 ± 14	10.1 ± 0.3	180 ± 60
PHQ[e]	280 ± 23	180 ± 25	2200 ± 200	106 ± 12	10.1 ± 0.3	—

[a] Measured at 37 °C in air-saturated samples containing 6 μM quinone, 1.0 mM ascorbate (NQ, PHQ) or 200 μM ascorbate (UQ-0) and 50 mM phosphate buffer (pH 7.4). [b] Same sample condition as in footnote a plus 17 mM of the corresponding lipid mixture. [c] Quinone octanol/buffer partition constant. [d] Semiquinone membrane buffer partition constants determined using eqn. (2) and 120 mM PC. [e] DTT (1.0 mM) used as reducing agent. [f] Estimate of the largest partition constant that can be calculated from a semiquinone spectrum corresponding to this semiquinone in 120 mM PC, assuming that a broad spectral component was still found in this spectrum even though no broad component was distinguishable. [g] Ref. 13.

Table 2 Effect of NaCl concentration on the ratio of the initial oxygen consumption rate in the presence of lipid to that corresponding to its absence at the same ionic strength.[a]

Quinone	DMPC/CTAB		DMPC		DMPC/DMPA	
	with NaCl	without NaCl	with NaCl	without NaCl	with NaCl	without NaCl
UQ-0	1.03 ± 0.15	0.98 ± 0.22	0.8 ± 0.2	1.0 ± 0.2	0.97 ± 0.17	1.02 ± 0.17
NQ	1.46 ± 0.25	2.8 ± 0.6	0.65 ± 0.13	0.67 ± 0.13	0.56 ± 0.1	0.61 ± 0.12
PHQ	3.0 ± 0.6	6.8 ± 1.0	0.25 ± 0.05	0.24 ± 0.05	0.21 ± 0.08	0.23 ± 0.09

[a] Measured at 37 °C in air-saturated samples containing 6 μM quinone, 1.0 mM ascorbate (NQ, PHQ) or 200 μM ascorbate (UQ-0) and 50 mM phosphate buffer plus 17 mM of the corresponding lipid mixture in the presence or absence of 65 mM NaCl. For example: the value of 0.56 for NQ in the presence of DMPC/DMPA with NaCl is obtained by dividing the R_{ox} corresponding to this sample by that of a sample containing NQ and 65 mM NaCl with no lipid.

Table 3 Hyperfine coupling constants for semiquinones under study here in nitrogen-saturated phosphate buffer (pH 7.4)

Semiquinone	a_H[a]/Gauss	a_H/Gauss
$PHQ^{\bullet-}$	1.41 (1H), 1.81 (1H), 1.67 (2H), 0.43 (2H), 0.30 (2H's)	1.73 (2H), 1.69 (2H), 0.41 (4H)[b]
$NQ^{\bullet-}$	3.18 (2H), 0.63 (4H)	3.11 (2H), 0.63 (2H), 0.55 (2H)[c]
UQ-$0^{\bullet-}$	2.44 (3H), 1.93 (1H), 0.03 (6 H)	2.34 (3H), 2.07 (1H), 0.03 (6H)[c]

[a] This work. [b] Ref. 35. [c] Ref. 23 (in alkaline aqueous solutions).

