Synthesis, redox and electrochemical properties of new anthraquinone-attached micelle- and vesicle-forming cationic amphiphiles

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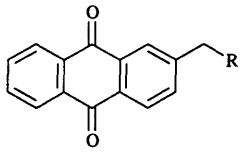
Three new cationic amphiphiles bearing anthraquinone moieties at the polar headgroup region were synthesized. The single-chain amphiphile, *N,N*-dimethyl-*N*-octadecyl-*N*-(9,10-dihydro-9,10-dioxoanthracen-2-ylmethyl)ammonium bromide 1, in the presence of cetyltrimethylammonium bromide upon dispersion in water gave co-micellar aggregates containing covalently attached anthraquinone residues at the polar aqueous interfaces. The other two double-chain amphiphiles, *N,N*-dioctadecyl-*N*-methyl-*N*-(9,10-dihydro-9,10-dioxoanthracen-2-ylmethyl)ammonium bromide 2 and *N,N*-dimethyl-*N*-(1,2-bispalmitoyloxypropanyl)-*N*-(9,10-dihydro-9,10-dioxanthracen-2-ylmethyl)ammonium bromide 3, however, on dispersion in aqueous media produced vesicular aggregates. The critical temperatures for the gel to liquid-crystalline-like phase transition processes for the vesicular systems were determined by following temperature-dependent changes in the ratios of keto–enol tautomeric forms of benzoylacetanilide doped within respective vesicular assemblies. The redox chemistry of the these supramolecular assemblies was also studied by following the time-dependent changes in the UV–VIS absorption spectroscopy in the presence of exogenous reducing or oxidizing agents. Electrochemical studies using glassy carbon electrodes reveal that redox-active amphiphiles adsorb on to the glassy carbon surfaces to form electroactive deposits when dipped into aqueous suspensions of either of these aggregates irrespective of the micellar or vesicular nature of the dispersions.

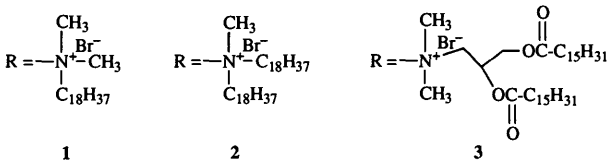
Introduction

Transduction and storage of energy in living systems involve biological redox reactions across bilayer membranes.[1] Electrochemical gradients across mitochondrial or thylakoid membranes are maintained by the transport of electrons and protons through the membrane bilayer *via* naturally occurring ubiquinones.[2] The electron-transfer steps in these ensembles are catalysed by membrane protein molecules, which also provide the required selectivity and asymmetric organization. However, the primary role of the membrane is to act as a device for energy-transduction by providing a control over to passive ion permeation leading to the generation of osmotic and ionic gradients.[3] Based on these considerations, several supramolecularly assembled, biomimetic, electroactive systems have been developed.[4–11]

Comparisons of the electroactive systems attached to organized assemblies that differ in terms of dynamics, supramolecular morphology and specific electrostatic features could be useful in the development of different matrices having practical ramifications. In this context, the elucidation of viable approaches to the generation of advanced electrochemical materials would be of practical utility. The potential targets include the generation of membranes that are capable of vectorial transmembrane charge separation for phototransduction or photostorage or even for molecular nanoelectronics.[12] Mechanistic requirements for these applications, however, differ widely.[13] Consequently, these require design and syntheses of different supramolecular, electroactive systems and their detailed characterization.

Due to our interest in supramolecular aggregates,[14] we became interested in the development of structurally simple, chemically stable, electroactive, amphiphilic molecular assemblies that contain *covalently attached* anthraquinone units at the level of the polar headgroups. Supramolecular assemblies of this kind attempt to model biological systems where the electron transfer processes of quinone or hydroquinone units play pertinent roles. Leidner and coworkers have reported[15] the synthesis of quinone functionalized phospholipid systems. Recently, a complete account of these studies has also been published.[16] Briefly, these lipids were *zwitterionic* in nature and required elaborate synthetic efforts. Vesicle formation from such systems needed prolonged sonication time. Perhaps due to the zwitterionic nature of the surface charge and bulky headgroup size, vesicular suspensions from these systems were also found to be turbid.[16] However, the corresponding *micellar* analogues and *cationic* vesicle forming counterparts were not synthesized and consequently comparative studies of quinone-bound micellar and vesicular systems were therefore not available. This point is of importance as reactivities at the micellar and vesicular interfaces are often not similar.[17] Micelles have high curvature and possess only one exposed, polar interface with bulk water. In contrast, vesicular assemblies contain inner and outer aqueous compartments providing scope for differential reactivities at the outer (exovesicular) and inner (endovesicular) surfaces.[18] Moreover, the inner and outer surfaces differ in curvature within the same vesicle. We thought that access to both micelle- and vesicle-forming anthraquinone-bound systems to a headgroup of similar charge types would be useful. These would allow the examination of the properties of newly developed systems under both micellar and vesicular conditions in a common electrostatic environment. In this paper, we report the synthesis of three new anthraquinone-bearing surfactants **1–3**, their micelle- and vesicle-forming behaviour and examination of their redox and electrochemical properties. The vesicular systems were also characterized in terms of their thermotropic phase-transition behaviour. Note that these two vesicle-forming systems **2** and **3** differ in terms of their molecular architecture. Thus, while **2** contains dialkylammonium ion units, **3** was constructed from a pseudoglyceryl backbone and therefore is architecturally closer to naturally occurring glycerolipid molecules.





