



ELECTROCHEMICAL BEHAVIOUR OF METHYLENE BLUE IN NON-AQUEOUS SOLVENTS



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ABSTRACT

The electrochemical behaviour of methylene blue in solution of non-aqueous solvents with different supporting electrolytes was studied by cyclic voltammetry. Dye electro-reduction presents two well-defined processes of monoelectronic charge transfer yielding a free radical in the first process and an anion in the second electron transfer. Free radical and anion are long living species in some of the studied media. Effects of supporting electrolyte and solvent on the peak potentials, the peak current functions and the reversibility of the charge transfer processes are reported. A dissociation equilibrium of the dye in solution of non-aqueous solvents and the acid or base added determine markedly the electrochemical responses. In the particular cases of KOH/DMF or EDA basic media the chemical formation of the stable methylene blue radical was detected and it was characterized by EPR spectroscopy. A general reaction scheme is proposed.

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1. INTRODUCTION

Methylene blue (3,7-bis(dimethylamino) phenothiazin-5-ium chloride, 1^+Cl^- in Fig. 1) is a cationic dye, widely used in various fields related to chemistry and biology [1–3]. Main uses of 1^+Cl^- are associated with the determination of glucose, oxygen and ascorbic acid [4,5]. Also, 1^+Cl^- is one of the materials of high consumption in the dye industry, being used for cotton and silk painting [6].

The electrochemical behaviour of 1^+Cl^- has been the subject of numerous investigations performed in aqueous solutions [7–17], but the behaviour in non-aqueous solvents has been insufficiently studied, and the published results are very scarce [18].

The electrode process of 1^+Cl^- in aqueous media is illustrated in Scheme 1. It has been shown that 1^+Cl^- undergoes a fast two-electron transfer process without an evident separate electron transfer step, with only one cathodic wave and only one anodic wave being observed by cyclic voltammetry [7]. However, *in situ* ESR results provide evidence for the formation of a radical intermediate (Scheme 1, 1^\bullet) [19]. The intermediate is a short-lifetime radical, which combines with a proton and suffers an

electron reduction, yielding the non-paramagnetic leucomethylene blue (1H).

In special cases, two electron transfer processes are detected in the growth of 1H films and their electro-dissolution on bare gold (Au) and sulphur modified gold (Au-S) electrodes in methylene blue solutions. These processes are attributed to 1H^{*+} and 1H formation in acid aqueous medium. The first cathodic process, at more positive potentials, is assigned to the reduction of 1^+Cl^- from solution with the formation of 1H. The second redox process, at more negative potentials, is due to the reduction of the dye adsorbed on the electrode surface with the formation of 1H [20].

It is well known that ionic dyes tend to aggregate in aqueous solutions, leading to the formation of dimers, and occasionally even higher order aggregates [21–23]. The aggregation of 1^+Cl^- in aqueous solution affects its colouristic and photo-physical properties, being therefore of special interest. However, is not possible to carry out a complete analysis of the results from studies on aggregation, since the influence of the medium, of the nature of the counter-ion, etc. has been sporadically investigated, and ambiguous interpretations are given.

We hypothesise that due to the particular structure of 1^+Cl^- (salt nature and aggregation possibilities) its electrochemical behaviour should be notably influenced by the composition of the medium in which the electrochemical reaction occurs. The

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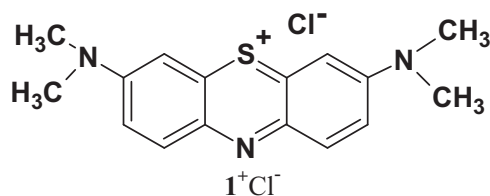


Fig. 1. Structure of methylene blue (1^+Cl^-).

understanding of the electrochemical behaviour of 1^+Cl^- in solution of non-aqueous solvents is interesting because it offers the possibility of transforming the molecule by particular ways of synthesis, that often are very complicated by traditional thermal routes [24]. It is known that the use of organic solvents has several advantages over water. The most accessible potential range in organic solvents allows easy detection of the processes of electro-reduction/oxidation in non-aqueous media that are hardly achievable in aqueous solutions.

This work focuses on the electrochemical reactivity of 1^+Cl^- in solution of non-aqueous solvents with a variety of supporting electrolytes, and in the presence of an anhydrous acid or a base added in order to ascertain if our proposed hypothesis is fulfilled.

2. EXPERIMENTAL

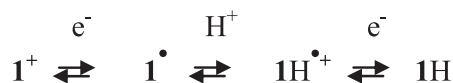
The investigation was performed on 1^+Cl^- in solution of six dipolar non-aqueous solvents, without and with H-bond ability, and with different strength as Lewis acids or bases [25], in presence of two supporting electrolytes (tetraethylammonium perchlorate (TEAP) and $LiClO_4$) in the absence and in the presence of an anhydrous acid (trifluoroacetic acid, TFA) or a base (KOH) added. Further, the studies were extended to other supporting electrolytes for acetonitrile (ACN), and ethanol (EtOH) solvents.

Solutions preparation and electrochemical experiments were carried out inside a glove box under dry nitrogen atmosphere. Non-aqueous solvents and inorganic compounds were purified by standard methods [26]. Solvents ACN, N,N-dimethylformamide (DMF), tetramethylurea (TMU), formamide (FM), EtOH and ethylenediamine (EDA) were dried with freshly activated 4 Å molecular sieves and stored in the dry glove box on freshly activated molecular sieve. 1^+Cl^- (Rhone Poulenc, Buenos Aires, Argentina) was successively re-crystallized from water and ethanol and its purity was thoroughly tested by thin layer chromatography.

Cyclic voltammetry (CV) experiments were performed in a conventional undivided gas-tight glass cell with dry nitrogen gas inlet and outlet. The working electrode (WE) was a 3 mm diameter vitreous carbon disk encapsulated in Teflon, and the counter-electrode (CE) was a 2 cm² Pt foil. An Ag^+ (0.1 M, ACN)/Ag reference electrode (to which all reported potentials are referred) was used. Prior to each electrochemical measurement the WE was polished with alumina up to 1 μm, and then thoroughly ultrasonically rinsed with water; final drying was performed with dry nitrogen gas.