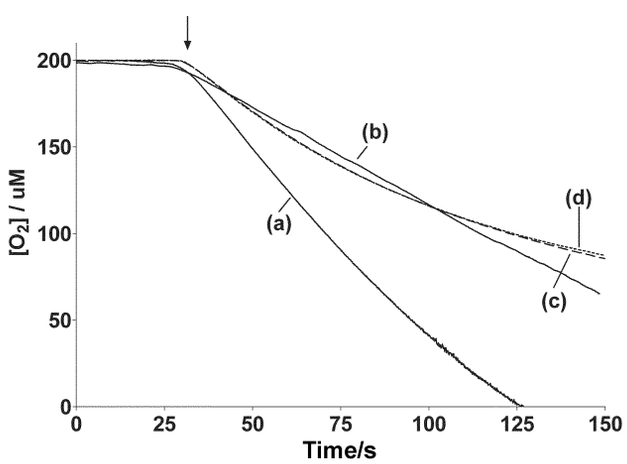


Fig. 2 Oxygen consumption occurring in air-saturated samples containing 50 mM phosphate buffer (pH 7.4), at 37 °C, and (a) 6 μM NQ, 20 mM of an equimolar DMPC/CTAB mixture and 1.0 mM ascorbate, (b) 6 μM NQ and 1.0 mM ascorbate, (c) 6 μM UQ-0, 20 mM of an equimolar DMPC/CTAB mixture and 200 μM ascorbate, (d) 6 μM UQ-0 and 200 μM ascorbate.

same type of behavior for UQ-0 was observed. Evidence which supports the predominance of electrostatic forces in causing these rate effects, in samples containing NQ and PHQ, when using positively charged lipid mixtures is the decrease in R_{ox} upon increasing the ionic strength with NaCl (Table 2). However, for samples containing these same quinones, essentially no change in R_{ox} was detected upon changing ionic strength in samples with negatively charged lipids. The latter is evidence that, in the case of negatively charged lipids, the magnitudes of the observed R_{ox} values depends more on the rate of quinone diffusion into the hydrophobic interior of the liposome than on semiquinone or ascorbate electrostatic repulsion with the membrane interface of DMPC/DMPA vesicle. No significant difference in R_{ox} change with increase in lipid concentration was detected for UQ-0 with or without added NaCl (Table 2). These observations correlate with semiquinone and quinone hydrophobicities, *i.e.* their membrane-buffer distribution coefficients (Tables 1 and 4). Thus, systems where quinones and semiquinones are hydrophobic display a larger effect on R_{ox} with membrane presence and charge. The high hydrophilicity of UQ-$0^{\bullet-}$ is also exemplified by the impossibility of detecting a broad EPR signal even at a concentration of 120 mM PC/CTAB, where the membrane-bound species is detected for $NQ^{\bullet-}$ and $PHQ^{\bullet-}$, Fig. 4. EPR hyperfine constants of the observed semiquinone spectra are depicted in Table 3.

It could be argued that the observed decrease in R_{ox}, either in the presence of DMPC or DMPC/DMPA could also be due to faster partition of quinones into the lipid phase with increasing lipid concentration, thus decreasing the rate of quinone reduction. However, essentially the same rate of quinone partition into DMPC/CTAB liposomes as that occurring with DMPC and DMPC/DMPA LUVs is expected for each quinone since these quinones are neutral species. Thus, hydrophobic quinones are available at the DMPC/CTAB liposome interface to serve as ascorbate oxidants. Since quinones are neutral species, these should also be available at the interface of both DMPC and DMPC/DMPA liposomes. Therefore, the larger R_{ox} values