1 2 3

Results and discussion

Synthesis

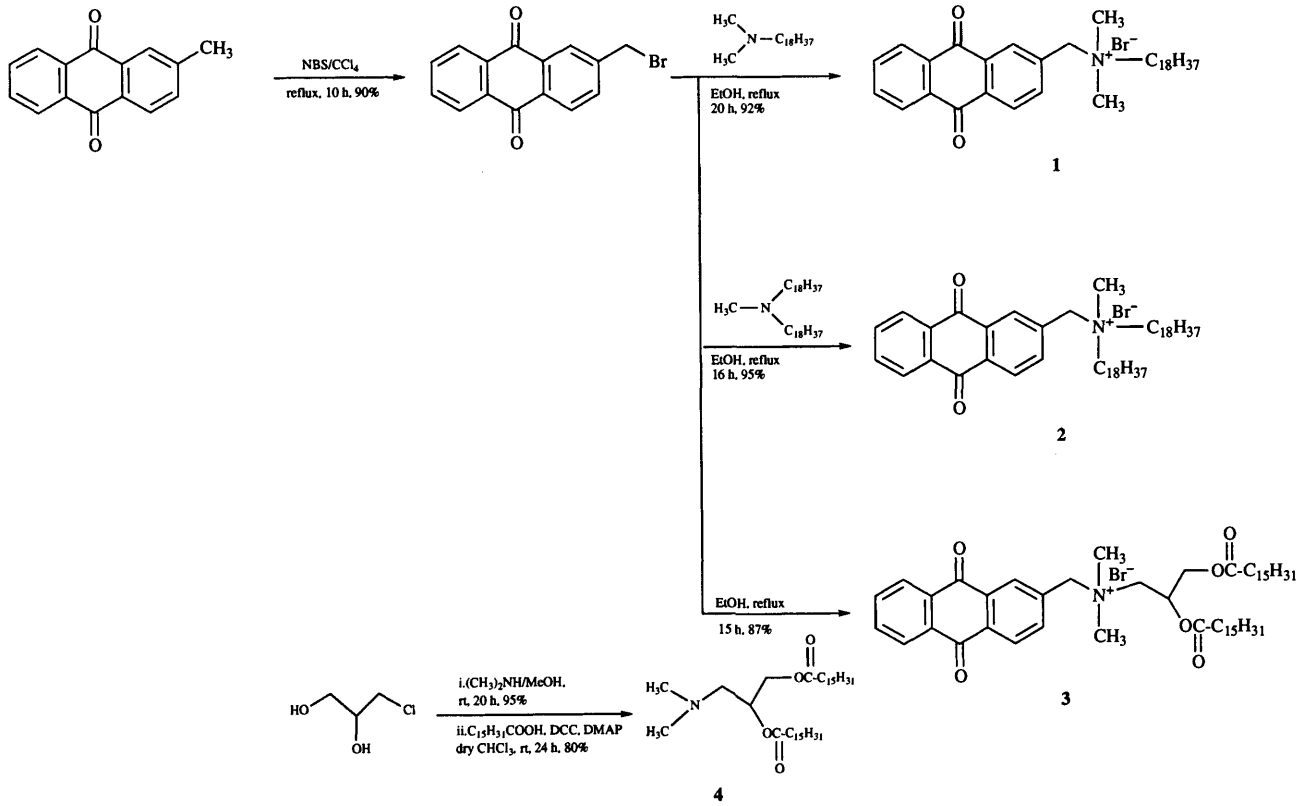
2-Methylanthraquinone was refluxed with an equimolar quantity of *N*-bromosuccinimide in anhydrous CCl_4 in the presence of a catalytic amount of benzoyl peroxide. This afforded 2-bromomethylanthraquinone in *ca.* 90% yield after chromatographic purification on a silica gel column using $CHCl_3$–MeOH (95:5) as eluent. Then, the single-chain anthraquinone amphiphile **1** was prepared by refluxing equimolar quantities of *N*-octadecyl-*N*,*N*-dimethylamine and 2-bromomethyl-9,10-anthraquinone in absolute ethanol for 20 h. The crude yellow solid thus obtained on evaporation of the solvent was purified by column chromatography over silica gel. This gave a pale-yellow, crystalline solid, **1**, in *ca.* 92% yield. Similarly 2-(bromomethyl)-9,10-anthraquinone upon refluxing with *N*-methyl-*N*,*N*-dioctadecylamine in dry EtOH yielded the amphiphilic anthraquinone **2** (*ca.* 95% yield) as a yellow, crystalline solid after column chromatography over silica gel. The other anthraquinone amphiphile **3** could be synthesized in the following steps. 3-Chloropropane-1,2-diol was first converted into the corresponding 3-*N*,*N*-dimethylamino-propane-1,2-diol by treatment with dimethylamine in dry methanol essentially following a published procedure.[19] The resulting 3-*N*,*N*-dimethylaminopropane-1,2-diol derivative was then acylated with two equivalents of palmitic acid in the presence of DCC and DMAP in dry $CHCl_3$. This gave 3-*N*,*N*-dimethylamino-1,2-bis(palmitoyloxy)propane **4** as a crystalline solid in *ca.* 80% yield. Quaternization of the amine **4** with 2-bromomethylanthraquinone in dry ethanol under refluxing condition (15 h) afforded *N*,*N*-dimethyl-*N*-(1,2-bispalmitoyl-oxypropanyl)-*N*-(9,10-dihydro-9,10-dioxoanthracen-2-yl-methyl)ammonium bromide **3** after purification (column chromatography) in *ca.* 87% yield as a pale-yellow, crystalline solid. All the new intermediates and final products were characterized by IR, ^1H NMR spectroscopy and elemental analyses. The overall synthetic sequences are summarized in Scheme 1.

Micelle and vesicle formation

The newly prepared anthraquinone amphiphiles readily formed translucent dispersions on sonication in water or in buffered aqueous media using an immersion probe [108 × 3 mm (diameter)] ultrasonic processor (Heat Systems) for few minutes at 25 W. All the micellar and vesicular solutions also appeared by light scattering at 400 nm to be size-stable for several hours. For specific details of their preparative protocol, see Experimental section.

Thermotropic phase-transition behaviour of the vesicular assemblies

As the properties of the vesicles could be modulated by variation of temperature, we first decided to characterize individual vesicular preparations by determining their critical vesicular phase-transition temperatures. The critical temperature (T_m) associated with the gel to liquid-crystalline phase transition process of holovesiclar **2** or **3** were individually determined by following the temperature-dependent keto–enol tautomerism[20] of different vesicle-doped benzoylacetanilide (BAA) samples. In this study, the temperature-dependent changes in the relative ratios of keto and enol tautomeric forms



