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Study of the interaction between oxygen and bile salts

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Abstract

The interaction between molecular oxygen and bile salts, previously observed using chemiluminescence techniques, is studied in this paper by electrochemical techniques to further highlight the nature of the interaction. A shift of half-wave potential of the first polarographic wave for the reduction of molecular oxygen was observed in solutions in the presence of bile salts. The shift could be related to different phenomena, such as adsorption of bile salt molecules on the mercury electrode, irreversibility of the oxygen reduction reaction, pH of the solution. Experimental results suggest the exclusion of the above mentioned processes and outline the occurrence of a direct interaction between oxygen and bile salts, where the hydrophobic face of bile salt monomers and/or small aggregates are involved, enhancing so dismutation of superoxide ion produced at the electrode. The presence of bile salts in solutions containing triphenylphosphine oxide, a hydrophobic surfactant, increases also the wave of reduction of molecular oxygen. As a consequence bile salts, beside the well-assessed physiological roles, can behave as oxygen carrier and as antioxidant, preventing the oxidation of biological compounds by superoxide ion.

Keywords: Oxygen; Bile salts; Polarography; Electro-generated superoxide ion; Antioxidant

1. Introduction

Bile acids are hydroxy steroids, biosynthesized from cholesterol in the liver. The molecule of a bile acid contains a steroid ring system and a branched short side chain. The chain terminates in a carboxyl group, which, in the physiological terms, can be conjugated with glycine or taurine through an amido moiety (Fig. 1F). Steroid rings carry hydroxyl groups and common bile acids differentiate for their number, position and orientation (Fig. 1A–E). In the ionized form (also widely reported with the term "bile salt", BS) they behave as physiological surfactants and form micelle-like aggregate above a narrow concentration range (critical micelle concentration, CMC). Their surface activity originates from a structural dissimilarity, because of the distribution of hydrophilic (hydroxy groups and the acidic side chain) and hydrophobic moieties in different faces of the steroid ring system. A hydrocarbon backbone builds up the so-called *beta face* that is unable to form H-bonds with water molecules, but represents a privileged site for the interaction with the hydrophobic portions of dissolved molecules [1]. For instance self-aggregation of BS above CMC involves the *beta face* [2].

Recently an unexpected role of bile salt towards the oxygen molecule was described by De Lange et al. [3]. They reported that glycocholic acid could behave as antioxidant or promoter of lipid peroxidation, according to the lipid substrate concentration. Afterwards other authors reported about ability of BS to scavenge superoxide anion generated by xanthine-xanthine oxidase [4], or their effects on the free radical generation, lipid peroxidation and the antioxidant defense system in the liver of rat [5], or as protective against oxidative injury induced by reactive oxygen species [6].

Moreover in previous papers [7,8], using a chemiluminescent technique, based on horse radish peroxidases and a luminol-oxidant enhancer reagent, we too observed that a representative series of free, glycine and taurine conjugated BS inhibit the steady state of a chemiluminescent reaction, where oxygen is involved. The extent of enhanced luminescence

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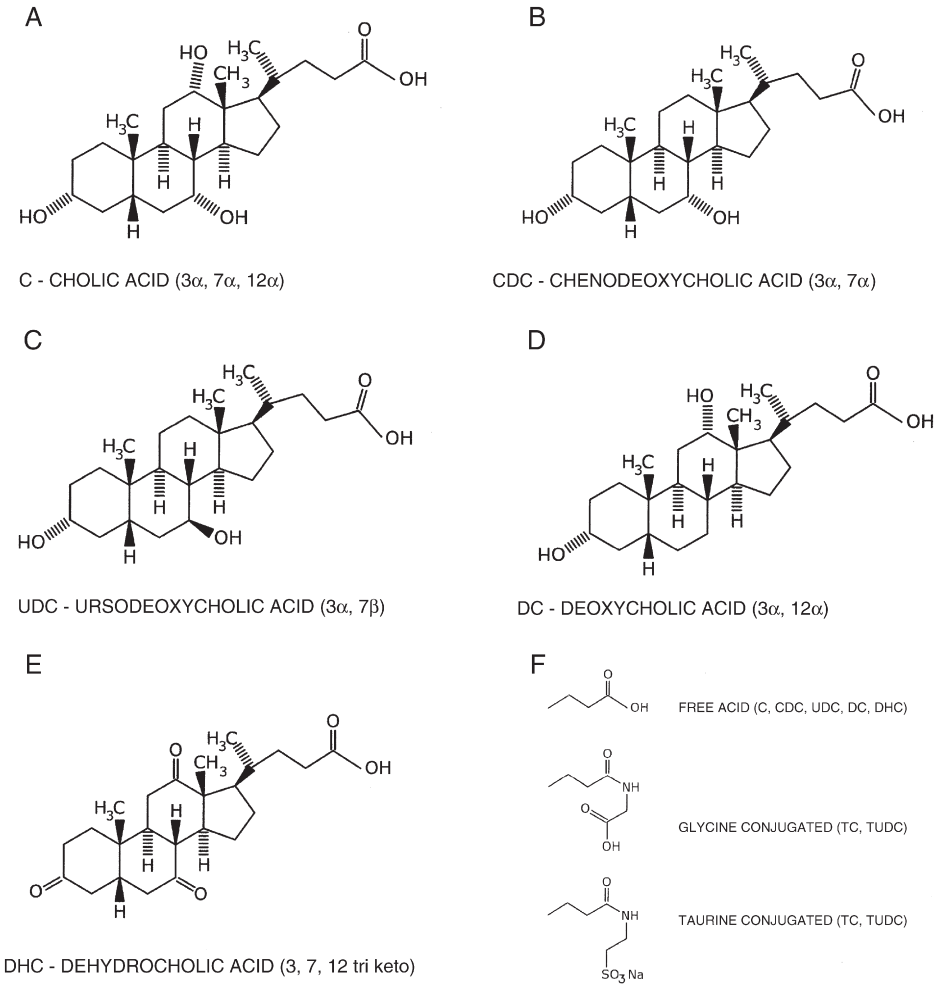

Fig. 1. Molecolar structure of the bile acids examined: the common name is shown together with the position and orientation of the steroid hydroxyl groups. The last window shows possible structures of the side chain.

inhibition is higher for BS with a large hydrophobic surface area and when hydroxy are replaced by keto groups. Also hepatic chemiluminescence in normal and oxidative-stressed rats was found influenced by bile acids [9].