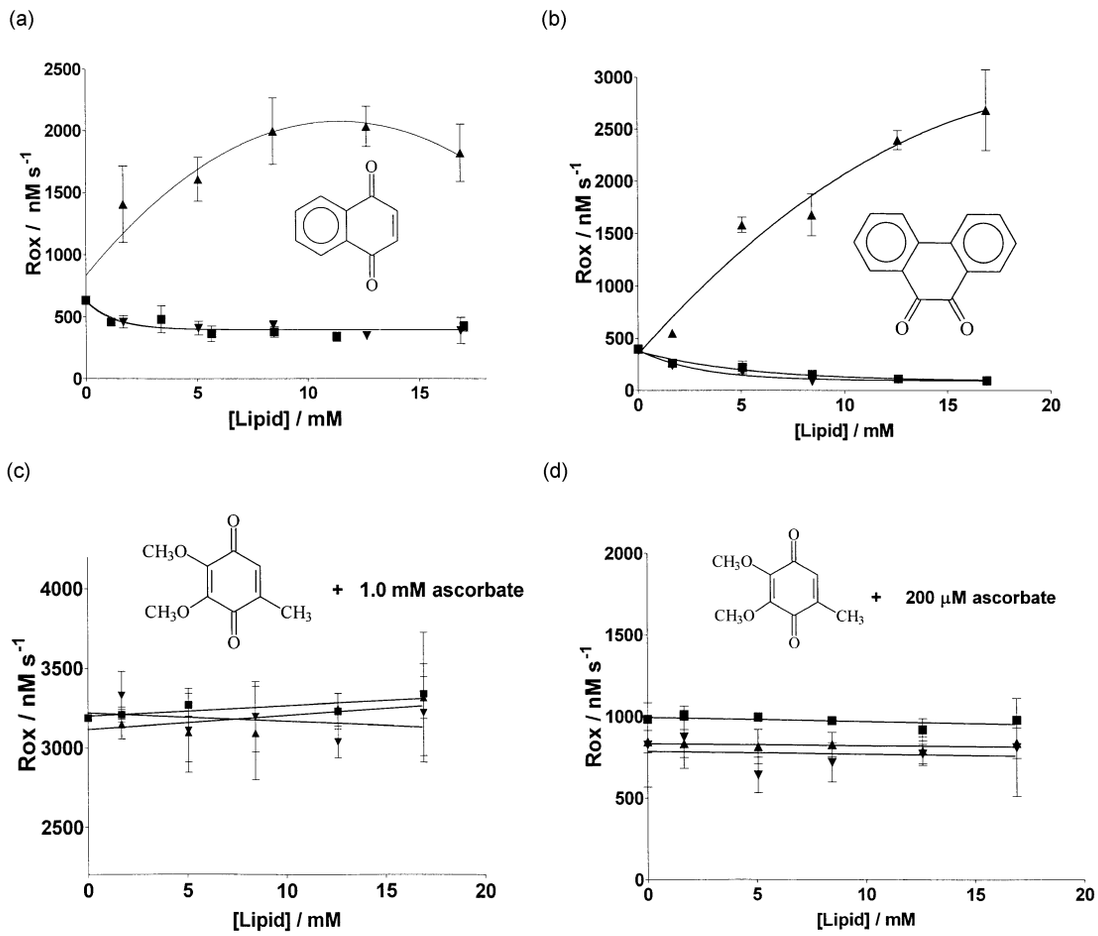


Fig. 3 R_{ox} dependence on LUVs electrical charge and concentration. Samples in (a)–(c) contain 6 μM quinone, 1.0 mM ascorbate, 50 mM phosphate buffer (pH 7.4) and equimolar amounts of DMPC with CTAB (▲), DMPA (▼) and no other type of lipid (■). Samples in (d) contains 6 μM of UQ-0 and the same lipid mixtures as stated for (a)–(c) but 200 μM ascorbate instead of 1.0 mM. Runs were made at 37 °C in air-saturated solutions.

Table 4 Semiquinone concentration (μM) in the presence or absence of 120 mM of lipid at pH 7.4 and 25 °C

Semiquinone	No lipid	PC	PC/DMPA	PC/CTAB
$PHQ^{\bullet -}$	0.8 ± 0.1	1.5 ± 0.7	0.06 ± 0.02	9.6 ± 0.9
$NQ^{\bullet -}$	2.0 ± 0.4	10 ± 2	0.13 ± 0.06	31 ± 8
$UQ\text{-}0^{\bullet -}$	5.0 ± 0.9	4.0 ± 1.2	3.2 ± 1.2	5.1 ± 1.3