Scheme 1

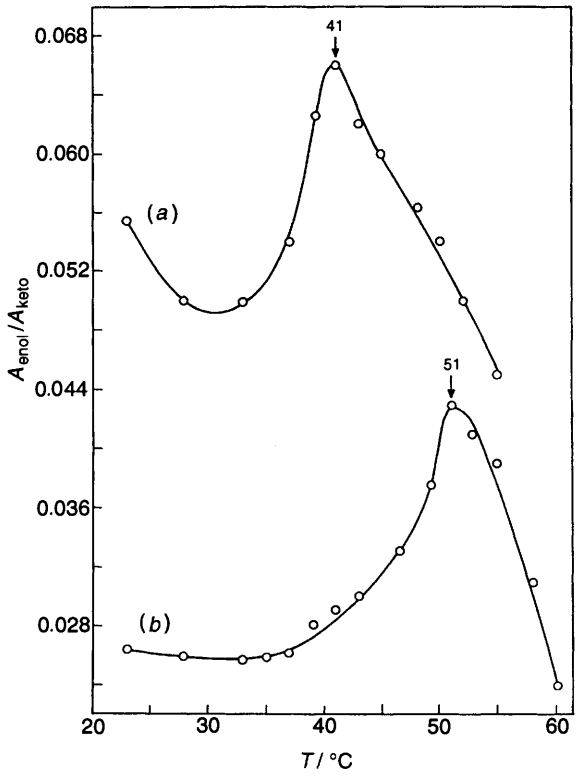


Fig. 1 (*a*) Plot of A_{enol}/A_{keto} due to vesicle-doped BAA *vs.* *T*/°C for **2** (5×10^{-4} M); (*b*) plot of A_{enol}/A_{keto} due to vesicle-doped BAA *vs.* *T*/°C for **3** (5×10^{-4} M)

of BAA was employed to measure T_m values. Since gel-like suspensions at high concentration, as required for differential scanning calorimetric measurement, from amphiphilic–anthraquinones **2** and **3** were not satisfactory, we have chosen the UV absorbance method[20] with BAA as a probe to measure the T_m values. In particular, we found that this method requires quite a low concentration of lipids or surfactants and thus allows the determinations of the phase-transition processes of different vesicular samples conveniently. Recently, we also found that the T_m values measured by this procedure agree quite well with those obtained by differential scanning calorimetric methods for a number of cationic lipids that contain aromatic units at different depths of the hydrophobic segments.[21] In comparison to other known methods for T_m determination, the present method is simple and does not require sophisticated instrumentation making it useful.

Typically in an aqueous ethanolic solution (10% EtOH, v/v), BAA was found to show only one absorption maximum at *ca.* 250 nm due to the presence of the *keto* form of the tautomers. In contrast however, in the liposomal suspension, absorptions due to both the *keto* (*ca.* 250 nm) and the *enol* forms (*ca.* 315 nm) of BAA could be seen. Thus, it appears that upon incorporation of BAA molecules into the vesicular bilayers, the keto forms tautomerize into enol forms. In other words, when BAA is introduced into the liposomal matrix, the keto form prefers to be in the bulk water while the enol form tends to remain within the vesicular mesophases. Since the bilayer packing loosens as the temperature of the vesicular system is increased, the ratio of the enol to keto absorbance (A_{enol}/A_{keto}) changes directly with temperature. Thus, when the temperatures (T) of vesicular samples were gradually increased from the ambient temperature at which the vesicles remained in a 'rigid' gel-like state either for vesicular **2** or **3**, the absorption intensity of the enolic form kept increasing with the simultaneous loss in the absorbance of the keto form. This monotonic rise in A_{enol}/A_{keto} as a function of *T* continues for both the vesicular **2** and **3** until at a particular temperature, the A_{enol}/A_{keto} ratio, instead of increasing further, started *decreasing* with increase in the temperature. The maximum temperature at which such a break is seen is taken as the apparent phase transition temperature for the system under examination. At temperatures *higher* than the phase transition of vesicles, the all-*trans* chain conformations of the lipids undergo rotational isomerization leading to kink formation and the BAA molecules under such situation exit from the bilayer matrix and are released into the bulk water leading to a continuous decrease in the A_{enol}/A_{keto} value at $T > T_m$. Representative plots of the ratio of the enolic to keto absorbance (A_{enol}/A_{keto}) with temperature showed maxima corresponding to the T_m values for that liposomal **2** and **3** (Fig. 1). Vesicular dispersions of amphiphile **2** gave a peak at *ca.* 41 (± 1) °C when A_{enol}/A_{keto} is plotted against the temperature [Fig. 1(*a*)]. The corresponding value found with liposomal **3** was *ca.* 51 (± 1) °C [Fig. 1(*b*)].

Redox chemistry at the micellar and vesicular surfaces

Anthraquinone-functionalized surfactant aggregates (micelles or vesicles) could be prepared by brief sonication of the aqueous dispersions as described previously. The anthraquinone bound at the surfaces of these supramolecular aggregates could be reduced readily by exogenous dithionite ($S_2O_4^{2-}$) or borohydride (BH_4^-) reagents. The reduced forms of these systems could be reoxidized by addition of $Fe(CN)_6^-$ or by exposure to aerial oxygen.

Fig. 2(*a*) illustrates the UV–VIS spectral response following the addition of a ten-fold molar excess over **1** (final concentration) of aqueous $S_2O_4^{2-}$ to a co-micellar [**1**, (5.9×10^{-5} M)/CTABr, (5.9×10^{-4} M)] solution buffered at pH 7.8 (phosphate) at 25 °C. The anthraquinone peak [λ_{max} *ca.* 328 (± 1) nm] decreased as the corresponding reduced hydroquinone peak [λ_{max} *ca.* 389 (± 1) nm] increased upon addition of $S_2O_4^{2-}$ until *quantitative* reduction of the fully exposed anthraquinone moieties at the micellar interfaces was achieved [Fig. 2(*a*)]. Importantly, the absorption maxima for the micelle-bound anthraquinones and the corresponding hydroquinones were in near agreement with that for *homogeneous* methanolic solutions of anthraquinone (λ_{max} *ca.* 325 nm) and anthrahydroquinone (λ_{max} *ca.* 382 nm).[22] The reduction of anthraquinones bound at the co-micellar surfaces followed an apparent first-order kinetic process (correlation coefficient *ca.* 0.999) with a half-life of *ca.* 52 s at 25 °C. A representative time-course of such a micellar reduction process is shown in Fig. 3 (curve 1). Thus, it is clear that the co-micellar **1**/CTABr could be reduced by $S_2O_4^{2-}$ in a *single*, quantitative, *kinetically monoexponential* process. Addition of ferricyanide or brief exposure to ambient oxygen readily regenerated the anthraquinone maxima (λ_{max} *ca.* 328 nm) (figure not shown) with concomitant disappearance of the anthrahydroquinone signatures.