Therefore to highlight this unforeseen role of the bile salts on the mechanism of inhibition of light emission or as antioxidants, we examined the interaction between oxygen molecule and BS by means of polarography, successfully used in these systems [10–14]. Sodium cholate molecule, with three alpha-oriented hydroxy groups in the 3, 7 and 12 positions of the steroid rings (Fig. 1A), was used for preliminary studies, while other BS were chosen for their different structural parameters (Fig. 1A–F).

Oxygen is an electroactive molecule and for this property it is a probe particularly suitable for polarographic measurements.

2. Experimental

2.1. Reagents

The following bile acids (as sodium salt, when commercially available): cholic (C) chenodeoxycholic (CDC), ursodeoxycholic (UDC), deoxycholic (DC), tauroursodeoxycholic (TUDC), taurocholic (TC), glycocholic (GC), glycodeoxycholic (GDC), dehydrocholic (DHC) acid were obtained from Sigma (St. Louis, Mo, USA): their structure is reported in Fig. 1A–F.

Sodium nitrate, sodium acetate, thallium (I) nitrate, sodium hydroxide, buffer solutions, and triphenylphosphine oxide (TPO) were analytical grade chemicals from Merck (Darmstadt, Germany). All the compounds were used as delivered.

Stock solutions were freshly prepared before the experiments, dissolving BS at increasing concentrations in 0.15 mol/L sodium nitrate, to avoid possible interferences with other ions; equivalent amount of NaOH was added in the case of free bile acid (CDC); sodium cholate (C) was also prepared in 0.1 mol/L NaOH or in buffer solution at pH 10 (boric acid/KCl/NaOH: Merck, Darmstadt, Germany). All the solutions were prepared using bidistilled water.

2.2. Apparatus

Polarographic current-voltage (DCP) and differential pulsed polarographic (DPP) curves were recorded by a polarograph

Amel, Model 466, using an electrolytic cell with a saturated calomel electrode as reference electrode (SCE) and all the potentials are referred to it. A dropping mercury electrode was used as a working electrode, at controlled time $t=2.0$ s, and a wired platinum was used as counter electrode. Measurements were carried out at 25.0±0.1 °C at atmospheric pressure that can be considered constant during the time of measurements.

2.3. Procedures

The polarographic current-voltage curves for the first reduction step of molecular oxygen (see below) were recorded in solutions, in the absence or in the presence of increasing BS concentration, saturated by air. To measure the *relative* oxygen concentration, we used the ratio ($\bar{i}_l/\bar{i}_d$) of the mean limiting current ($\bar{i}_l$) of reduction of molecular oxygen in the presence of BS to the mean limiting current diffusion controlled ($\bar{i}_d$), recorded in the absence of BS. To measure the extent of the interaction between oxygen and BS we used the shift of the half-wave potential for the reduction of oxygen, in terms of difference ($\Delta E_{1/2}/V$) between the values measured in the absence and in the presence of increasing BS concentration. The electrode potential is measured versus SCE.

To follow the reaction between superoxide ion and BS, polarograms of reduction of the oxygen dissolved at atmosphere pressure were carried out at different pH, in the presence of TPO, a hydrophobic surfactant, at increasing BS concentrations.

3. Results and discussion

3.1. The polarographic reduction of O_2

Oxygen molecule is naturally present in the solutions kept in contact with atmosphere and solubility ranges from 2.10^{-4} mol/L in water to $1.1.10^{-3}$ mol/L in an aprotic solvent, such as dimethylformamide (DMF) [15].

It is well known [16] that in aqueous solutions the electroreduction of molecular oxygen occurs at the mercury electrode, by two bi-electronic consecutive electrode processes, leading to H_2O_2 (or its parent anion O_2H^-) and to H_2O (or OH^-) respectively.

The first step is the formation of the superoxide ion O_2^- by reduction of molecular oxygen [13]:

$$O_2 + e^- \rightarrow O_2^- \text{ fast} \quad (1)$$

This reaction becomes reversible at pH>12.

Since the direct reduction of superoxide ion O_2^- to O_2^{2-} is energetically forbidden, a further reduction of O_2^- can occur after its transformation into $O_2H\bullet$, by the reaction with water molecules, adsorbed at the mercury electrode, acting as proton donor:

$$O_2^- + H_2O \leftrightarrow O_2H\bullet + OH^- \text{ slow} \quad (2)$$

The species $O_2H\bullet$ can undergo dismutation:

$$2O_2H\bullet \leftrightarrow H_2O_2 + O_2. \quad (3)$$

The overall post-electron transfer reaction (Eq. (2)+Eq. (3)) is described by the following equation:

$$2O_2^- + 2H_2O \leftrightarrow H_2O_2 + O_2 + 2OH^- \quad (4)$$

The dismutation reaction (Eq. (3)) is known to be very slow at high pH.

The net electrochemical process (Eq. (1)+Eq. (2)+Eq. (3)), relative to the first polarographic wave for the reduction of molecular oxygen, is given by:

$$O_2 + 2H_2O + 2e^- \rightarrow H_2O_2 + 2OH^- \text{ (1st polarographic wave)} \quad (5)$$

In aprotic solvents (such as DMF) the only species formed during the first electron transfer is O_2^-: this reaction is known to be reversible in these media (Eq. (6)). A chemical reaction involving the superoxide ion in these media could be protonation by a weak acid (e.g. a phenol, HB):

$$O_2 + e \leftrightarrow O_2^- \quad (6)$$

$$O_2^- + HB \leftrightarrow O_2H\bullet + B \quad (7)$$

$$2O_2H\bullet \leftrightarrow O_2 + H_2O_2 \quad (8)$$

or even by a supporting electrolyte, such as tetraethyl ammonium ion (TEAP), which, in the absence of stronger acids, acts as proton donor and it was reported [11] that it converts into ethylene and triethylamine: but this last reaction is very slow.

