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Study on the aggregation and electrochemical properties of Rose Bengal in aqueous solution of cyclodextrins

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Abstract

The interactions of Rose Bengal (RB) with α-cyclodextrin (α-CD), hydroxypropyl-β-cyclodextrin (HP-β-CD), hydroxypropyl-γ-cyclodextrin (HP-γ-CD), heptakis(2,3,6-tri-*O*-methyll)-β-cyclodextrin (TM-β-CD) were studied in aqueous solutions of 0.1 M $KClO_4$ and 0.1 M $LiClO_4$ by vis absorption, fluorescence spectroscopy as well as electrochemical measurements at 298 K. The spectrophometric results indicate that RB is included in all β- and γ-CDs forming complexes with a stoichiometry 1:1 whose stability is slightly higher in $KClO_4$ than in $LiClO_4$ solutions. The complex stability constants determined for salt-containing CD solutions are lower than those for water solutions. The complexation of RB with β- and γ-CD and the differences between the complexes obtained in the presence of the two salts were confirmed by an electrochemical study.

Keywords: Rose Bengal; Aqueous solutions; Cyclodextrins; Inclusion complexes; $KClO_4$; $LiClO_4$

1. Introduction

The use of Rose Bengal (RB) as photosensitizer in dye sensitizer photochemical cells has been subject to extensive studies [1,2]. The tendency of this bis-anionic dye to aggregate in solution at high concentrations restricts the widespread use of RB in solution, because the formation of aggregates impairs the photochemical response [3]. The aggregation is particularly important in salt solution; in fact it is well known that the maximum concentration at which RB can be considered as monomer significantly decreases upon the addition of salt. A strategy to prevent the dye's self aggregation can be its introduction in the solution of cyclodextrins, cyclic oligosaccharides which have the ability to include molecules of organic compounds into their cavities. As evidenced by the results of our previous studies [4,5] some of these macrocycles, interacting selectively with monomer RB, are able to shift the equilibrium monomer-aggregates towards the formation of the monomeric form, producing an increase of the dye efficiency and protecting it against possible processes of photooxidation. In order to obtain a more detailed comprehension on the mechanism involved in such processes, we have extended the study on the effect of cyclodextrins and salts on the aggregation and electrochemical properties of RB to the case of cyclodextrins differently modified in the presence of $LiClO_4$ and $KClO_4$.

2. Materials and methods

α-Cyclodextrin (α-CD), hydroxypropyl-β-cyclodextrin (HP-β-CD) DS=5.6, hydroxypropyl-γ-cyclodextrin (HP-γ-CD) DS=4.8, heptakis(2,3,6-tri-*O*-methyll)-β-cyclodextrin (TM-β-CD) were purchased from Aldrich. RPE ACS D(+)-glucose was purchased from Carlo Erba; Rose Bengal (RB), $LiClO_4$ and $KClO_4$ were purchased from Fluka. The molecular structures of RB and used CDs are reported in Fig. 1. All chemicals were used as received. A solution of 0.1 M $LiClO_4$ and a solution of 0.1 M $KClO_4$ were prepared with doubly distilled water and used as solvents in the preparation of the RB stock solutions. The solutions of RB and CD or glucose were prepared dissolving weighted amounts of CD or glucose in a 5-ml of a RB aqueous stock solution pipetted into 10 ml calibrated