Attempts to prepare either holovesicular **2** or **3** or covesicular dispersions with dioctadecyldimethylammonium bromide (DODAB) at the same pH using a phosphate buffer solution, however, did not produce samples of satisfactory optical stability to allow for spectrophotometric studies. For this reason, covesicles of **2** were generated in a Tris-buffered solution at pH 7.8, using DODAB as a host surfactant. Notably, these vesicular preparations remained size-stable for several hours and provided sufficient optical stability to allow them to be examined by UV–VIS absorption spectroscopy. Addition of a ten-fold molar excess (final concentration) of the dithionite (with respect to **2**) to the covesicular **2** (5.8×10^{-5} M)/DODAB (5.8×10^{-4} M) in Tris-buffered aqueous media (pH 7.8, 0.01 M KCl) at 25 °C (*i.e.* below the phase-transition temperature, *ca.* 40 °C) resulted in a rapid but incomplete reduction of anthraquinone moieties (λ_{max} *ca.* 332 nm) (four trials). Representative spectra for this experiment are given in Fig. 2(*b*). Fig. 2(*c*) shows the reaction of a ten-fold molar excess (final concentration with respect to **3**) of aqueous dithionite to a

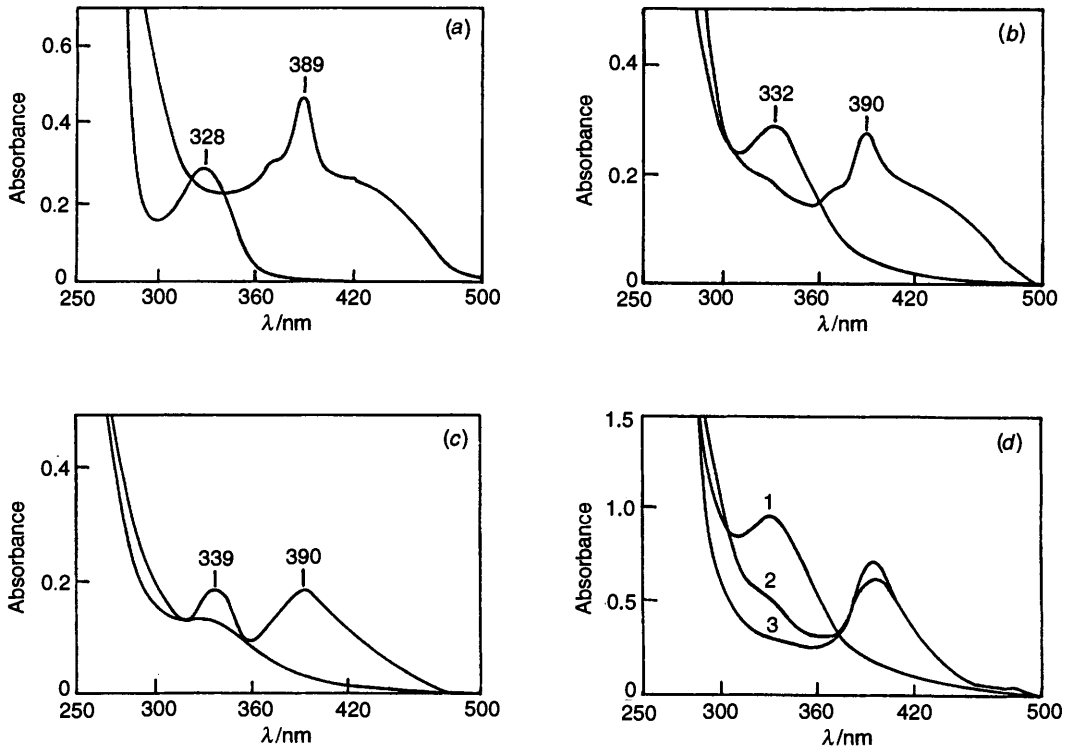


Fig. 2 UV–VIS spectrophotometric response of amphiphilic anthraquinones **1**–**3** upon addition of external $S_2O_4^{2-}$ or BH_4^-. (*a*) Absorbance of micellar solution of amphiphile **1** (5.9×10^{-5} M) and CTABr (5.9×10^{-4} M) in phosphate buffer pH 7.8 before addition (328 nm) and after addition (389 nm) of $S_2O_4^{2-}$ (final conc. 5.9×10^{-4} M) at 25 °C. (*b*) Absorbance of vesicular solution of amphiphile, **2** (5.8×10^{-5} M) and DODAB (5.8×10^{-4} M) in Tris buffer pH 8 (0.05 M) before addition (332 nm) and after addition (390 nm) of $S_2O_4^{2-}$ (final conc. 5.8×10^{-4} M) at 25 °C. (*c*) Absorbance of amphiphile **3** (4×10^{-5} M) in Tris buffer pH 8 before addition (339 nm) and after addition (390 nm) of $S_2O_4^{2-}$ (final conc. 4×10^{-4} M) at 25 °C. (*d*) Absorbance of amphiphile **2** (1.94×10^{-4} M) and DODAB (1.9×10^{-3} M) in Tris buffer pH 8, trace 1, before addition; trace 2, after addition of $NaBH_4$ (final conc. 1.9×10^{-3} M) both at 25 °C and upon heating the same reaction mixture to > 52 °C (trace 3).

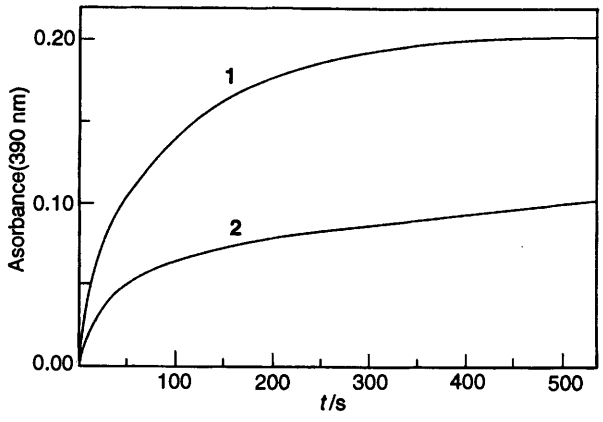


Fig. 3 Absorbance at 390 nm *vs.* *t*/s for reduction of anthraquinone moieties bound to co-micellar **1**/CTABr (2.5×10^{-5} M/2.5×10^{-4} M) (curve 1) and for the reduction of accessible anthraquinone from covesicular **2**/DODAB (2×10^{-5} M/2×10^{-4} M) (curve 2) at bulk aqueous (external) pH of 7.8 following addition of ten-fold molar excess of $S_2O_4^{2-}$ in each case. All the anthraquinone surfactant concentrations were confirmed from their extinction coefficients obtained and time-course studies were initiated after appropriate baseline corrections.

holovesicular **3** (4×10^{-5} M) solution buffered at pH 8 (Tris) at 25 °C. The anthraquinone peak due to vesicular **3** at λ_{max} *ca.* 339 nm decreased as the corresponding reduced anthrahydroquinone units were produced with a maximum at *ca.* 390 nm as was observed in the previous instance. Thus, below the phase-transition temperature, only a partial reduction could be seen (five trials). Note, however, when either of these vesicular reactions were carried out in the presence of 1×10^{-3} M Triton-x-100, the reduction went to virtual completion (98–99%).