The first polarographic wave, relative to the first electrochemical process for the reduction of oxygen, can be used in both cases to follow the behavior of the oxygen in the presence of interacting substrates, such as, in present paper, a bile salt.

The second electrode process occurs at more electronegative potential and it is not important for present purposes.

3.2. The reduction of O_2 in the presence of sodium cholate

Mean limiting current ($\bar{i}_d$) and half-wave potential ($E_{1/2}/V$) related only to the first wave of the oxygen reduction in aqueous solution were recorded in the presence of increasing concentrations of sodium cholate ($\bar{i}_l$).

The dependence of mean current, as $\bar{i}_l/\bar{i}_d$, and of the half-wave potential, as $\Delta E_{1/2}/V$, on sodium cholate concentrations for aqueous solutions, previously stirred for 18 h, is shown in Fig. 2A. Since half-wave potentials shift to more negative values, $\Delta E_{1/2}/V$, as defined, is always negative. According to the results, the plot can be divided into three parts.

$\Delta E_{1/2}/V$ for oxygen reduction increases with sodium cholate concentration, starting from the lowest value examined; whereas $\bar{i}_l/\bar{i}_d$ shows a slight decrease as sodium cholate concentration increases: this was observed up to 8.10^{-3} mol/L (Range I). At this value the current reaches a constant value. $\Delta E_{1/2}/V$ remains constant from 8.10^{-3} to 3.10^{-2} mol/L (Range II) and then increases again (Range III). No examination was carried at concentration>0.1 mol/L for solubility problems.

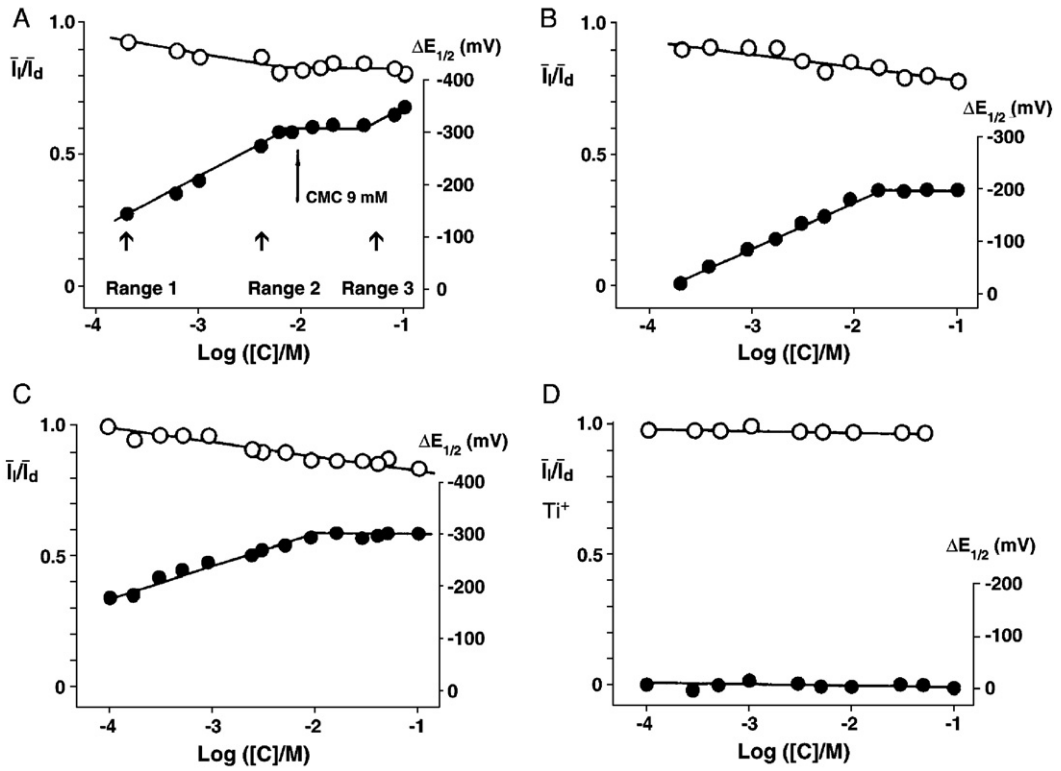

Fig. 2. Dependence of the limiting current $\bar{i}_l/\bar{i}_d$ (○, left scale) and of the half-wave potential $\Delta E_{1/2}$/V (●, right scale) for the reduction of the dissolved oxygen on the logarithm of the concentration of sodium cholate: A — in $NaNO_3$ 0.15 mol/L aqueous solution; B — in NaOH 0.1 mol/L aqueous solution; C — in buffer solution pH 10; D — for the reduction of 2.10^{-4} mol/L Tl^+ in $NaNO_3$ 0.15 mol/L aqueous solution. Range I, II and III are indicated in A together with CMC values (9.10^{-3} mol/L).

Because these effects could be generated either by adsorption phenomena, or by the irreversibility of the O_2 reduction or by change of pH or by some interaction between O_2 and cholate anions, we carried out a series of supplementary tests.

We repeated the measurements in 0.1 mol/L NaOH solution, where the reduction of the oxygen is reported to be reversible [12] and where the pH of the solution can be considered constant and independent of the electrode process. In this case (Fig. 2B) current linearly decreases with increasing cholate concentration; while half-wave potential shifts, as previously observed, up to $1.2.10^{-3}$ mol/L and then remains constant.