observed for hydrophobic quinones in the presence of DMPC/CTAB LUVs should be caused by a factor which is not available in neutral or negatively charged liposomes, *i.e.* either a larger ascorbate concentration localized at the DMPC/CTAB membrane interface as compared to the aqueous phase and other lipids interface or an increase in the quinone redox potential due to semiquinone stabilization at the positively charged interface (see below). Since hydrophobic quinones such as NQ and PHQ, but not hydrophilic quinones such as UQ-0, will also concentrate at the membrane at the expense of the aqueous phase, a rate enhancement is only observed for this type of quinone in the presence of DMPC/CTAB LUVs. A similar effect of bilayer charge on the rate of ascorbate oxidation by the α-tocopheroxyl radical was previously detected.[26] At physiological pH, ascorbate is an anion (pK_a = 4.0 at 25 °C at I = 0.1[27] and 3.6 in hexadecyltrimethylammonium chloride micelles[26]) which is then electrostatically attracted to the DMPC/CTAB interface but not attracted or repelled from the DMPC and DMPC/DMPA interfaces, respectively. These effects described above seem to be more important in this system than the role of a larger oxygen concentration at the lipid phase.[12] The observed decrease in R_{ox} with increasing DMPC or DMPC/DMPA concentration in the case of the hydrophobic quinones can be explained by a reduced accessibility to ascorbate by the quinone in the lipid phase.

As mentioned above, another possibility which needs to be considered in the effect of membrane charge on R_{ox} is the role of possible changes on the quinone one-electron redox potential in the presence of the membranes under study here. In a previous work, it was shown that semiquinone disproportionation corresponding to hydrophobic semiquinones was strongly diminished in the presence of positively, and enhanced in the presence of negatively, charged liposomes. A similar observation is made in this work with the semiquinone disproportionation of the two hydrophobic semiquinones ($NQ^{\bullet -}$ and $PHQ^{\bullet -}$) while for the hydrophilic semiquinone ($UQ\text{-}0^{\bullet -}$) essentially no effect on the semiquinone disproportionation equilibrium is observed, Table 4. The disproportionation Gibbs free energy, ΔG^0_1, is a function of differences in the redox potentials, $E(Q/Q^{\bullet -})$ and $E(Q/QH_2)$ (eqn. (3)).

$$\Delta G^0_1 = -2F\{E(Q/QH_2) - E(Q/Q^{\bullet -})\} \quad (3)$$

Thus, assuming that $E(Q/QH_2)$ is not too dependent on membrane charge, as it should be for $E(Q/Q^{\bullet -})$, then a more positive ΔG^0_1 could also be caused by a more positive $E(Q/Q^{\bullet -})$ value. Since, larger R_{ox} values are detected for quinones with less negative E^1_7 values,[24] in accordance to the Marcus theory for electron transfer, another possible contribution to the observed increase in R_{ox} in the presence of DMPC/CTAB could be a more positive value for the hydrophobic quinone one-electron redox potential produced by the stabilization of the semiquinone at the positively charged interface. Conversely,