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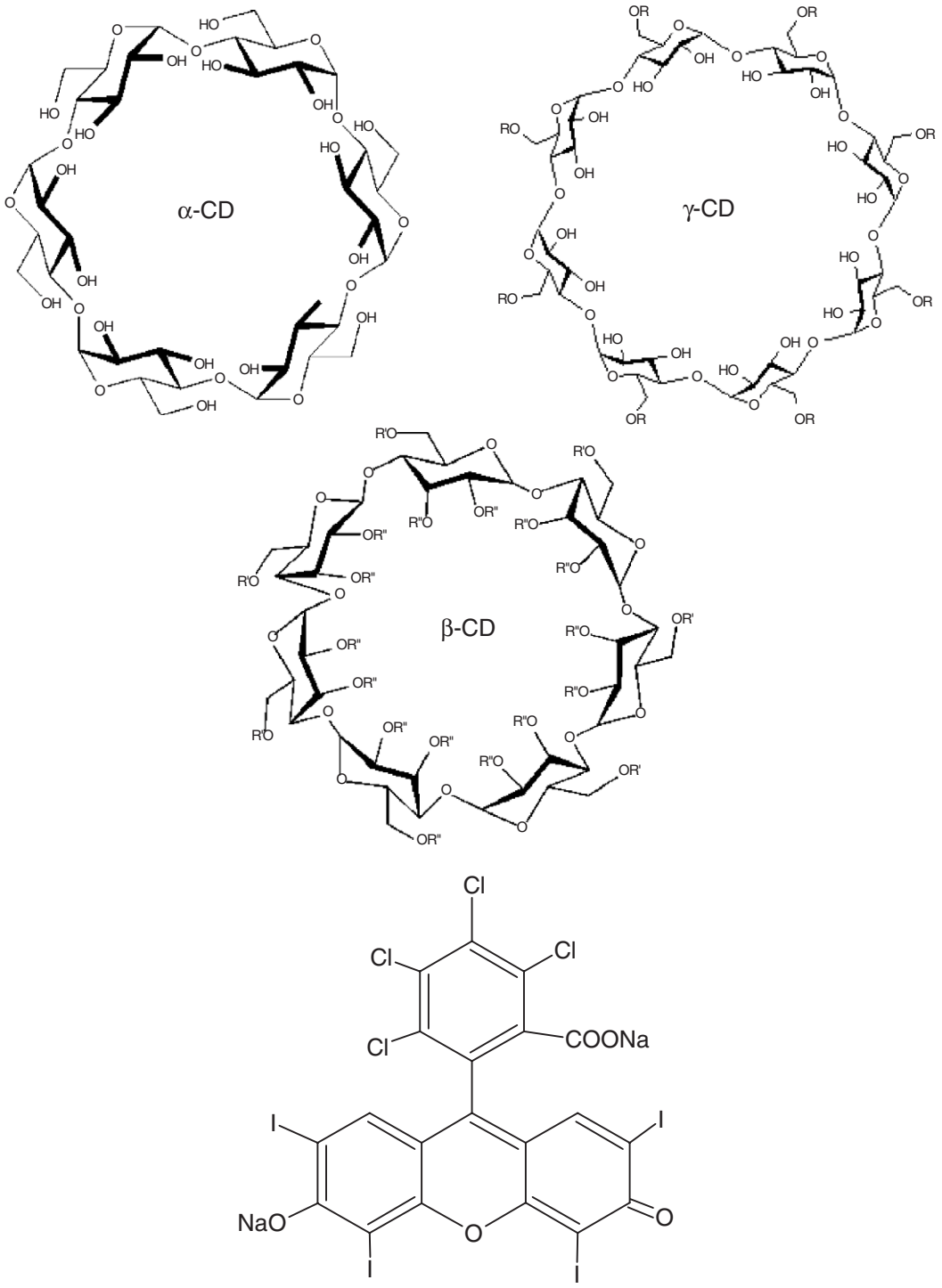

Fig. 1. Molecular structures of RB, α-CD, HP-β-CD (R'=2-hydroxypropyl), TM-β-CD (R', R'', R'''=methyl) and HP-γ-CD (R=2-hydroxypropyl).

flasks and diluted to volume with the salt 0.1 M solution. This procedure ensured a constant concentration of RB both in the absence and in the presence of the various CD or glucose concentrations.

Visible absorption spectra were recorded using a Varian CARY/3 spectrophotometer. Fluorescence measurements were carried out using a Varian Cary Eclipse fluorescence spectrophotometer exciting at 550 nm. Electrochemical experiments were performed in a standard three-electrode cell used as working electrode HMDE. Voltammograms were recorded by using the AUTOLAB PGSTAT10 potentiostat interfaced with a personal computer. All experiments were performed at 298 K.