Polarograms were also carried out in the presence of increasing cholate concentrations in buffer solution at pH 10. Fig. 2C shows that electrochemical parameters are similar as those in 0.1 mol/L NaOH solution.

As a result it appears that the shift of the half-wave potential towards more negative values for the reduction of oxygen, in the presence of increasing cholate concentrations, does not change when the reaction occurs reversibly or not, as it occurs at lower pH values; and when pH remains constant, as in 0.1 mol/L NaOH or in solution buffered at pH 10 (Fig. 2B,C), or not, as in 0.15 mol/L $NaNO_3$ solution (Fig. 2A).

To check that the shift is not related to passivation of the electrode by adsorbed cholate, we examined the reduction of Tl^+ ion as depolarizer, at increasing cholate concentrations in the absence of O_2. In fact Tl^+ ions, which are reversibly reduced at the electrode, are known not to form complexes with sodium cholate [6]. Measurements were carried out in de-aerated solutions containing 2.10^{-4} mol/L Tl^+ and 0.15 mol/L $NaNO_3$ solution indicated that both polarographic currents and half-wave potential for the reduction of Tl^+ ions are not affected in the whole cholate concentration range (Fig. 2D). This proves that the electrode is not passivated by the presence of the bile salt, as also previously demonstrated [6] and that the reduction of oxygen in the presence of cholate is not affected by this phenomenon.

Measurement of oxygen reduction was also carried out in solutions at increasing concentration of sodium acetate to control the effect of the carboxylate group and of the change of the pH near the electrode by the electrode process, since acetate anions do not form micelle aggregates. The measurements (not reported here) indicate that the process of reduction of oxygen is not affected by the presence of sodium acetate.

From these tests it can be reasonably assumed that the shift observed in Range I of Fig. 2A is related to a possible interaction in the aqueous solution between the cholate anion and the oxygen molecule and cannot be attributed to side effects, such as adsorption of the bile salt to mercury electrode, pH of the solution or irreversibility of the electrode process.

Polarograms for the oxygen reduction in different media ($NaNO_3$ 0.15 mol/L; TPO 10^{-3} mol/L, pH 10 buffer; NaOH 0.1 mol/L aqueous solutions; in the absence and in the presence of increasing concentration of sodium cholate) are shown in Fig. 3A–C.

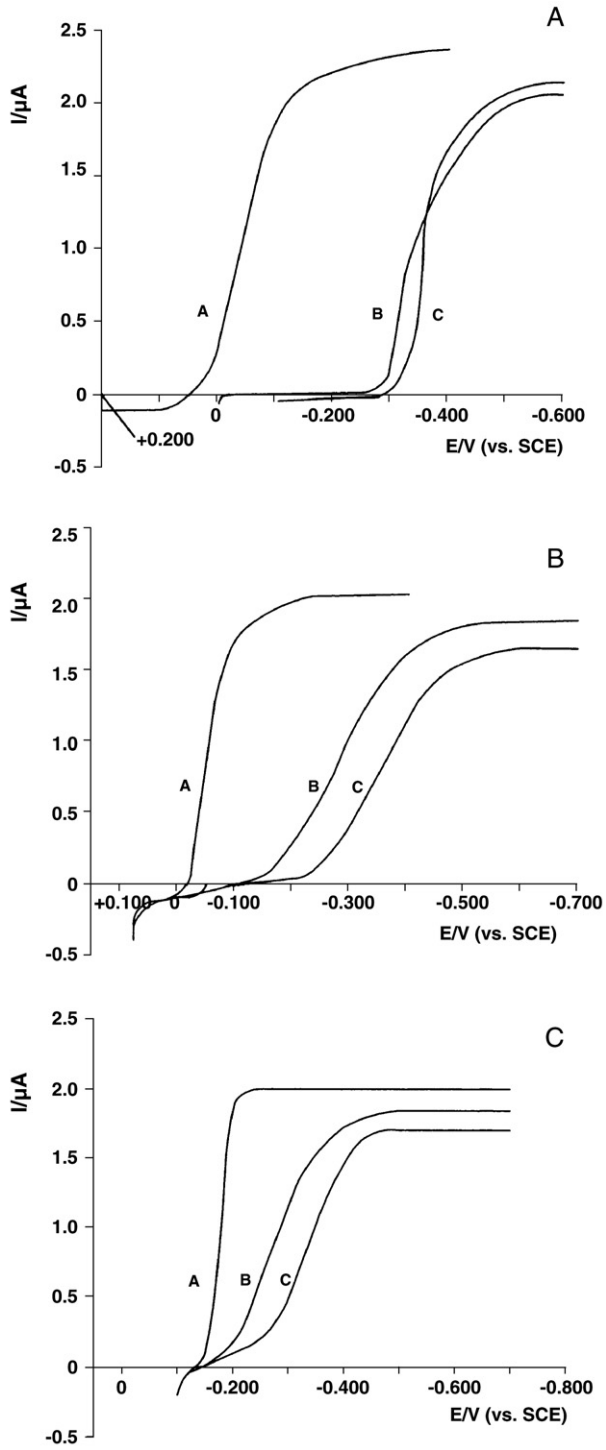

Fig. 3. Envelop of i_{max} of polarographic curves associated to oxygen reduction: A, in $NaNO_3$ 0.15 mol/L aqueous solution; B, idem, in the presence of 10^{-2} mol/L sodium cholate; C idem, in the presence of 10^{-1} mol/L sodium cholate.

3.3. The reduction of O_2 in the presence of different BS

Measurements were also carried out on solutions of increasing BS concentrations differing from cholate for the number, position, orientation of hydroxy groups and nature of the side chain; also a semi-synthetic tri-keto compound was tested: CDC, UDC, DC, TUDC, TC, GC, GDC, DHC.

Plots obtained of $\bar{i}_l/\bar{i}_d$ and the $\Delta E_{1/2}/V$ values vs the increasing concentrations of the different BS are reported in Figs. 4 and 5.