Peak current intensities for the first electro-reduction process (I_{cp1}) increased linearly with the square root of the potential sweep rate (ν) in the range 0.050–0.300 V/s.

EPR spectra were obtained at room temperature with a Bruker EMX-Plus spectrometer, using the 9.7 GHz frequency (X band).



Scheme 1. Electrode process of 1^+Cl^- in aqueous media.

Uv–vis absorption spectrophotometric measurements were recorded using a Shimadzu UV – 1800 spectrophotometer.

3. RESULTS

The CV behaviour, mainly the reduction process, of 1^+Cl^- in solution of the different solvents and supporting electrolytes investigated without and with acid or base added is qualitatively described below. Quantitative results are shown in Tables 1–4. Rationalization of the results is under the subtitle: 4. Discussion.

3.1. Cathodic behaviour

3.1.1. 1^+Cl^- in ACN solution

1^+Cl^- in ACN solution with TEAP as supporting electrolyte was electro-reduced in CV experiments in two separated quasi-reversible one electron transfer steps (cathodic peak 1/anodic peak 1, cp1/ap1, and cathodic peak 2/anodic peak 2, cp2/ap2) with cathodic peak potentials $E_{cp1} = -0.73$ V and $E_{cp2} = -1.54$ V (Fig. 2, dotted line). CV experiments performed with different supporting electrolytes show that there is an effect of the supporting electrolyte on the separation between cathodic peaks ($\Delta E = E_{cp1} - E_{cp2}$) and on the peak current intensities measured for the first charge transfer (Fig. 2, full line and Table 1). The effect of the initial concentration of 1^+Cl^- ($[1^+Cl^-]_o$) in ACN solution with TEAP or $NaClO_4$ as supporting electrolyte on the current function ($cf = I_p/\nu^{1/2} [1^+Cl^-]_o$) for the first electro-reduction step (cf_{cp1}) of 1^+Cl^- is shown in Fig. 3.

When the electro-reduction of 1^+Cl^- was measured in solution of ACN with TEAP in the presence of TFA or KOH, a changed CV behaviour was observed. Some examples of such behaviours are shown in Fig. 4. In presence of added TFA (Fig. 4 dotted line, e.g. molar ratio = $R_{acid} = [TFA]/[1^+Cl^-]_o \approx 67$) only one electro-reduction peak at $E_{cp1,acid} = +0.030$ V was observed. R_{acid} ratio value had effect on $E_{cp1,acid}$ and on the peak current intensity ($I_{cp1,acid}$) (results not shown). $I_{cp1,acid}$ increased up to reach a maximum value and $E_{pc1,acid}$ shifted toward more positive potentials with increasing R_{acid} . In presence of added KOH only one electro-reduction peak with a greater $I_{cp1,base}$ was observed at more cathodic potential than in the absence of base. $E_{cp1,base}$ shifted towards more cathodic values and $I_{cp1,base}$ increased with the increase of the molar ratio $R_{base} = [KOH]/[1^+Cl^-]_o$. A typical example of this behaviour is shown in Fig. 4 (dashed line), for $R_{base} \approx 91$ ($E_{cp1,base} = -1.24$ V). The addition of KOH to the initial blue solution of 1^+Cl^- in ACN/TEAP electrolytic solution caused a colour change of the solution. For $R_{base} > ca 2$, the initial blue solution acquired an intense purple colour.

Measured CV for a solution of 1^+Cl^- in ACN with $LiClO_4$ instead of TEAP as supporting electrolyte is shown in Fig. 5. 1^+Cl^- electro-reduction (Fig. 5, full line) occurs at $E_{cp1} = -0.73$ V (quasi-reversible) and $E_{cp2} = -0.99$ V (irreversible), being the more

Table 1

Effect of the supporting electrolyte on the current function for 1^+Cl^- first electro-reduction step (cf_{cp1}). Solvent: ACN; $[1^+Cl^-]_o$: 11 mM; scan rate: 0.2 V s⁻¹.

Supporting electrolyte (conc.)	cf_{cp1}^a ($AV^{-1/2} s^{1/2} M^{-1} 10^{-3}$)	$\Delta E = E_{cp1} - E_{cp2}$ (mV)
TBAP (0.34 M)	12	780
TEAl (0.10 M)	13	840
TEAP (0.10 M)	14	820
NaI (0.10 M)	14	550
TEAP (0.10 M) + $LiNO_3$ (25 mM)	21	–
KSCN (0.10 M)	21	660
$Mg(ClO_4)_2$ (0.10 M)	36	0
$LiClO_4$ (0.10 M)	43	270
$NaClO_4$ (0.10 M)	52	580

TEAP: tetraethylammonium perchlorate; TBAP: tetrabutylammonium perchlorate.

^a Current function ($cf = I_p/\nu^{1/2} [1^+Cl^-]_o$).

Table 2Current function for 1^+Cl^- first step electro-reduction in several solvents for two supporting electrolytes. $[1^+Cl^-]_0$: 5 mM.