3. Results and discussion

In water the absorption spectra of RB is characterized by a maximum at 548.10 nm and a shoulder at about 510 nm. The relative intensity of shoulder to the peak is usually used as a

measure of the aggregation of RB in solution [6,7]. In water, at a concentration of 2.5×10^{-5} M, this relative intensity, 0.37, indicates that RB is mainly present as monomer. In 0.1 M salt solutions, both for $LiClO_4$ and for $KClO_4$, the spectra of RB are shifted about 0.5 nm and are characterized by higher values of the relative intensity of the shoulder to the peak evidencing a partial aggregation of the dye in agreement with other studies on RB in salt solutions [8,9]. The addition of CD to aqueous salt solutions causes a bathochromic shift of the RB spectra similar to that already observed in water and ascribed to the formation of inclusion complexes between RB and CDs [4]. The shift, $\Delta\lambda=\lambda_{max}-\lambda_o$, as a function of CD concentration is reported in Fig. 2, where λ_{max} and λ_o indicate the wavelengths of the maximum absorbance of RB in salt solution with and without CDs, respectively. In order to distinguish between the spectral modifications produced by the inclusion of RB in the CDs cavity from other effects, like the presence of nonspecific interactions between the dye and the CDs or changes in the solvent properties, in the same Fig. 2 the $\Delta\lambda$ values risen by the addition of increasing amounts of D-(+)-glucose are also reported. Experimental data show the addition of increasing amounts of any saccharide causes a red shift and therefore, a positive $\Delta\lambda$ whose values, as a function of saccharide concentration, depends on its nature. In particular, amounts of glucose comparable to the amounts of CDs used gives rise only to a red shift lower than 1.5 nm. Similarly $\Delta\lambda$, only slightly higher than glucose, have been obtained in the case of α-CD. These results indicate that the spectral modification observed in the presence of α-CD are mainly due to effects similar to those produced by glucose and therefore not associated to the formation of inclusion complexes as already observed by Flamigni [10]. Much higher $\Delta\lambda$ have been observed in the case of the addition of increasing amounts of β- and γ-CDs to the RB salt solutions. The HP-γ-CD produces the sharpest rise of $\Delta\lambda$ at increasing of CD concentration, followed by the TM-β-CD and then by the HP-β-CD. The values of $\Delta\lambda$ and their dependence on the CD concentration indicate the formation of inclusion complexes with different stabilities. The formation of inclusion complexes between RB and β- and γ-CDs were already observed in water [4,10]. Clues on the formation of complexes are also provided by the decrease of the relative intensity of the shoulder to the peak observed at increasing of CD concentration of HP-β-CD, TM-β-CD and HP-γ-CD. No changes were observed in the presence of increasing amounts of glucose and a slight increase in the presence of high concentration of α-CD. The molecular dimensions of β- and γ-CDs allow the inclusion of only the RB monomer; therefore, the formation of complexes, promoted by an increase of the CD concentration, is expected to give rise to a shift of the equilibrium monomer/aggregate of RB towards the monomeric form. The different behaviour observed between glucose and α-CD at high concentration indicates that, at such concentrations, the changes in the solvent properties produced by the presence of CDs cannot be completely mimed by glucose.

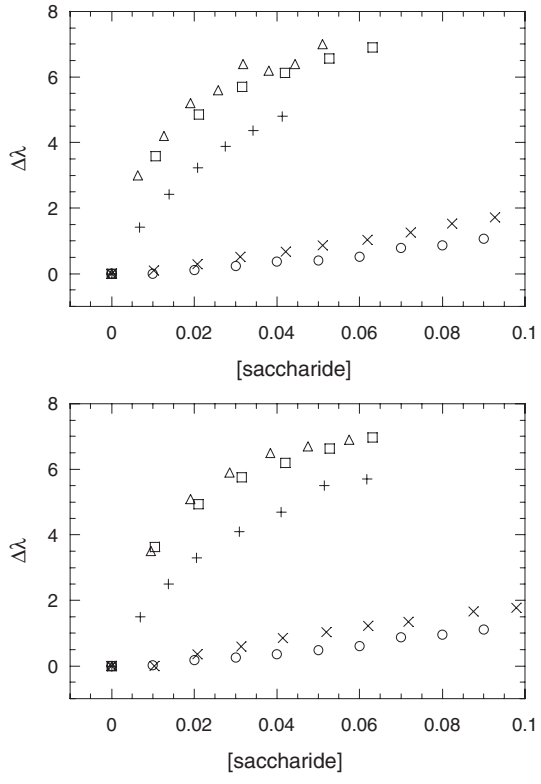

Fig. 2. Experimental shift of the wavelengths of the absorption maximum of RB 2.5×10^{-5} M, $\Delta\lambda=\lambda_{max}-\lambda_o$, as a function of the concentration of (O) glucose, (×) α-CD, (+) HP-β-CD, (□) TM-β-CD, (Δ) HP-γ-CD in 0.1 M $KClO_4$ (top) and 0.1 M $LiClO_4$ (bottom). λ_{max} and λ_o indicate the wavelengths of the maximum absorbance of RB in salt solution with and without CDs, respectively. The concentrations of CD are expressed as mol/l whereas the concentration of glucose is expressed as g/ml.