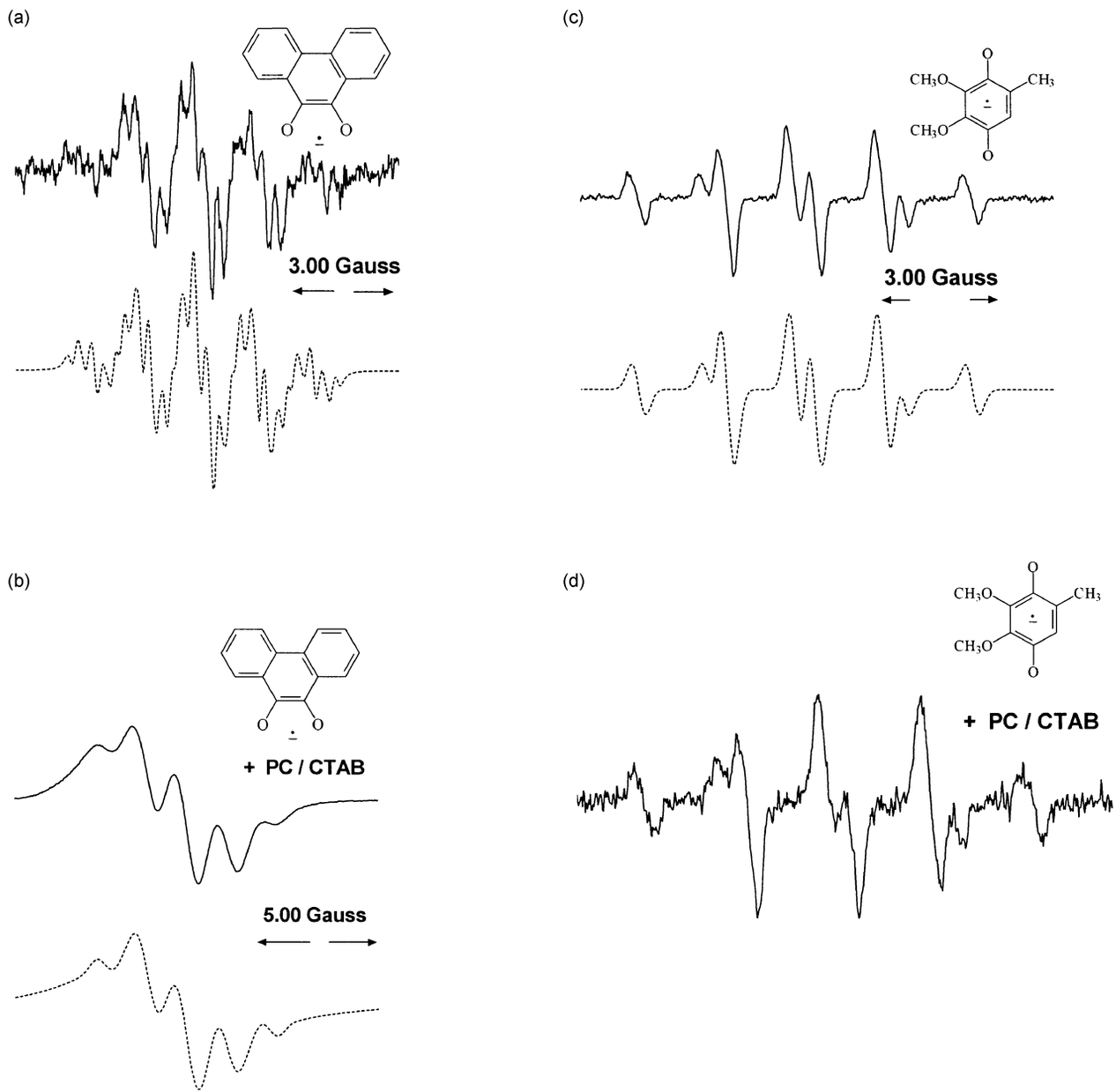


Fig. 4 EPR spectra corresponding to (a) $PHQ^{\bullet -}$ in a N_2-saturated solution in the absence of lipids at pH 7.4, (b) $PHQ^{\bullet -}$ in a N_2-saturated solution containing MLVs of 120 mM PC/CTAB (1:1 mol:mol), (c) UQ-$0^{\bullet -}$ in a N_2-saturated solution with no lipids and (d) UQ-$0^{\bullet -}$ in a N_2-saturated solution containing 120 mM PC/CTAB (1:1 mol:mol). Spectra with dotted lines are computer simulations.

more negative ΔG^0_1 values, as those detected for hydrophobic semiquinones in negatively charged membranes, could imply a more negative $E(Q/Q^{\bullet -})$ value. A similar explanation was given for the observed increase in the oxidation rates of *n*-alkyl-1,4-dihydronicotinamides by the lipophilic quinone, avarone, in the presence of CTAB micelles, *i.e.* semiquinone stabilization due to interaction of this species with CTAB.[28] In fact, comparison of the electrochemical reduction of avarone in the presence of CTAB micelles with the same process in a homogeneous water–ethanol mixture shows an anodic shift of the quinone one-electron reduction potential in the presence of CTAB which evidences the role of CTAB positive charge in stabilizing the avarone semiquinone and, thus increasing the quinone redox potential.[29]