Solvent (η)	ϵ	DN	AN	TEAP (0.1 M)				LiClO ₄ (0.1 M)			
				cf_{cp1}^a	n^b	ΔE^c (mV)	$cf \times \left(\frac{\eta_{solv}}{\eta_{ACN}}\right)^{1/2}$	cf_{cp1}^a	n^b	ΔE^c (mV)	$cf \times \left(\frac{\eta_{solv}}{\eta_{ACN}}\right)^{1/2}$
FM (3.302)	109.5	24	40	63.0	1	0	187	52.0	1	0	154
DMF (0.796)	36.7	26	16	52.0	2	780	76	48.0	2	670	70
TMU (2.31)	23.6	31	9	9.0	2	780	22	11.0	2	800	27
ACN (0.375)	37.4	14	19	19.0	2	790	19	43.0	2	250	43
EtOH (1.08)	24.3	19	37	8.5	2	230	14	8.2	2	120	14

 η : viscosity (cP). The current functions were normalized for the correction the viscosities relative to ACN viscosity (8th and 12th columns).^a cf_{cp1} : current function ($AV^{-1/2}s^{1/2}M^{-1}10^{-3}$) in solvent.^b Observed number of processes of electro-reduction.^c $\Delta E = E_{cp1} - E_{cp2}$.**Table 3**Ratios of current functions measured for 1^+Cl^- electro-reduction in several solvents, in the presence of acid or base, for two supporting electrolytes. $[1^+Cl^-]_0$: 5 mM. Concentrations of added acid or base are between parentheses.

Solvent	TEAP (0.1 M)		LiClO ₄ (0.1 M)	
	cf_{acid}/cf_{cp1} ([TFA]/mM)	cf_{base}/cf_{cp1} ([KOH]/mM)	cf_{acid}/cf_{cp1} ([TFA]/mM)	cf_{base}/cf_{cp1} ([KOH]/mM)
FM	0.8 (44)	0.9 (133)	1.2 (63)	0.9 (170)
EtOH	1.7 (200)	2.5 (19)	1.8 (28)	4.6 (74)
DMF	1.8 (320)	0.7 (38)	1.7 (76)	1.0 (42)
TMU	2.2 (110)	2.6 (31)	2.1 (170)	1.8 (51)
ACN	4.4 (340)	2.3 (93)	1.8 (110)	1.4 (160)

cathodic process the most affected for the change of the cation of the supporting electrolyte. The addition of KOH to the system 1^+Cl^- in solution of ACN with LiClO₄ caused several changes in the CV according to the concentration of the base added that were similar to the changes observed with TEAP. At the lower molar ratios R_{base} (e.g. R_{base} ca 4) the solution turned violet-coloured and only a cathodic peak was observed at $E_{cp1,base} = -0.93$ V (Fig. 5, dotted line). With the increase of R_{base} this peak shifted to more cathodic potentials. For higher ratios R_{base} (e.g. R_{base} ca 32) a peak of great intensity at $E_{cp1,base} = -1.29$ V was developed (Fig. 5, dash dotted line) with a simultaneous change of the solution colour from the initial blue to purplish red. The CV electro-reduction behaviour of 1^+Cl^- in ACN solution with LiClO₄ in the presence of added TFA was similar to that measured with TEAP as supporting electrolyte.

To learn more about the effect of the supporting electrolyte on 1^+Cl^- in solution of non-aqueous organic solvents, Uv-vis spectra were measured for the dye in ACN solution without and with TEAP or NaClO₄. The spectra (Fig. 6) show that the position of the

maxima of the absorption bands ($\lambda_{max} = 290$ and 650 nm) are not modified by the presence of the supporting electrolytes, but the absorbances are increased, being the change more pronounced in presence of NaClO₄ than with TEAP.

3.1.2. 1^+Cl^- in DMF solution

The electro-reduction behaviour of 1^+Cl^- in DMF solution with TEAP as supporting electrolyte was in some aspects similar to that observed for the dye in solution of ACN. The voltammogram of 1^+Cl^- in DMF/TEAP electrolytic solution showed two quasi-reversible processes at $E_{cp1} = -0.74$ and $E_{cp2} = -1.51$ V (Fig. 7, full line). In presence of added KOH (R_{base} ca 4) the blue coloured solution of 1^+Cl^- /DMF/TEAP turned greenish brown and the CV showed two nearby electro-reduction peaks at -1.45 and -1.60 V (Fig. 7, dotted line).

The effect of the supporting electrolyte on the electrochemical behaviour of 1^+Cl^- in DMF is shown in Fig. 8 (full line) for LiClO₄ used instead of TEAP. The CV in presence of LiClO₄ shows two peaks at $E_{cp1} = -0.73$ (reversible) and $E_{cp2} = -1.41$ V (irreversible). In presence of KOH (R_{base} ca 8) the blue solution of 1^+Cl^- /DMF/LiClO₄ turned of an intense violet colour, and the two cathodic peaks cp1 and cp2 observed in DMF-LiClO₄ system were replaced for a single irreversible peak at -1.41 V with a current intensity approximately twice that the measured in absence of base (Fig. 8, dotted line). The anodic sweep (inset in Fig. 8) shows a reversible couple centred at -0.70 V. A stable paramagnetic radical species was detected by ESR of the 1^+Cl^- /LiClO₄/DMF solution in presence of KOH (R_{base} ca 8). The spectrum is shown in Fig. 9, together with the fit. The hyperfine couplings (hfc) obtained from the fit are listed in the figure caption. A similar spectrum, with similar hfc, was obtained for 1^+Cl^- /TEAP/DMA solution

Table 4Current functions, peak potential for the 1^+Cl^- first electro-reduction process, and gap between E_{cp2} and E_{cp1} in several supporting electrolytes. $[1^+Cl^-]_0$: 5 mM; solvent: ethanol; [supporting electrolyte] = 0.1 M; sweep rate: 0.2 V s⁻¹.