Careful examination of either of these time-courses reveals that reduction processes on vesicular systems were not monophasic. A representative time-course plot is given in curve 2 of Fig. 3, for the reduction of covesicular **2** (2×10^{-5} M)/DODAB (2×10^{-4} M) solution in the presence of 2×10^{-4} M dithionite. As is obvious from the plot, the kinetics in vesicles are biphasic. This became apparent when the points of this time-course were fitted either with a single-exponential or with a double-exponential analysis. The single-exponential fit was found to be quite poor, clearly indicating that curve 2 followed a double-exponential process. Taking these factors into account, the following explanation appears likely: (*a*) the kinetics in vesicular systems are biphasic; (*b*) the first, fast process accounts for the reduction of the exovesicular layer exposed to the reagents in bulk aqueous solution and according to the above-mentioned interpolation, this amounts to *ca.* 36% of the total; (*c*) the slow process, which is already detectable in curve 2, accounts for the remaining *ca.* 64% of the substrates confined within endovesicular sites. Since the vesicles were shown to be multilamellar on the basis of the transmission electron microscopic (TEM) results, the outer layer exposed to the dithionite in bulk aqueous solution should account for less than 50% of the total theoretical absorbance. The above conclusions are further supported by the fact that when the reaction involving vesicular systems were done in the presence of a ten-fold molar excess of dithionite and in the presence of Triton-x-100, quantitative reductions were seen. Similar examination of the time-course (not shown) of the reduction of vesicular **3** revealed the biphasic nature of the kinetics also. Based on a double exponential fit, the first, fast kinetic phase accounted for *ca.* 38% and the residual *ca.* 62% of anthraquinones is reduced at a much lower rate. We believe that the slower reduction of the anthraquinones at the inner 'protected' vesicular sites originates presumably from flipping rather than permeation of the dithionite (see below).

The half-lives for the exovesicular kinetic phases with either of

the above cited instances involving covesicular **2**/DODAB or holovesicular **3** were also interpolated and the $t_{\frac{1}{2}}$ values were found to be 16.5 and 17 s, respectively. When compared with the half-lives for the dithionite-mediated reduction on corresponding micellar systems, these rates are faster over the same on micelles by a factor of three. This could be due to the use of different buffers in micellar conditions as opposed to vesicular reactions. Although present findings are new, manifestations of biphasic kinetics in vesicular reactions are known in other systems also.[18,23] $S_2O_4^{2-}$ ions do not appear to permeate freely through transmembrane barriers composed of either **2**/DODAB or **3** bilayers. Hence, the reduction of the quinone moieties could not take place monoexponentially at 25 °C on vesicular matrices. This temperature is well below the phase-transition temperature for either of these vesicular systems. Below the phase-transition temperatures, a similar lack of $S_2O_4^{2-}$ permeation across vesicular bilayers has also been observed by other groups during their investigations with $S_2O_4^{2-}$-induced reduction of the encapsulated Ellman's reagent with the DODAB[24,25] vesicular bilayers. Interestingly the residual unreacted anthraquinones (*ca.* 64% for **2**/DODAB and *ca.* 62% for **3**) confined at the *inner* vesicular positions could also be eventually reduced although at a much lower rate below their phase-transition temperatures. Unfortunately, however, the slower kinetic phases of the reduction process with either of the vesicular systems could not be followed with adequate precision. This was due to attendant increases in the optical scattering of the vesicular reaction mixtures and consequent phase separation. Presumably, the increase in turbidity could be a consequence of an inter-vesicular fusion[26] process promoted by $S_2O_4^{2-}$ ions. Fusogenic properties of other anions of similar structure *e.g.* SO_4^{2-} towards cationic DODAB vesicles have been reported by Fendler *et al.*[27] Attempts to estimate the rate of the reduction process at the temperatures above the phase-transition temperature (41 °C) resulted in a phase separation.

With anthraquinone functionalized, *zwitterionic phosphatidylcholine* systems, however, Leidner and co-workers found[16] that $S_2O_4^{2-}$ ions were able to permeate vesicular bilayers making the reduction of vesicular anthraquinones quantitative. This result is in disagreement with the presently described vesicular systems. We believe that *anionic* $S_2O_4^{2-}$ ions most likely form tight ion-pairs with the *cationic* vesicular systems examined herein. Under such circumstances, it becomes difficult for $S_2O_4^{2-}$ ions to cross the transmembrane gradients imposed by covesicular **2**/DODAB or holovesicular **3**. Similar inabilities of $S_2O_4^{2-}$ ions to permeate vesicular bilayers below the phase-transition temperatures have also been reported by others during their studies with $S_2O_4^{2-}$-promoted reduction of cationic vesicle entrapped Ellman's reagents.[24,25]

The transparent optical nature of BH_4^- prompted us to employ it as a reducing agent for examination of reactivities of the covalently bound vesicular anthraquinone systems. Unfortunately, the use of BH_4^- for monitoring of such processes by optical spectroscopy was rendered ineffective for several reasons. At *ca.* pH 8, a solution of BH_4^- was found not to be very stable especially at elevated temperature; the situation was aggravated by steady production of bubbles in the reaction mixture. Such inherent instability precluded a precise determination of the time-course of BH_4^--mediated reduction processes of vesicular anthraquinone systems. After several trials, we found that a formulation containing *ca.* 2×10^{-3} M BH_4^- was reasonably stable to allow the examination of the anthrahydroquinone formation from covesicular **2**/DODAB at 25 °C and pH 8 by spectrophotometry.