All the salts examined show an $E_{1/2}/V$ shift to more negative values at increasing BS concentrations. The shift stops around the CMC value (indicated by an arrow in the figures) and $\Delta E_{1/2}/V$ afterwards remains constant. It can be appreciated that all BS behave similarly, even though at range III it is not always well defined. The passage from range I to range II (corresponding to CMC) is made quite evident by the change of the slope: as a consequence this technique could be proposed as a simple and non-invasive method to determine CMC values for BS (Table 1). This is not true for the dehydrocholic (DHC), which is known to self-aggregate only at much higher concentration [1]. Because of problems of solubility the measurements were stopped at concentration 1.10^{-2} mol/L. In this case the shift is constant for the whole range of DHC concentrations examined. Table 1 shows the $\bar{i}_l/\bar{i}_d$ values for all BS examined at 5.10^{-2} mol/L concentration. Also CMC values are reported in Eq. (1) and compared with the values graphically obtained from the plots in Figs. 4 and 5.

3.4. The effect of bile salts on O_2^- reactivity

To further examine the effect of sodium cholate on the oxygen reduction we repeated measurements in the presence of triphenylphosphine oxide (TPO), a hydrophobic surfactant strongly adsorbed on the electrode surface.

In the presence of TPO, the reduction of the superoxide ion O_2^-, produced at the electrode, is inhibited, since adsorbed protogenic water is absent. As a consequence hydration of the superoxide ion O_2^- on the electrode surface (Eq. (2)) does not occur, preventing the formation of the hydroperoxide radical [17–19].

In these conditions the limiting current should drop to a half of the value measured in the absence of TPO, because the only reaction possible is described by Eq. (1) and the reduction of O_2^- to H_2O_2 (Eq. (3)) does not occur. This could be also observed provided that pH is high enough to make negligible the effect of the disproportion reaction (Eq. (3)), which is known to be pH dependent. Otherwise due to the occurring disproportion reaction, the current ranges between 1/2 and 1 of the limiting value, depending on the pH of the solution. The stability of O_2^- ions in fact increases with pH, half-life values $t_{1/2}$ range from 0.2 s at pH = 10.2 to 2 s at pH 12 and 50 s at pH 14 [12]. This means that at high pH values, the species O_2^- can be considered stable, at least with respect to the polarographic dropping period.

We therefore measured the mean limiting polarographic currents in these conditions, that is in buffer at pH 10.0 and in 0.1 mol/L NaOH solution, and repeated the measurements in the presence and in the absence of 10^{-3} mol/L TPO and in the presence of increasing concentrations of sodium cholate.

As expected in 0.1 mol/L NaOH solution in the absence of cholate, we found: $\bar{i}_l = \bar{i}_d$, while in the presence of TPO: $\bar{i}_l = \bar{i}_d/2$, where $\bar{i}_l$ and $\bar{i}_d$ are the mean current values in the presence and in the absence of TPO respectively. At pH 10 an intermediate value was found: $\bar{i}_l = 0.60\bar{i}_d$ (Fig. 6A).

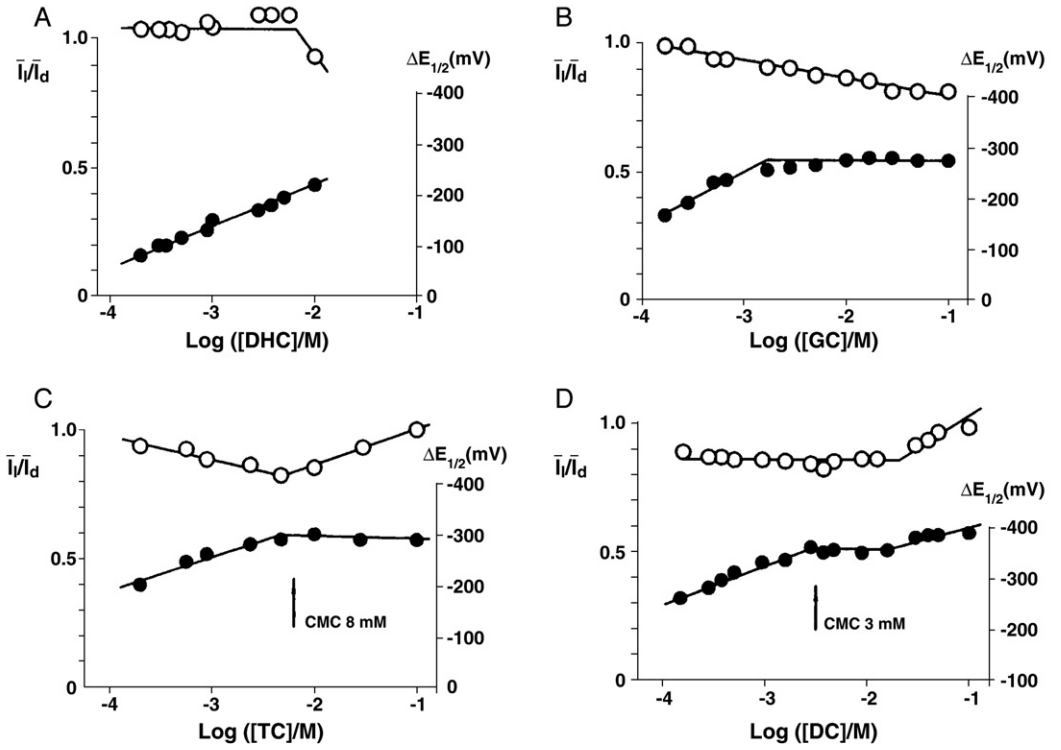

Fig. 4. Dependence of the limiting current $\bar{i}_l/\bar{i}_d$ (○, left scale) and of the half-wave potential $\Delta E_{1/2}/V$ (●, right scale) for the reduction of the dissolved oxygen in $NaNO_3$ 0.15 mol/L aqueous solution, on the logarithm of the concentration of: A — DHC; B — GC; C — TC; D — DC. The arrow indicates the CMC value.