Supporting electrolyte	cf_{cp1} ($AV^{-1/2}seg^{1/2}M^{-1}10^{-3}$)	number of electro-reduction processes observed (n).	E_{cp1} (V)	$\Delta E = E_{cp1} - E_{cp2}$ (mV)
TEAP	8	2	-0.76	230
TEACl	67	2	-0.76	260
LiClO ₄	9	2	-0.66	120
NaClO ₄	11	2	-0.66	140
LiSCN	16	2	-0.68	130
KSCN	22	2	-0.67	190
KI	25	2	-0.68	170
NaI	27	2	-0.76	190
LiI	28	2	-0.70	180
LiBr	40	2	-0.74	210
LiCl	41	2	-0.75	220
LiNO ₃	42	2	-0.68	190
TEABr	42	2	-0.67	220

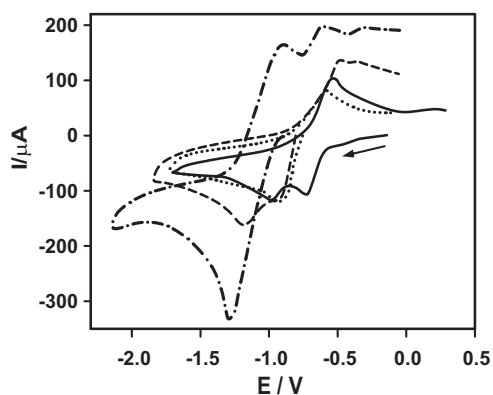


Fig. 5. (—) CV of 1^+Cl^- with: (●●●) 0.022 M KOH; (---) 0.10 M KOH; (-●-) 0.16 M KOH. $[1^+Cl^-]_0$: 5.1 mM; solvent: ACN; sweep rate: $0.2 V s^{-1}$. Supporting electrolyte: 0.1 M $LiClO_4$. The base was added from dilution of a 3.4 M KOH in ethanol stock solution.

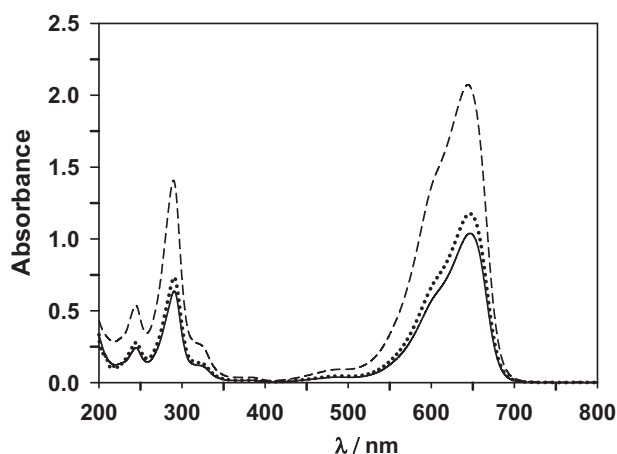


Fig. 6. (—): UV-vis spectra of 1^+Cl^- ; (●●●●●) with 0.10 M TEAP and (-●-) with 0.10 M $NaClO_4$. $[1^+Cl^-]_0$: 52 μM; solvent: ACN. The solutions were prepared from dilution of a 3.10 mM 1^+Cl^- in ACN stock solution.

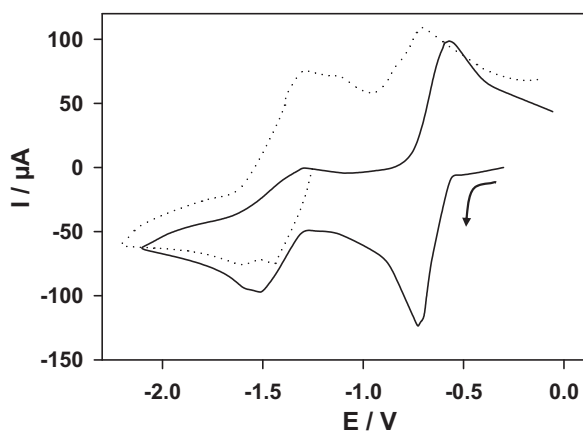


Fig. 7. Cyclic voltammograms of 1^+Cl^- (5.02 mM) in DMF solution (—); with added KOH (0.019 M): (●●●●●). Sweep rate: $0.2 V s^{-1}$. Supporting electrolyte: 0.1 M TEAP. The base was added from dilution of a 3.4 M KOH in ethanol stock solution.

rate constant for the dissociation of 1^+Cl^- is lower than the scan rate [27], the extension of the dissociation of 1^+Cl^- provides an *effective concentration* of 1^+ that determines the peak current intensity for the first and consequently for the second electro-

reduction process. A greater degree of association decreases the amount of 1^+ susceptible to be reduced.

The reversibility of the first step of charge transfer implies stability of the radical 1^* (see Scheme 3). The radical stability is associated with its structure and with the reactivity of the reaction medium. The delocalization of the unpaired electron in 1^* onto the aromatic rings and the presence of the electronegative N heteroatom in *benzitic* position justify the radical stability. The absence of a strong proton donor contributes to the radical stability in solution unlike what is observed in aqueous media (see Scheme 1) [7,19].

The initial concentration of 1^+Cl^- has also an effect on the cf_{cp1} (Fig. 3). cf_{cp1} decreases with the increase of the initial concentration of the dye, and seems to reach a constant value for the higher concentrations of 1^+Cl^- . For low concentrations of substrate, the dissociation equilibrium of 1^+Cl^- (C_{eq} in Scheme 2) is shifted to the right, thus increasing the relative concentration of free 1^+ to be electro-reduced.

4.1.1. Supporting electrolyte effect

The effect of the supporting electrolyte on the electrochemical behaviour of 1^+Cl^- has been studied in more detail in two solvents: ACN and EtOH.

4.1.1.1. 1^+Cl^- in ACN solution. The results in Table 1 show that there is a dependence of the cf_{cp1} with the nature of the cation and the anion of the supporting electrolyte. Na^+ or Li^+ cations produce a substantial increase in cf_{cp1} with respect to TEA^+ or TBA^+ cations (e.g.: $cf_{cp1,NaClO_4}/cf_{cp1,TEAP} = 3.7$, see Table 1). An increase in cf_{cp1} was also observed when $LiNO_3$ was added to a solution of 1^+Cl^- in ACN with TEAP ($cf_{cp1,LiNO_3-TEAP}/cf_{cp1,TEAP} = 1.5$, see Table 1). The effect of the anion of the supporting electrolyte on cf_{cp1} is seen for example by comparing the values for cf_{cp1} corresponding to experiments performed with $NaClO_4$ or NaI ($cf_{cp1,NaClO_4}/cf_{cp1,NaI} = 3.7$, see Table 1).