The comparison between data obtained in the solutions of the two salts indicates that in $KClO_4$ solutions the complexes have higher binding constants than in $LiClO_4$ solutions because the increase of $\Delta\lambda$ in the presence of the first salt is higher than in the presence of the other. Also the faster decrease of the relative intensity of the shoulder to the peak at increasing β- and γ-CDs in $KClO_4$ than in $LiClO_4$ solutions suggests the same conclusion.

Because the dye is partially aggregated at the RB concentration used in the absorbance measurements, the observed spectral modification was not used to evaluate the binding constants as done for the same dye in water [4]. The quantitative study of the interactions between RB and CDs in salt solutions was done by fluorescence measurements. The concentration of the RB solution used in this study, 7×10^{-6} M, was chosen in order to have RB mainly as monomer and to avoid fluorescence quenching. In Fig. 3 the emission spectra of RB in salt solutions of α-CD and HP-γ-CD are reported. It is possible to observe that the increase of α-CD concentration does not produce any increase in fluorescence intensity, thus confirming the non-inclusion of RB in α-CD. Otherwise an increase of fluorescence intensity is obtained at increasing CD concentration, as general behaviour, for β- and γ-CDs in both $KClO_4$ and $LiClO_4$ solutions.

The binding constants for the inclusion complexes of RB with HP-β-CD, TM-β-CD and HP-γ-CD were calculated by