Since ascorbate is a negatively charged reducing agent and, thus, it is attracted to the DMPC/CTAB interface, we also used DTT as the reducing agent. The pK_a value of this reducing agent is 10.1,[30] *i.e.* four orders of magnitude less acidic than ascorbate. Therefore, the fraction of anionic reducing agent in this case is negligible at physiological pH. However, essentially the same membrane charge effects as those observed in the case of ascorbate were also detected with DTT when used as a reducing agent for PHQ in air-saturated solution, Fig. 5. Similar effects were previously observed in the reduction of isoalloxazines by 1,4-butanedithiol, *i.e.* reaction rates were enhanced in the presence of CTAB micelles and decreased in the presence of anionic and non-anionic micelles, even though the pK_a of 1,4-butanedithiol is 10.7.[31] The rate of oxidation of a 1,4-butanedithiol by flavin measured in that work was found to be enhanced by 18-fold in the presence of CTAB.[31] This fact was explained as an "unusual" activation of thiolate anions derived from the formation of a "hydrophobic ion pair" between the surface cation and the thiolate anions, even though the thiolate concentration in the aqueous phase at pH 7.4 should be 1/2000 of that of the protonated dithiol. Thus, the possible enhanced stabilization of flavin semiquinones after interacting with CTAB should also be considered as an explanation for the CTAB effect on flavin reduction/dithiol oxidation as we propose for the hydrophobic semiquinones under study here in the presence of DMPC/CTAB. *However, since absolute reactivities of the thiolate and thiol of DTT are not known in this system, it may still be possible that the pK_a of the dithiol is*

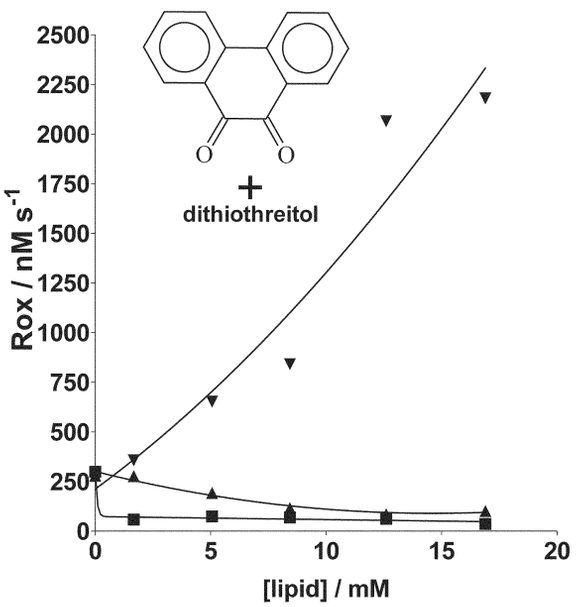


Fig. 5 R_{ox} dependence on LUVs electrical charge and concentration. Samples contain 6 μM PHQ, 1.0 mM dithiothreitol, 50 mM phosphate buffer (pH 7.4) and equimolar amounts of DMPC with CTAB (▼), DMPA (▲) and no other type of lipid (■).

depressed at the interface and, thus, the thiolate species is reacting, despite its low concentration in the aqueous phase, in a similar manner as ascorbate.

Very small rates of oxygen consumption were detected in our work when mixing 6 uM of either NQ or UQ-0 with DTT in the absence of lipids and, thus, a study on the reactivity of DTT with UQ-0 and NQ in the presence of lipids was omitted. This difference in behavior could be ascribed to additional retarding paths in the consumption of reducing equivalents from DTT due to the possibility of conjugates formation between DTT and both NQ and UQ-0, but not with PHQ, in a similar fashion as reported for reactions between naphthoquinone and benzoquinone derivatives with glutathione and other thiols.[32–34] In fact, PHQ catalyzed the most rapid oxidation of dihydrolipoamide among several substituted and non-substituted quinones.[34]

In summary, the quinone-mediated ascorbate oxidation rate, in the case of the hydrophobic quinones PHQ and NQ, is affected by the presence of charged or neutral phospholipid membranes. No effect on this rate is noticed if the hydrophilic quinone, UQ-0, is present in the same environments. Essentially, the same types of effects are observed for the PHQ-mediated DTT oxidation rate.

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