The current function changes are explained by the shift of the equilibrium C_{eq} in Scheme 2. When TEAP supporting electrolyte is changed by $NaClO_4$, the replacement of chloride (Cl^-) in 1^+Cl^- by perchlorate (ClO_4^-) (probably by $NaCl$ precipitation in the non-aqueous solvent) produces the less associate species $1^+ClO_4^-$ and the *effective concentration* of 1^+ to be reduced is increased. However, when the anion of the supporting electrolyte is iodide ion (NaI or $TEAI$), cf_{cp1} has a value similar to that obtained with TEAP (see Table 1) and the anion effect seems does not to exist. This apparently negligible anion effect on cf_{cp1} is explained as a result of the formation of 1^+I^- a more associated species which leads to a decreased *effective concentration* of 1^+ and compensates the change produced by the Na^+ cation. At difference of iodide, perchlorate ion is regarded to have almost no complex formation ability because of its excellent three-dimensional symmetry. Also, tetraalkylammonium ions (such as Et_4N^+) in solution may cause no chemical interactions through coordination or hydrogen bonding since the coordination numbers of the central elements in the species (ions) are fully satisfied, apart from their excellent three-dimensional symmetries.

Uv-vis spectra of 1^+Cl^- in ACN solution without and with added TEAP or $NaClO_4$ are shown in Fig. 6. The shape of the spectrum did not change with the addition of the inorganic salts, but absorbance values were markedly increased in the presence of $NaClO_4$. Uv-vis results are in agreement with CV results, provided the absorbance is caused principally or totally by 1^+ and not by (1^+Cl^-) .

There is not a significant effect of cations and anions of supporting electrolytes on the peak potential for the first electro-reduction process (E_{cp1} ca $-0.7 V$, reversible in all cases). However, for the second process the effect of the cation is important (e.g.: $E_{cp2} = -1.30 V$ (irreversible) for $NaClO_4$ (Fig. 2, full line) and $-1.60 V$

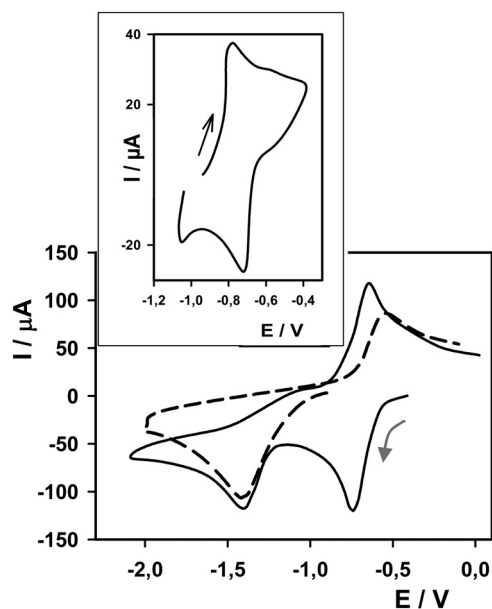


Fig. 8. CV of 1^+Cl^- (5.02 mM) in DMF solution (—); with added KOH (42 mM): (---). Sweep rate: 0.2 Vs^{-1} ; supporting electrolyte: 0.1 M LiClO_4 . The base was added from dilution of a 0.34 M KOH in ethanol stock solution. The offset shows an anodic sweep carried out from the rest potential of solution with 42 mM KOH.

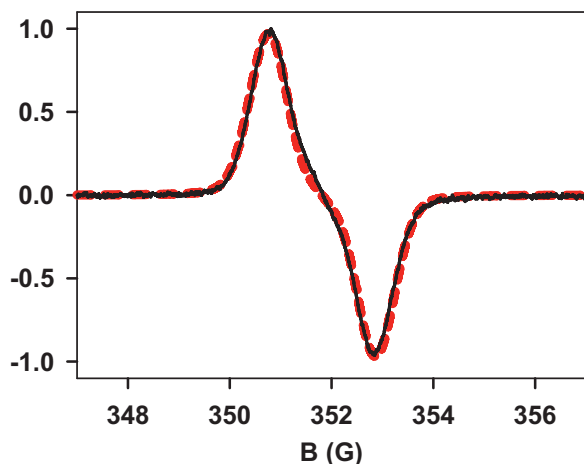


Fig. 9. (—): experimental ESR spectrum of 1^+Cl^- (5.02 mM) in DMF, 32 mM KOH, and 0.1 M NaClO_4 ; (---): simulated spectrum. The hyperfine coupling parameters giving the best fit are 0.70 mT (1N, heterocycle), 0.15 mT (2N, dimethylamine groups), 0.26 mT (2H), 0.15 mT (12H, methyl groups), and 0.06 mT (2H).

(reversible) for TEAP (Fig. 2, dotted line), in ACN solvent. The supporting electrolyte anion has an almost insignificant effect on $E_{\text{cp}2}$ as may be seen in Table 1 by comparing ΔE values for NaI and NaClO_4 (ΔE : 550 and 580 mV, respectively), and for TEAP and TEAI (ΔE : 820 and 840 mV, respectively).

$E_{\text{cp}1}$ remains practically unchanged with the supporting electrolyte. This behaviour is explained considering the species (1^+ and 1^*) involved in the first electron transfer reaction. The absence of negative charges in these species makes their interactions with acid metal cations less likely.