Fig. 2(*d*) shows the reduction of **2**/DODAB covesicular solution by UV-transparent BH_4^-. Trace 1 in Fig. 2(*d*) shows the UV–VIS spectrum of **2**/DODAB [**2** (1.9×10^{-4} M)/DODAB (1.9×10^{-3} M)] covesicular solution at 25 °C. Addition of BH_4^- (final concentration: 1.9×10^{-3} M) to this covesicular solution resulted in the spectrum 2, as shown in Fig. 2(*d*), where *ca.* 35 ± 2% of the anthraquinones (λ_{max} *ca.* 330 nm) got reduced to their corresponding anthrahydroquinones (λ_{max} *ca.* 391 nm). Heating this sample above the phase-transition temperature, *i.e.* > 52 °C, resulted in further *ca.* 20% reduction of anthraquinones [spectrum 3, Fig. 2(*d*)]. Since different bilayer membranes[15,16] have been shown to be practically impermeable to BH_4^- ions the additional reduction achieved upon heating could be presumably brought about by 'flipping' of anthraquinones that are covalently bound to inner endovesicular multilamellar membrane surfaces rather than by permeation of BH_4^-. Kinetic examination after prolonged exposure of BH_4^- to covesicular **2**/DODAB at > 52 °C was not successful due to rapid decomposition of BH_4^- in the cuvette and consequent bubble formation. Nevertheless, BH_4^- serves as a useful reactant for differentiation of anthraquinone units that lie at the exovesicular surface as opposed to the ones confined at the inner positions. Similar behaviour was observed with anthraquinone-attached dipalmitoylphosphatidyl choline liposomal systems also.[16]

A primary approach to studies of ubiquinone function has been the comparative evaluation of functional and structural properties of differently localized quinones of variable prenyl chain lengths. From the combined UV–VIS spectroscopic data on the extent and apparent kinetic information available with the present set of systems it may be possible to put our results into perspective. It appears that anthraquinones bound at the polar interfaces (with a short spacer in between) simulate readily reducible, short-chain ubiquinones such as UQ_2 or UQ_3 which are also located sufficiently close to the outer surface.[2c] We are now planning to design new anthraquinone-attached lipids with variable spacer chain length between the anthraquinone unit and the charged head group to allow looping back of the anthraquinone moieties at various depths of the membrane interior.

Electrochemistry of micellar and vesicular anthraquinones

We were interested in studying the electrochemical behaviour of the newly synthesized quinone-functionalized surfactant aggregates in water on appropriate electrode surfaces. In order to obtain such information, CV experiments employing anthraquinone-bearing surfactant aggregates were carried out. In CV, the potential of a stationary working electrode is linearly changed with time starting from a potential where no reaction takes place at the electrode and changing to potentials where reduction or oxidation reaction of a redox-active system occurs.[28] After following the potential region in which electrode reactions take place, the direction of the linear sweep is reversed and even the reaction involving the intermediates and products generated at the electrode surfaces during the forward scan can often be detected.

The CVs of the co-micellar solution of amphiphilic anthraquinone **1** with CTABr (30 mol% of **1**) were recorded in aqueous 0.1 M KCl solution. The CVs were measured by dipping the glassy carbon electrode into the corresponding optically translucent co-micellar solution at ambient temperature (*ca.* 25 °C) at different scan rates. The CV response of a vesicular **2**/DODAB suspension (30 mol% **2**) at a glassy carbon electrode shows the anthraquinone/hydroquinone couple of *ca.* −0.6V *vs.* standard calomel electrode (SCE) (figure not shown). The covesicular **3**/DODAB (30 mol% **3**) dispersion in aqueous 0.1 M KCl solution shows a reduction peak and the corresponding oxidation spike upon scan reversal. The CVs were obtained with both the covesicular **2**/DODAB and **3**/DODAB solutions at increasing scan rates (50, 100, 200 and 300 mV s^{-1}) above the respective phase-transition temperatures. Below their phase transition in the rigid gel states, the CVs were irreproducible. The nature of the voltammetric waves in all the three cases and their dependence on scan rates suggest a possible involvement of an electrocatalytic step. It was also

noticed that the voltammetric waves at *ca.* −0.6 and *ca.* −0.7 V, respectively, persist even after the electrode is rinsed and placed in an aqueous 0.1 M KCl solution devoid of either of the vesicles (**2**/DODAB and **3**/DODAB) (figure not shown). A similar phenomenon was also observed with anthraquinone-functionalized phosphatidylcholine liposomes.[15] The surface coverages of the anthraquinone amphiphilic molecules on the glassy carbon electrode and detailed characterization of such electroactive deposits are in progress.

Experimental

General methods

Melting points were measured using open capillary tubes and are uncorrected. IR spectra were recorded using a Perkin-Elmer 781 IR spectrophotometer. ^{1}H NMR spectra were obtained on either Bruker WH-270 (270 MHz) or Jeol FX-90Q (90 MHz) spectrometers. The chemical shifts for ^{1}H NMR spectra are reported in parts per million (ppm) relative to $SiMe_4$ as internal standard and *J* values are reported in Hz. Spectral splitting patterns are designated as s, singlet; d, doublet; dd, double doublet; d of d, doublet of doublet; t, triplet; m, multiplet and br, broad. Elemental analyses were performed by using a Carlo-Erba 1106 analyser. UV–VIS absorption spectra were recorded using a Shimadzu UV 2100 spectrophotometer equipped with thermoelectric temperature-controlled cell holder, TCC-260 and TCC-controller. Vesicles obtained from the amphiphilic-AQ were prepared by sonication method using immersion probe [108 × 3 mm (diameter)] sonicator at 25 W, model XL-2020, Heat systems, ultrasonic processor. Column chromatography was performed using silica gel of 60–120 mesh (Merck chemicals). CV was performed by using a BAS-100A electrochemical work station interfaced with a DMP-40 digital plotter.

Unless noted otherwise, all the starting materials were obtained from commercial suppliers and used without further purification. 2-Methylanthraquinone, palmitic acid, dicyclohexylcarbodiimide (DCC), 4-dimethylaminopyridine (DMAP): Aldrich, *N*-bromosuccinimide (NBS): Ranbaxy, benzoylacetanilide (BAA), dimethylamine: Merck. DODAB was prepared from stearyl bromide: Aldrich, following a reported method.[29] Solvents were freshly distilled prior to use.