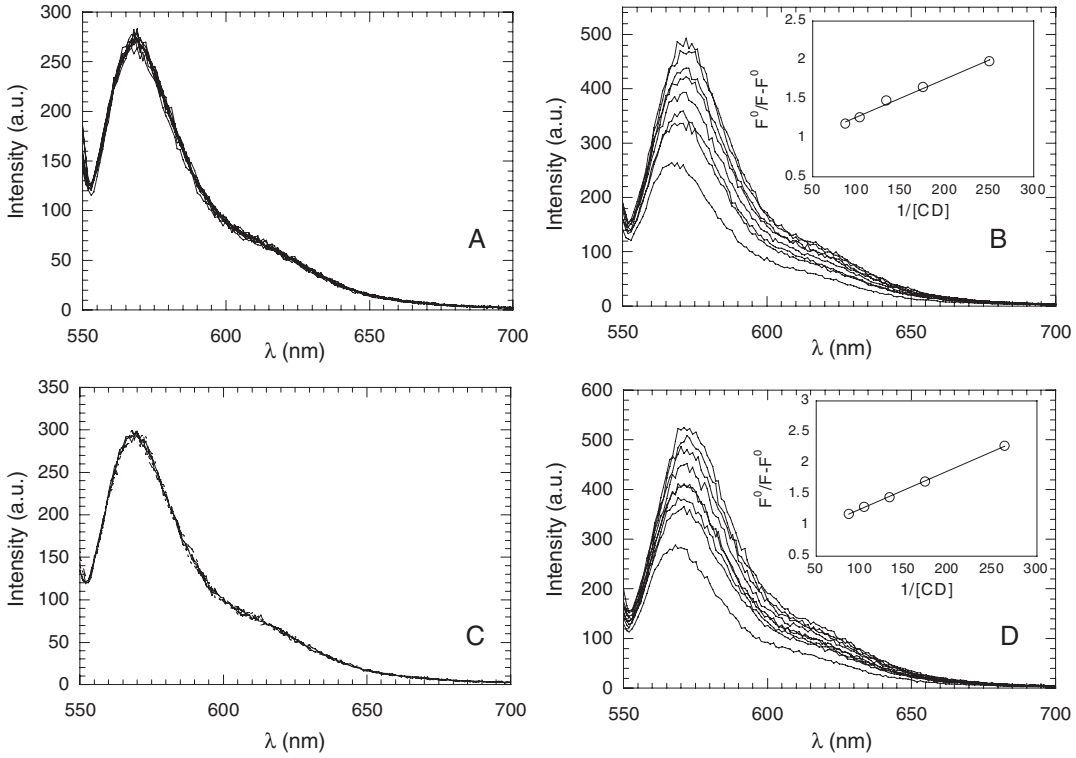

Fig. 3. Fluorescence spectra of RB, 7×10^{-6} M, at different concentrations of α- CD (A) and HP-γ-CD (B) in a solution of 0.1 M $KClO_4$ and of α-CD (C) and HP-γ-CD (D) in a solution of 0.1 M $LiClO_4$. Inset: Benesi-Hildebrand plots of $F^0/(F-F^0)$ vs. 1/[HP-γ-CD] in solution of $KClO_4$ (B) and in solution of $LiClO_4$ (D).

applying the modified Benesi-Hildebrand treatment to the fluorescence measurements in the following form [11]:

$$\frac{F^0}{F-F^0}=\frac{1}{A}+\frac{1}{AK[\mathrm{CD}]^n}$$

where K is the binding constant, F^0 is the initial fluorescence intensity of free RB, F is the maximum fluorescence intensity of the RB-CD inclusion complexes at the [CD] cyclodextrin concentration, A is a constant, and n is the number of binding sites. The insets of Fig. 3 are the plots of $F^0/(F-F^0)$ vs. 1/[HP-γ-CD] in solution of $KClO_4$ and in solution of $LiClO_4$. Similar plots were also obtained in the case of HP-β-CD and TM-β-CD. The linearity of the plots $F^0/(F-F^0)$ vs. 1/[CD] obtained in the case of β- and γ-CDs in both salt solutions reflects the formation of complexes 1:1 between the dye and the CDs as already observed in water [4]. The binding constants evaluated in $KClO_4$ and $LiClO_4$ solutions are reported in Table 1.

The values of the binding constants confirm the already advanced hypothesis of a higher stability of the complexes obtained in $KClO_4$ solutions and indicate some differences compared to that observed in water. In salt solutions the binding constants are lower than in water; the CD able to form the most stable inclusion complexes is still the γ-CD whereas the order of stability of the two β-CDs is inverted: the methylated CD complexes RB better than the HP-β-CD. While the difference between the values obtained with β- and γ-CDs can be directly ascribed to the different cavity size confirming the results already obtained in water [4], the difference between that obtained in water and that obtained in salt solutions and the differences between the two salts require more consideration.

Table 1
RB/CD binding constants in $KClO_4$ and $LiClO_4$ solutions

	K (M^{-1}) in $KClO_4$ solutions	K (M^{-1}) in $LiClO_4$ solutions
HP-β-CD	40	35
TM-β-CD	80	75
HP-γ-CD	160	120

In general the presence of electrolytes in solution can affect the interaction between two molecules and therefore their ability to bind by means of different mechanisms. Ions in solution produce changes in the dielectric constant and in water activity; in the case of charged molecules, ions can also be directly involved in the formation of non-covalent bindings [12]. This contribution, which becomes important in the case of polyelectrolyte molecules, as for example nucleic acids, for the systems under examination can be neglected [13]. The increase of the dielectric constant of the medium, caused by the addition of $KClO_4$ or $LiClO_4$, makes the electrostatic contribution, which is one of the non-covalent interactions generally involved in the formation of inclusion complexes, less important [14]. In water the higher stability of the complexes with HP-CDs compared to those with methylated CDs suggested that the occurrence of hydrogen bonds between the hydroxypropyl moiety of CD and one of the electron acceptor moieties of RB have an important role. The presence of salts makes the effect of the formation of hydrogen bonds less