The shift of $E_{\text{cp}2}$ is higher the stronger as a Lewis-acid is the cation of the supporting electrolyte. The observed effect implies a stabilization of the electrogenerated base 1^- though the formation of complexes with acid metal cations. The localization and availability of the negative charge in 1^- contributes favourably

to an increased interaction. The presence of the electronegative heterocyclic N atom in 1^- mainly confines the negative charge on it and the charge density is greater than if the negative charge would be dispersed in the aromatic rings and the interaction with cations would be less effective. Moreover, the localization of the charge on the N atom allows simultaneous aromaticity of both phenyl (Scheme 3). The extent of the association of 1^- with the cation increases with increasing positive charge density on the cation and establishes the increasing order of association and the anodic potential shift as $(\text{Et})_4\text{N}^+ < \text{K}^+ < \text{Na}^+ < \text{Li}^+ < \text{Mg}^{2+}$. This process of association and stabilization also affect the reversibility of the second electron transfer. For the weakest Lewis acid and barely complexing cations (R_4N^+) this process is reversible (e.g. Fig. 2, dotted line), while for the stronger Lewis acid cations the formed complexes should be so strongly bonded that the second electrochemical reaction becomes irreversible (e.g. Fig. 2, full line).

4.1.1.2. 1^+Cl^- in EtOH solution. Table 4 shows $\text{cf}_{\text{cp}1}$, $E_{\text{cp}1}$ and peak potential separation ($\Delta E = E_{\text{cp}1} - E_{\text{cp}2}$) values for the electro-reduction of 1^+Cl^- in ethanol solvent in the presence of a variety of supporting electrolytes. There is an effect of the supporting electrolyte on $E_{\text{cp}1}$ and $E_{\text{cp}2}$, but the shift of $E_{\text{cp}2}$ is higher than the corresponding to $E_{\text{cp}1}$ (ca. 100 mV for all studied electrolytes). Electrolyte cations have not an important effect on the potential peaks as for example is seen in iodide and perchlorate series (see Table 4 iodide series, LiI: $\Delta E = 180 \text{ mV}$; NaI: $\Delta E = 190 \text{ mV}$; KI: $\Delta E = 170 \text{ mV}$; perchlorate series, TEAP ($\Delta E = 230 \text{ mV}$) > NaClO_4 ($\Delta E = 140 \text{ mV}$) > LiClO_4 ($\Delta E = 120 \text{ mV}$)). The strong solvation of 1^- species by EtOH (solvent with high Gutmann acceptor number, AN: 37, and H-bond donor ability) diminishes the interaction of 1^- with the electrolyte cation, hence the shift of $E_{\text{cp}2}$ s are, in general, smaller than in ACN. The anion effect on ΔE can be seen in the lithium series: LiClO_4 ($\Delta E = 120 \text{ mV}$) < LiSCN ($\Delta E = 130 \text{ mV}$) < LiI ($\Delta E = 180 \text{ mV}$) < LiNO_3 ($\Delta E = 190 \text{ mV}$) < LiBr ($\Delta E = 210 \text{ mV}$) < LiCl ($\Delta E = 220 \text{ mV}$). Small anions form more strong bounds with Li^+ cations. This behaviour has an effect on the proportion of free Li^+ that is complexing with 1^- species. Supporting electrolyte effect on the electrochemical behaviour of 1^+Cl^- in EtOH solution is different to that measured in ACN solvent. The first electro-reduction peak of the dye in ACN solution is not shifted by changing the supporting electrolyte, but it is shifted in EtOH solvent. $E_{\text{cp}2}$ is affected by the supporting electrolyte anion in EtOH but is unchanged in ACN. The supporting electrolyte effect on $E_{\text{cp}1}$ in EtOH is due to the different solvation of anions combined with the positive charge involved in stage E_1 (Scheme 2). The $\text{cf}_{\text{cp}1}$ s ranged from 8 with TEAP up to 67 with TEACl (Table 4). These different values are attributed to the same causes that for ACN solutions. However, 1^+Cl^- in EtOH solution showed a clearly different electrochemical behaviour for some electrolytes. $\text{cf}_{\text{cp}1}$ s measured with LiClO_4 or NaClO_4 in ethanol solutions are relatively smaller (vs. TEAP) than in ACN solvent ($\text{cf}_{\text{cp}1, \text{LiClO}_4, \text{EtOH}} / \text{cf}_{\text{cp}1, \text{TEAP, EtOH}} < \text{cf}_{\text{cp}1, \text{LiClO}_4, \text{ACN}} / \text{cf}_{\text{cp}1, \text{TEAP, ACN}}$). It seems that there is a greater effect of the anions in ethanol than in ACN solution. Due to the strong solvation of anions, especially for the smaller ones as Cl^- , in ethanol (very poor solvation in ACN), the reaction C_{eq} (Scheme 2) is displaced to the right side with respect to a large anion (ClO_4^-), which is less solvated.

4.1.2. Solvent effect

Experiments performed with the same supporting electrolyte are taken into account to analyse the solvent effect. CV results in Table 2 show a solvent effect on the number of peaks and/or on $\text{cf}_{\text{cp}1}$. With TEAP or LiClO_4 as supporting electrolyte (see Table 2, n, 6th and 10th columns, respectively) two reduction processes were detected for EtOH, ACN, TMU and DMF, and only one process for FM. Solvent effect on the potential separation $\Delta E = E_{\text{cp}1} - E_{\text{cp}2}$ is explained by considering the strength of the solvents as Lewis

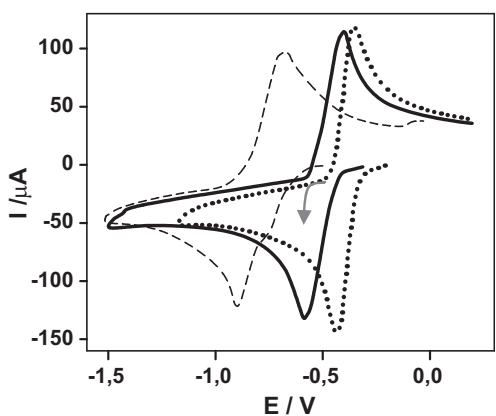


Fig. 10. Cyclic voltammograms of 1^+Cl^- (5.08 mM) in FM solution (—) with: 0.11 M KOH (---); 0.22 M TFA (●●●). Supporting electrolyte: 0.1 M $LiClO_4$. Sweep rate: $0.2 V s^{-1}$. The base was added from dilution of a 3.4 M KOH in ethanol stock solution.