Synthesis: preparation of 2-(bromomethyl)-9,10-anthraquinone

To a mixture of 2-methyl-9,10-anthraquinone (4.4 g, 0.02 mol) and NBS (3.56 g, 0.02 mol) in freshly distilled dry CCl_4 (50 ml) was added a catalytic amount of benzoyl peroxide (*ca.* 50 mg) and the mixture refluxed on a heated water bath for 10 h. The colourless succinimide produced floated on the surface of the solution and was filtered off. Solvent was removed under vacuum and the crude yellow solid thus obtained was purified by silica gel column chromatography using $CHCl_3$–MeOH (95:5) as eluent. Mp 198–200 °C (lit.,[30] mp 199–201 °C); yield, 5.4 g (90%).

N,N-Dimethyl-*N*-octadecyl-*N*-(9,10-dihydro-9,10-dioxo-anthracen-2-ylmethyl)ammonium bromide 1

To a solution of *N*-octadecyl-*N,N*-dimethylamine (297 mg, 1 mmol) in absolute ethanol (20 ml) was added 2-(bromomethyl)-9,10-anthraquinone (300 mg, 1 mmol). The reaction mixture was refluxed for 20 h. The solvent was evaporated and the crude solid thus obtained was purified by passing through a silica gel chromatographic column using $CHCl_3$–MeOH (95:5) as eluent. Pale-yellow crystalline solid of **1** was obtained in *ca.* 92% yield. Mp 199–201 °C. ν(neat)/cm^{-1} 1660 (quinone carbonyl), 1580, 1460, 1280. δ_H($CDCl_3$, 270 MHz) 0.87 (t, 3 H, *J* 6.8), 1.23 (br m, 26 H), 1.36 (crude t, 2 H), 1.78–1.88 (m, 4 H), 3.42 (s, 6 H), 3.62 (t, 2 H, *J* 8.7), 5.43 (s, 2 H), 7.77–7.80 (m, 2 H, Ar-H), 8.19–8.43 (m, 5 H, Ar-H) (Found: C, 70.20; H, 8.79; N, 2.26. $C_{35}H_{52}NO_2Br$ requires C, 70.21; H, 8.75; N, 2.33%).

N,N-Dioctadecyl-*N*-methyl-*N*-(9,10-dihydro-9,10-dioxo-anthracen-2-ylmethyl)ammonium bromide 2

A mixture of *N,N*-dioctadecyl-*N*-methylamine (270 mg, 0.5 mmol) and 2-(bromomethyl)-9,10-anthraquinone (150 mg, 0.5 mmol) in absolute ethanol (20 ml) was refluxed for 16 h. The solid obtained upon evaporation of the solvent and purification by silica gel column chromatography using $CHCl_3$–MeOH (95:5) gave **2** as a pale-yellow crystalline solid. Mp 138–140 °C. Yield, 375 mg (95%). ν(neat)/cm^{-1} 1670 (quinone carbonyl), 1580, 1460, 1320, 1280. δ_H($CDCl_3$, 270 MHz) 0.87 (t, 6 H, *J* 6.7), 1.25–1.37 (br m, 56 H), 1.80 (br m, 8 H), 3.28 (s, 3 H), 3.44 (t, 4 H, *J* 8.0), 5.30 (s, 2 H, Ar-CH_2), 7.80–7.81 (m, 2 H, Ar-H), 8.20–8.32 (m, 4 H, Ar-H), 8.43–8.46 (d, 1 H, Ar-H, *J* 8.1) (Found: C, 74.47; H, 10.57; N, 1.88. $C_{52}H_{86}NO_2Br$ requires C, 74.60; H, 10.35; N, 1.67%).

N-(1,2-Bispalmitoyloxypropanyl)-*N*-(9,10-dihydro-9,10-dioxo-anthracen-2-ylmethyl)-*N,N*-dimethyl ammonium bromide 3

3-(*N,N*-dimethylamino)propane-1,2-diol. A solution of 3-chloropropane-1,2-diol (1.5 g, 1.13 ml, 14 mmol) in dry methanol (total volume 35 ml) in a sealed tube was cooled at −5 °C. Dry dimethylamine gas was gently passed through the stirred methanolic solution until the volume of the resulting solution was raised to *ca.* 41 ml. The stirred solution was then kept at room temperature for 20 h. The solvent was removed under vacuum in a rotary evaporator at room temperature. The amine hydrochloride thus formed was treated with a stoichiometric amount of sodium ethoxide at 0 °C. This resulted in the precipitation of sodium chloride which was filtered off. Removal of the ethanol from the filtrate under vacuum gave a light yellowish, dense oily mass, 3-dimethylaminopropane-1,2-diol. Yield, 1.57 g (95%).[19] The spectroscopic properties (IR, ^{1}H NMR) of this material were in agreement with the literature.[19]

(±)-1,2-Bis(palmitoyloxy)-3-(dimethylamino)propane, 4. To a stirred solution of 3-dimethylaminopropane-1,2-diol (240 mg, 2 mmol), palmitic acid (1.07 g, 4.2 mmol) and DCC (865 mg, 4.2 mmol) in freshly distilled dry $CHCl_3$ (30 ml) was added DMAP (100 mg) at room temperature and the mixture stirred for 24 h. The dicyclohexylurea precipitated was filtered off and the solvent from the filtrate was removed under vacuum. The solid thus obtained was purified by passing through a silica gel column using $CHCl_3$–MeOH (95:5) as eluent. Evaporation of the solvent gave **4** as a colourless solid, mp 48–50 °C. Yield, 950 mg (*ca.* 80%). δ_H($CDCl_3$, 90 MHz) 0.88 (t, 6 H, *J* 8), 1.22 (br m, 48 H), 1.42–1.65 (m, 4 H), 2.28 (s, 10 H), 2.40–2.56 (d of d, 2 H, *J* 7.2 and 2.4), 4.1–4.3 (two dd, 2 H, *J* 7.2 and 3.5), 5.1–5.26 (m, 1 H).