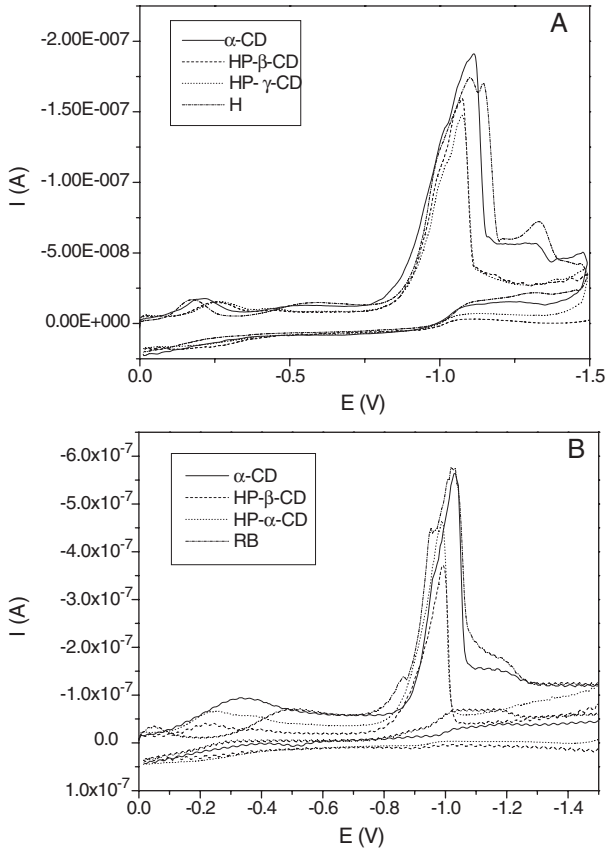

Fig. 4. Cyclic voltammograms of a RB (2×10^{-5} M) solution in aqueous 0.1 M $KClO_4$ (A) and $LiClO_4$ (B) containing different CDs (0.04 M).

important. In addition to the decrease of the dielectric constant there is also the chaotropic effect produced by the anion ClO_4^- which is a water structure breaker [15]. The result is that, in salt solution, TM-β-CD forms more stable complexes with RB than the HP-β-CD because the interactions between the methylated CD and RB are mainly hydrophobic. A possible explanation of the observed slight difference in stability of the complexes in $KClO_4$ and in $LiClO_4$ solutions can be found in the different position of the two cations in the lyotropic series. Considering the analogy between the interactions responsible for protein structure and those involved in inclusion of guest in CDs, it is possible to suppose that Li^+, which is a better denaturant of proteins than K^+ [12] having an higher charge density, gives rise to a slight decrease of complex stability.

The formation of inclusion complexes can be evidenced also by using a completely different analytical technique, such as Cyclic Voltammetry. In Fig. 4(A and B) the voltammograms relative to a 2×10^{-5} M RB solution containing different CDs in the two salt solutions are reported. It is necessary to outline that the electrochemistry of RB is quite complicated since the electrochemical reduction is followed by chemical reactions, which are not well known [16–19]. Nevertheless it is possible to identify two peaks due to the reduction of furan and pyron rings [16] at about −0.052 V and −1.018 V in $LiClO_4$, while $KClO_4$ solutions are located at −0.165 V and −1.124 V. The presence of CDs influences the electrochemical behaviour determining a shift of the peak II toward more positive potential values, together with a pronounced decrease of the current peak. In particular the peak II potential is shifted to −1.088 V in HP-β-CD and to −1.066 V in HP-γ-CD for $KClO_4$ solutions, whereas in $LiClO_4$ solutions the peak shifts to −0.978 V in HP-β-CD and to −0.963 V HP-γ-CD. This displacement of the reduction peak potential along with the reduction of the current peak are indicative of the formation of inclusion complexes, as well documented in literature. In particular the positive shift of the redox potential in presence of CD can be attributed to the inclusion of the guest into the hydrophobic apolar cavity of the beta and gamma CDs. In fact it is well known that a decreasing of the solvent polarity results in a shift of the redox potential of the molecule toward a more positive potential [20]. The current intensity decrease of the CD-RB solution can be attributed to the shielding effect of the CD which reduces the exposition of the molecule to the electrode surface and to the higher size of the complexes which decrease the diffusion coefficient of the RB molecule.

4. Conclusions

In $KClO_4$ and $LiClO_4$ 0.1 M solutions, RB is includes in all β and γ CD forming complexes with a stoichiometry of 1:1. The binding constants are lower than in water and depend differently on the CD nature from that observed in water. The complexes are more stable in $KClO_4$ solutions than in $LiClO_4$ ones and therefore the disaggregation process of the dye is more effective in the solution of the former salt. The complexation of RB with β- and γ-CD and the differences between the complexes obtained in the presence of the two salts have been confirmed by an electrochemical study.

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