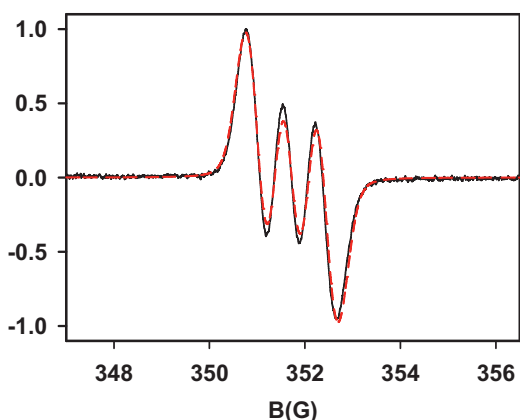
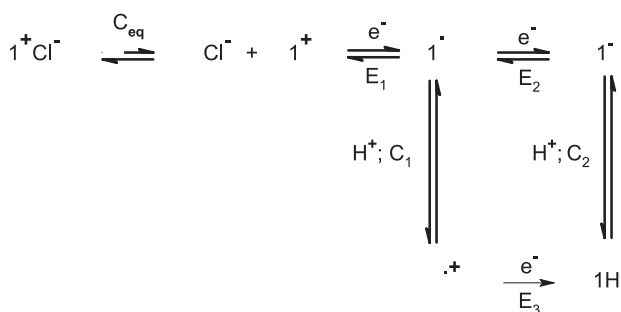


Fig. 11. (—), experimental ESR spectrum of 1^+Cl^- (5.0 mM) in EDA solution and 0.10 M $LiClO_4$ measured 25 min after the preparation of the solution; (---), simulated spectrum. The hyperfine coupling parameters giving the best fit are 0.70 mT (1N, heterocycle), 0.11 mT (2N, dimethylamine groups), 0.18 mT (2H), 0.075 mT (12H, methyl groups), and 0.04 mT (2H).



Scheme 2. General electrochemical scheme proposed for the electro-reduction of 1^+Cl^- in non-aqueous solvent solution in the absence and presence of a proton donor.

acids or bases. Empirical parameters such as the Gutmann acceptor number (AN) are used as a quantitative measure of the Lewis acidity of the solvents [28]. The effect of the AN on ΔE for the studied solvents with TEAP is also shown in Fig. 12. Solvents with small AN value (TMU, DMF, ACN) have little influence on the stabilization of 1^- anion. Therefore, the experimentally measured E_{cp2} is in the zone of more cathodic values. Conversely, as consequence of the stabilization of 1^- for the solvent with high AN (EtOH) a less ΔE is observed, while for FM with the higher AN only

one electro-reduction peak is observed. The Li^+ cation effect must be considered with $LiClO_4$ as supporting electrolyte (Table 2), and therefore in general, ΔE s are lower than with TEAP. TMU solvent has a low AN but a high donor number (DN: quantitative measure of Lewis basicity), then TMU has ability to solvate Li^+ cation and thus the stabilization of 1^- anion for this metal cation is affected and similar values for ΔE result for both supporting electrolytes.

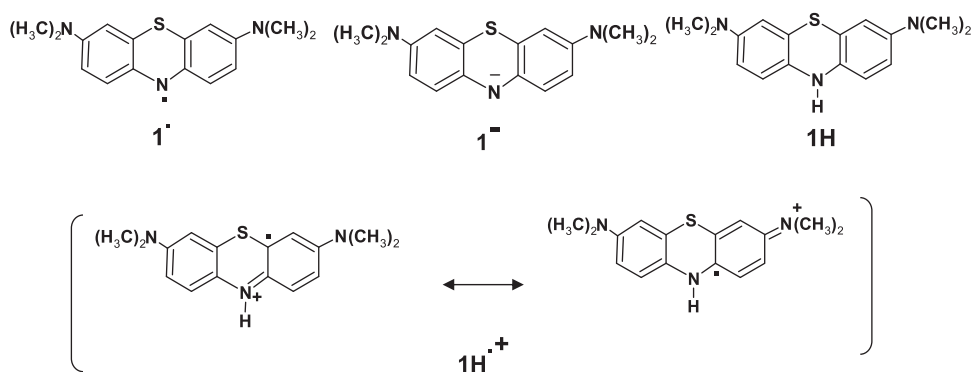
As discussed above, the supporting electrolyte effect (see subsection 4.1.1.) on E_{cp1} (E_1 , Scheme 2) is not very significant. The solvent effect on E_{cp1} is also not important. This behaviour is understood assuming a dispersion of the positive charge on the dye molecule by resonance. Therefore, the interaction of the anions and/or solvents with 1^+ species will be negligible. Spectroscopic and electrochemical results support this assumption (see below in this subsection). Although E_{cp1} (step E_1 , Scheme 2) is not affected by the investigated supporting electrolytes and solvents, \mathcal{C}_{cp1} and also \mathcal{C}_{cp2} (step E_2 , Scheme 2) are considerably influenced (Tables 1, 2, 4). The above mentioned ionic association in the molecule of the dye (1^+Cl^-) in non-aqueous solvents, that is to say, the homogeneous equilibrium reaction preceding the step E_1 (see C_{eq} , Scheme 2) accounts for the observed behaviour.

The hypothesis of 1^+Cl^- association in non aqueous solvents is supported by the results obtained in CV anodic potential sweeps (Supplementary Material). 1^+Cl^- electro-oxidation is mainly due to chloride oxidation. Chloride oxidation in the absence of 1^+Cl^- is less anodic than in presence of the dye. The free non-associated Cl^- ion is more easy to be electro-oxidized than chloride anion associated with 1^+ . The (1^+ anion $^-$) association was reported for 1-tetraphenylborate system (1^+TPB^-) in aqueous solution [29], therefore, and with more reason, (1^+ anion $^-$) ionic association will operate between anions and cations in non-aqueous solvents of lower solvation ability.