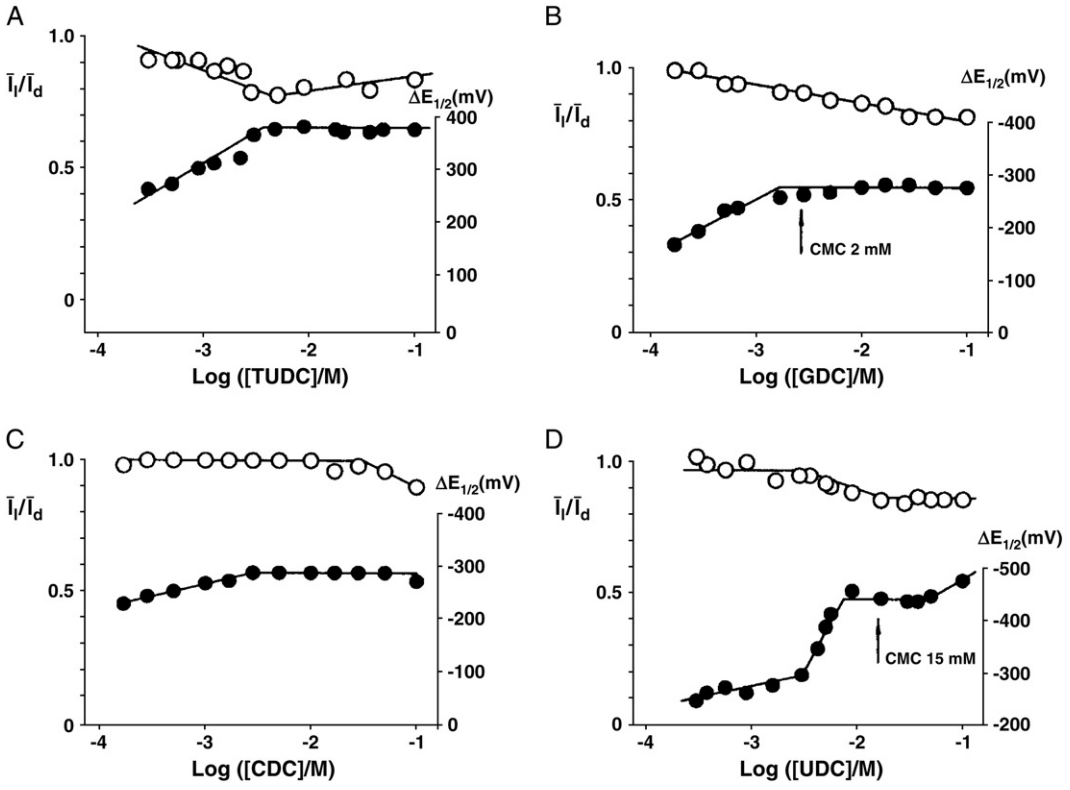

Fig. 5. Dependence of the limiting current $\bar{i}_l/\bar{i}_d$ (○, left scale) and of the half-wave potential $\Delta E_{1/2}/V$ (●, right scale) for the reduction of the dissolved oxygen in $NaNO_3$ 0.15 mol/L aqueous solution, on the logarithm of the concentration of: A — TUDC; B — GDC; C —CDC; D — UDC. The arrow indicates the CMC value.

Table 1
$\bar{i}_l/\bar{i}_d$ values for the disproportion of the superoxide ion O_2^- in the presence of 5.10^{-2} mol/L bile salt

Bile Salt	$(\bar{i}_l/\bar{i}_d)$	range I/range II (mM)	CMC (in NaCl 0.15 M) (mM)
Cholate	1.3		11
Glycocholate	1.6	3	40
Taurocholate	1.5	5	4
Chenodeoxycholate	1.2	5	3
Ursodeoxycholate	1.2	7	15
Tauroursodeoxycholate	1.3	3	2
Deoxycholate	1.2	3	3
Glycodeoxycholate	1.2	3	2
Dehydrocholate	1.1	-	

Addition of increasing concentrations of sodium cholate up to 5.10^{-2} mol/L did not give rise to any change in 0.1 mol/L NaOH solution in the presence of TPO (Fig. 6B). In these conditions O_2^- has much longer life time and therefore not detectable by polarography. In buffer pH 10 (in the presence of TPO) results are different. The addition of sodium cholate in the range 10^{-3}–5.10^{-2} mol/L increases the ratio $\bar{i}_l/\bar{i}_d$ (Fig. 6A).

This indicates that cholate anions are not able to increase the dismutation reaction of O_2^- at pH=13.0, but also that the bile salt is not preferentially adsorbed with respect to the TPO. In fact if BS would be preferentially adsorbed with respect to TPO, the electrode would be no more passivated and the current should increase and reach the value produced by the direct bi-electronic reduction of O_2.

To further confirm this last phenomenon, we examined the systems in buffer at pH 10, in the presence and in the absence of TPO, at increasing cholate concentration by Differential Pulse Polarography (DPP). TPO shows three adsorption peaks at +0.070, −0.010 and −1.650 V [20]. These peaks practically are not affected by the addition of the BS in the range 3.10^{-3}–1.10^{-2} mol/L. This clearly excluded the possibility of a preferential adsorption of sodium cholate with respect to TPO.

The same polarographic measurements of reduction of oxygen were repeated in a buffer solution at pH 10, in the absence and in the presence of TPO and in the presence of concentrations between 10^{-4} and 5.10^{-2} mol/L of all the BS examined. Results agree with those previously reported [8] and suggest that hydrophobic BS affect more the ratio $\bar{i}_l/\bar{i}_d$ and especially at lower concentrations; while the three hydroxy BS show stronger effect at higher concentrations. Hydrophilic BS increase the $\bar{i}_l/\bar{i}_d$ ratio in the concentration range 1.10^{-2} and 5.10^{-2} mol/L, while hydrophobic BS maintain constant this ratio in the same concentration range. In fact hydrophobic BS are at concentrations higher than their CMC, even if the values of the CMC in the medium around the electrode are not necessary the same as those found in aqueous solvent.