***N*-(1,2-Bispalmitoyloxypropanyl)-*N*-(9,10-dihydro-9,10-dioxoanthracen-2-ylmethyl)-*N,N*-dimethylammonium bromide, 3.** To a solution of (±)-1,2-bispalmitoyloxy)-3-dimethylaminopropane, **4** (300 mg, 0.5 mmol) in absolute ethanol (20 ml), 2-(bromomethyl)-9,10-anthraquinone (150 mg, 0.5 mmol) was added and refluxed for 15 h. The solid obtained on evaporation was purified by passing through a silica gel column using $CHCl_3$–MeOH (94:6) as eluent. The quaternary anthraquinone salt **3** was obtained as a pale-yellow crystalline solid, mp 115–117 °C. Yield, 390 mg (87%). ν(neat)/cm^{-1}, 1730 (ester carbonyl), 1660 (quinone carbonyl), 1580, 1450, 1280. δ_H($CDCl_3$, 270 MHz) 0.87 (t, 6 H, *J* 6.7), 1.22 (br m, 44 H), 1.6–1.7 (m, 8 H), 2.28–2.37 (m, 4 H), 3.38–3.48 [2s, 6 H, N(CH_3)$_2$], 3.85–3.92 (d of d, 1 H, *J* 14.5 and 8), 4.1–4.2 (d of d, 1 H, *J* 13.2 and 5.5), 4.54 (m, 2 H), 5.32–5.43 (dd, 1 H, *J* 12), 5.75 (s, 2 H), 7.81 (d, 2 H, *J* 9.6 Hz), 8.22 (m, 2 H), 8.35 (s, 2 H), 8.42 (s, 1 H) (Found: C, 67.79; H,

9.12; N, 1.77. $C_{52}H_{82}NO_6Br \cdot 1.5H_2O$ requires C, 67.58; H, 9.27; N, 1.51%).

Redox measurements with aqueous aggregates

Preparation of aqueous dispersions. Optically clear micellar solution of the amphiphile **1** was prepared by mixing 0.1 mmol of **1** and 0.9 mmol of CTABr in a phosphate buffer (pH 7.8) using deionized water (millipore) followed by sonication of the mixture using an immersion probe sonifier (Heat systems) for 2–3 min at room temperature. In the case of double-chain quinone amphiphiles **2** and **3**, vesicles were prepared by dissolving an appropriate mixture of **2** or **3** with or without DODAB in ethanol (20 μl) and followed by their injection into separate aqueous solutions (10 ml) containing Tris buffer at pH 8. The dispersion was sonicated above the phase-transition temperature (*ca.* 60 °C) for 2–3 min to obtain clear vesicular samples.

Based on TEM studies (Jeol 200-CX, TEM instrument, 0.5% uranyl acetate), we found that vesicle sizes ranged from 1000–1200 Å in the case of covesicular **2**/DODAB. With holovesicular **3**, the vesicular sizes were larger and ranged from *ca.* 1450–1600 Å. It was also apparent that in either instance, the nature of the organization was predominantly multilamellar.

Redox reactions with surfactant aggregates: estimation of quinone reduction and the corresponding rates. The amphiphilic quinone-bearing organized assemblies can be reduced and reoxidized rapidly and reversibly by adding appropriate chemical reagents. The reducing agents sodium dithionite or sodium borohydride were separately used to convert anthraquinone to anthrahydroquinone. The concentrations of anthraquinone-attached surfactants in micellar or vesicular dispersions were calculated from their extinction coefficients, $\varepsilon = 5100$ dm^3 mol^{-1} cm^{-1} at *ca.* 328 nm for **1**, $\varepsilon = 4950$ dm^3 mol^{-1} cm^{-1} at *ca.* 332 nm for **2** and $\varepsilon = 5000$ dm^3 mol^{-1} cm^{-1} at *ca.* 339 nm for **3**. The time course of the reduction of the anthraquinone species attached at the micellar or vesicular media was followed in a Shimadzu UV-2100 spectrophotometer after appropriate baseline correction. The cuvettes in both of the sample and reference compartments were thermostatted at specified temperatures using a thermoelectric temperature controller (TCC-260). The reactions were initiated under nitrogen by rapid mixing (< 5 s) with a Hamilton syringe.

Phase-transition temperature (T_m)

The thermotropic phase-transition temperatures (T_m), for the transition from the 'frozen' gel-like state to the 'fluid' liquid-crystalline state, were determined by following the temperature-dependent keto–enol tautomerism of vesicle-doped benzoylacetanilide (BAA) following a literature procedure.[20,21]

Sample preparation for phase-transition studies. Typically either of the amphiphiles **2** or **3** (5×10^{-5} M) were first dissolved in separate ethanolic solution of BAA (2×10^{-3} M), 100 μl, and the resulting solutions were injected rapidly into 10 ml of warm deionized water kept in a beaker. The suspensions were then sonicated at *ca.* 60 °C using an immersion probe sonicator [108 × 3 mm (diameter)], model XL-2020, ultrasonic processor (Heat systems) for 2–3 min. Upon cooling, 1 ml of translucent liposomal samples were added to the UV sample cell; the reference cell contained a liposomal sample of identical concentration that did not contain any BAA. The temperature of the cuvette chamber was first allowed to equilibrate and then the UV absorption spectra were recorded in the range 200–500 nm at every given temperature. The intensities of the absorbances due to the keto and enol forms of BAA at 250 and 315 nm were measured at each temperature. Typically at an interval of every 2 °C, the UV–VIS spectra were recorded up to 65 °C. Plots of the ratio of enol to keto absorbance, A_{enol}/A_{keto}, with temperature gave the T_m for the specific liposomal system.

Electrochemical studies. Sample preparation

The clear micellar and vesicular solution of the amphiphilic anthraquinones **1**–**3** for electrochemical study were prepared as follows. Co-micellar solution of **1** was prepared by dissolving the requisite amount of the anthraquinone, **1** (0.3 mmol) in 10 μl of EtOH and the ethanolic solution was rapidly injected into a clear micellar solution of CTABr (0.7 mmol) prepared in 10 ml of 0.1 M KCl solution. The dispersion was then briefly sonicated using the immersion probe sonicator for 2–3 min. The co-micellar **1** thus obtained was used immediately for electrochemical experiments. The covesiculars of **2**/DODAB and **3**/DODAB were prepared as follows. Requisite quantities of **2** or **3** were separately weighed and dissolved in 10 μl EtOH and then injected into separate 10 ml vesicular solution of DODAB in a 0.1 M KCl solution (7×10^4 M) such that the final concentrations of **2** or **3** in the resulting covesicular solution were 3×10^{-4} M. The resulting mixture was then sonicated using probe sonifier for 5–6 min > 60 °C. This produced translucent liposomal solutions which were used immediately for electrochemical studies.

Cyclic voltammetry

All CVS were performed at specified temperatures using a three electrode configuration, composed of glassy carbon as the working electrode (glassy carbon of 9 mm^2 surface area), platinum wire as an auxiliary electrode and KCl SCE.

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