All these results support the conclusion that all BS examined are able to affect the behavior of the oxygen molecule in solution, not only modifying the reduction potential of the oxygen molecule to O_2^-, but also affecting its disproportion reaction, behaving thus as antioxidants.

3.5. Nature of the interacting species

According to the results discussed above, a general equation can be proposed for the binding of O_2 to a bile salt anion:

$$BS + O_2 \leftrightarrow BS.O_2 \quad (10)$$

where $BS.O_2$ simply indicates the species resulting from the interaction, without any particular meaning for the stoichiometry or the nature of binding. As the concentration of BS increases, the equilibrium is shifted rightwards. This is paralleled by a shift of the $\Delta E_{1/2}/V$, indicating increasing difficulty for O_2 to be reduced. The decrease of $\bar{i}_l/\bar{i}_d$ can suggest, when occurring, either that the dissociation equilibrium is slowly established, compared to the time of measurement, or that the diffusion coefficient of the species in these conditions is lowered, because of higher mass of micelles with respect to BS monomers.

In the range III, which corresponds to the micelle solution [21], $\Delta E_{1/2}/V$ remains constant. Changes of the electrochemical parameters with BS concentration indicate that BS monomers could be responsible for the interaction. The interaction would

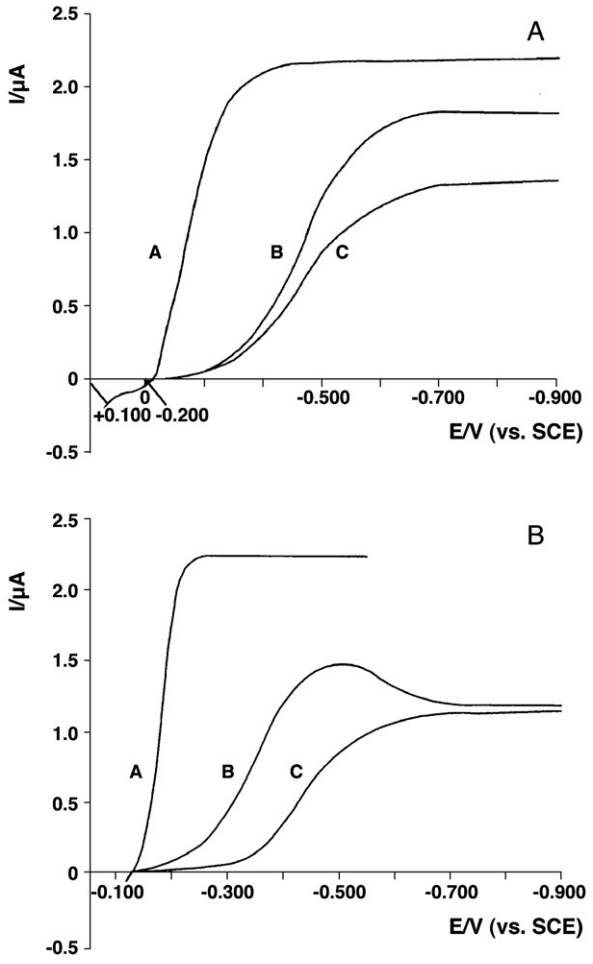

Fig. 6. Envelop of i_{max} of polarographic curves associated to oxygen reduction: A — (A) in buffer solution at pH 10; (B) idem, in the presence of 10^{-3} mol/L TPO; (C) idem, in the presence of 10^{-3} mol/L TPO and 10^{-2} mol/L sodium cholate. B — (A) in NaOH 0.1 mol/L aqueous solution; (B) idem, in the presence of 10^{-3} mol/L TPO; (C) idem, in the presence of 10^{-3} mol/L TPO and 10^{-2} mol/L sodium cholate.

occur in solutions of bile salt at sub- and pre-micelle concentrations, when mainly monomers are present. In fact starting from CMC, the monomer concentration (also called intermicellar concentration) is constant [22]. At concentrations higher than CMC, the added BS does not contribute to increase the monomer concentration. Monomers are involved into the formation of new micelle aggregates or into increasing the aggregation number of pre-existing micelles, a BS solution being a polydispersed system [23], and are no more available to interact with oxygen. Furthermore, since aggregation of BS is thought to occur back-to-back [2], this suggests that oxygen somehow interacts with the hydrophobic portion of the steroid nucleus present on the *beta face* of BS monomer. When two BS monomers start to aggregate and form a dimer, which represents the first step of the aggregation for these surfactants, the hydrophobic region of the steroid nucleus is brought far from the contact with the medium and unavailable for the interaction with other oxygen molecules, but trapping those just previously *"bounded"*. Since oxygen is a hydrophobic molecule, whose solubility increases more than tenfold passing from water to common organic solvents [16], some affinity of oxygen molecule towards the hydrophobic portion of BS is expected. To support this idea is the fact that the BS side chain appears not important with respect to BS/oxygen interaction, since only the structure of the steroid ring beta-face is involved to complex oxygen.

4. Conclusions

The interaction between molecular oxygen and BS, observed in solution, makes more difficult the reduction of oxygen and leaves this molecule less available to oxidation reactions. The interaction not only shifts the reduction potential of the oxygen to more negative values, but also affects the reaction pathway of the superoxide ion, since shifts the disproportion reaction of O_2^- rightwards, according to Latimer diagram, and lowers its concentration. The presence of a third range, in cholate solutions, can be attributed to the formation of micelles with a higher aggregation number. The more hydrophobic are the molecules and the more important are the phenomena showed. The shift of $E_{1/2}$ is more accentuated and stronger is the increase of the mean limiting current in the presence of TPO for BS concentrations lower than CMC. This is consistent with bioluminescence results: luminescence is inhibited in the presence of BS and inhibition is higher for di-hydroxy compared to tri-hydroxy BS, i.e. with larger hydrophobic surface area, or when hydroxy groups are replaced by keto groups.

All these results provide a confirmation of the antioxidant and oxygen carrier behavior of bile salts and offer the researchers in this field a possible explanation of their findings.

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References

[1] A. Fini, A. Roda, Interazione tra anioni biliari e cationi metallici, in: A. Roda, R. Pellicciari (Eds.), Acidi Biliari: Ricerca e Applicazioni Terapeutiche, Momento Medico, Bologna, 1991, pp. 89–106.

[2] M.C. Carey, Measurements of the physical-chemical properties of bile salt solutions, in: L. Barbara, R.H. Dowling, A.F. Hofmann, E. Roda (Eds.), Bile Acids in Gastroenterology, MTP Press Limited, Lancaster, 1982, pp. 19–56.

[3] R.J. DeLange, A.N. Glazer, Bile acids: antioxidants or enhancer of peroxidation depending on lipid concentration, Arch. Biochem. Biophys. 276 (1990) 19–25.

[4] P. Ljubuncic, O. Abu-Salach, A. Bomzon, Ursodeoxycholic acid and superoxide anion, World J. Gastroenterol. 11 (2005) 875–878.

[5] V.U. Buko, O.Y. Lukivskaya, L.V. Zavodnik, V.V. Sadovnichy, N.E. Petushok, N.D. Tauschel, Antioxidative effects of ursodeoxycholic acid in the liver of rats with oxidative stress caused by gamma irradiation, Ukr. Biohim. Z. 74 (2002) 88–92.

[6] H. Mitsuyoshi, T. Nakashima, Y. Sumida, T. Yoh, Y. Nakajima, H. Ishikawa, K. Inaba, Y. Sakamoto, T. Okanoue, K. Kashima, Ursodeoxycholic acid protects hepatocytes against oxidative injury via induction of antioxidants, Biochem. Biophys. Res. Commun. 24 (1999) 537–542.

[7] A. Roda, P. Pasini, M. Guardigli, M. Baraldini, M. Musiani, M. Mirasoli, Bio- and chemi-luminescence in bioanalysis, Fresenius' J. Anal. Chem. 366 (2000) 752–759.

[8] A. Roda, C. Russo, P. Pasini, F. Piazza, G. Feroci, L.J. Kricka, M. Baraldini, Antioxidant properties of bile salt micelles evaluated with different chemiluminescent assays: a possible physiological role, J. Biolumin. Chemilumin. 13 (1998) 327–337.

[9] T. Nakashima, N. Matsumoto, Y. Nakajima, H. Ishikawa, H. Mitsuyoshim, K. Inaba, M. Sakai, Y. Sakamoto, M. Matsumoto, T. Shima, K. Kashima, T. Kitayoshi, N. Shimamoto, Bile acids influence hepatic chemiluminescence in normal and oxidative-stressed rats, J. Gastroenterol. Hepatol. 13 (1998) 81–87.

[10] G. Feroci, A. Fini, P. Zuman, Polarographic study of the interaction of cholate aggregate with Cu^{2+}, Pb^{2+} and Cd^{2+} ions, Bioelectrochem. Bioenerg. 29 (1992) 91–102.

[11] G. Feroci, A. Fini, G. Fazio, P. Zuman, The role of reaction conditions in the interaction of cadmium (II) ions with cholate anions, J. Colloid Interface Sci. 166 (1994) 180–190.

[12] A. Fini, G. Feroci, G. Fazio, A. Roda, P. Zuman, Solution behavior of bile salts in the presence of divalent heavy metal cations, Curr. Top. Solution Chem. 1 (1994) 69–80.

[13] G. Feroci, A. Fini, G. Fazio, P. Zuman, Interaction between dihydroxy bile salts and divalent metal ions studied by polarography, Anal. Chem. 67 (1995) 4077–4085.

[14] G. Feroci, A. Fini, G. Fazio, P. Zuman, Effect of divalent transition metal ions on the aggregation of trihydroxy bile salts, J. Colloid Interface Sci. 178 (1996) 339–347.

[15] G. Feroci, S. Roffia, On the reduction of oxygen in dimethylformamide, J. Electroanal. Chem. 71 (1976) 191–198.

[16] D.T. Sawyer, Redox thermodynamics for oxygen species: effects of media and pH, Oxygen Chemistry, University Press, Oxford, 1991, pp. 19–51, Chapter 2.

[17] B. Kastening, G. Kazemifard, Elektrochemische Reduktion von Sauerstoff zum Superoxid-Anion in wässiger Lösung, Ber. Bunsenges. Phys. Chem. 74 (1970) 551–556.

[18] J. Chevalet, F. Rouelle, L. Gierst, J.P. Lambert, Electrogeneration and some properties of the superoxide ion in aqueous solutions, J. Electroanal. Chem. 39 (1972) 201–216.

[19] J. Divisek, B. Kastening, Electrochemical generation and reactivity of the superoxide ion in aqueous solutions, B, J. Electroanal. Chem. 65 (1975) 603–621.

[20] T. Ferri, L. Campanella, B.M. Petronio, R. Sbardellati, Behaviour study of some cholic acids at dropping mercury electrode using differential pulse polarography, Bioelectrochem. Bioenerg. 19 (1988) 263–275.

[21] A. Roda, F. Hofmann, K. Mysels, The influence of bile salt structure on self association in aqueous solutions, J. Biol. Chem. 258 (1983) 6362–6370.

[22] D. Attwood, T. Florence, Surfactant Systems: Their Chemistry, Pharmacy and Biology, Chapman and Hall, London, 1983, pp. 72–123, Chapter 3.

[23] P. Mukerjee, J.R. Cardinal, Solubilization as a method for studying self-association: solubility of naphthalene in the bile salt cholate and the complex pattern of aggregation, J. Pharm. Sci. 65 (1976) 882–886.