In aqueous solution, the two bands in the visible region are mainly assigned to monomer (644 nm) and dimer (shoulder at 605 nm) equilibrium. As the shape of the spectrum measured in our work is the same that in aqueous solution, an alternative explanation would in principle seem possible but we consider it less likely. Many works [23,30–33] analyse the structure of 1^+Cl^- in aqueous solution, where 1^+Cl^- would be totally dissociated into 1^+ and Cl^- (Scheme 2, C_{eq} completely displaced to the right side). The general hypothesis that emerges from those works is the existence of various states of aggregation whose presence is predominantly inferred from spectroscopic data. The Uv–vis behaviour (increased absorbance and the not altered shape of the spectrum) measured in our work in the presence of the electrolytes could be rationalized by considering that the possible formation of aggregates is not altered by the presence of the electrolytes. That the electrolytes do not modify the aggregates formation in solution would be a rare behaviour. Therefore, we consider that a change in the aggregates formation equilibrium position is an unreasonable explanation for our Uv–vis results.

The solvent effect on the \mathcal{C}_{cp1} (E_1 , Scheme 2) is also evident (Table 2). However the relationship of the observed \mathcal{C}_{cp1} with some characteristic parameters of the solvents is complicated. Nevertheless, there is a rough dependence of the \mathcal{C}_{cp1} s for 1^+Cl^- /solvent/ $LiClO_4$ and 1^+Cl^- /solvent/TEAP systems with the dielectric constant (ϵ) of the solvent: \mathcal{C}_{cp1} increases with the increase in ϵ , because C_{eq} (Scheme 2) is shifted to the right side.

Certainly, solvent and supporting electrolyte effects occur simultaneously on the electrochemical behaviour of 1^+Cl^- in non aqueous solvent solutions, and as a result a quantification of these effects on the base of characteristic parameters of the solvent is difficult. Moreover the situation becomes more complicated by the presence of two simultaneous electro-reduction stages for the solvent FM. Another difficulty lies in the fact that for electrochemical measures is only possible to use solvents of medium and high



Scheme 3. Formal structures for the species, 1^{\bullet} , $1^{\bullet-}$, $1H^{\bullet+}$ and $1H$ in Scheme 2.

polarity. A more complete study should include solvents with low polarity. However it is important to observe that if 1^{\bullet} must be electro-generated to be used as a reagent in other reaction, FM solvent must not been employed.

4.1.3. CV electro-reduction of $1^{\bullet}Cl^-$ in non-aqueous solvent in the presence of added acid or base

The addition of an anhydrous acid or a base drastically changed the voltammetric behaviour of $1^{\bullet}Cl^-$ in solution of the non aqueous solvents (Figs. 4, 5, 7, 8, 10 and Table 3).

4.1.3.1. In presence of acid. CVs for $1^{\bullet}Cl^-$ in ACN solution measured in the presence of an excess of TFA, to ensure pseudo first order for protonation reactions (see Scheme 2), did not change upon further additions of acid (typical examples are shown in Fig. 4). When the excess of acid was added to a solution of $1^{\bullet}Cl^-$ in a solvent/supporting electrolyte particular system, only one new electro-reduction peak was observed, shifted to a more anodic potential than E_{cp1} measured in the absence of acid. This peak should correspond to a two-electron global electro-reduction process (more about this subject will be discussed below in this subsection). The voltammetric behaviour of $1^{\bullet}Cl^-/TFA$ system should therefore take into account the general Scheme 2. We discard previous protonation of 1^{\bullet} because TFA in non-aqueous solvent is not so strong acid as in water (e.g. $pK_{a,ACN,25^{\circ}C} = 12.65$, [34]; $pK_{a,water,25^{\circ}C} = 0.50$ [35]). Protonation of 1^{\bullet} with HCl in aqueous solution was reported [36].

The formal structures for the species, 1^{\bullet} , $1^{\bullet-}$, $1H^{\bullet+}$ and $1H$, involved in Scheme 2 are the following (see Scheme 3, all structures shown are resonance hybrids, as $1H^{\bullet+}$; more stable resonance structures are shown) by

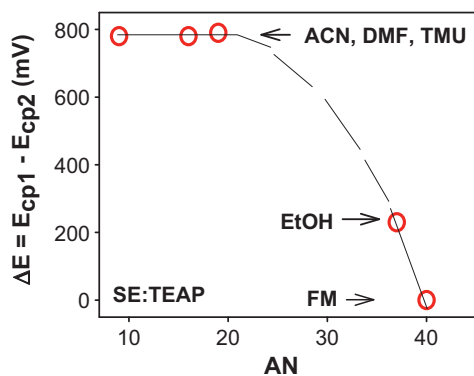


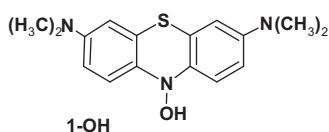
Fig. 12. Plot of the peak potential separation for the two electro-reduction of $1^{\bullet}Cl^-$ versus acceptor number of solvent. Supporting electrolyte: 0.1 M $LiClO_4$. Sweep rate: $0.2 V s^{-1}$.

The greater stability of the anionic species $1^{\bullet-}$ with respect to the radical-cation $1H^{\bullet+}$, and the charges involved in step C_2 would justify the steps $E_{1,rev} - E_{2,rev} - C_2$ as the most probably for electro-reduction of $1^{\bullet}Cl^-$ in non aqueous solution in the presence of acid.

The magnitude of the anodic potential shift for the new electro-reduction peak observed in the presence of added TFA is rationalized taking into account the following several factors:

- The typical shift due to $E_{rev} - C$ (Scheme 2: $E_{1,rev} - E_{2,rev} - C_2$ mechanism). In ref [37] (Figs. 12.3.10 and 12.3.11) it is shown that the anodic shift increases with the increase of the homogeneous rate constant (at a given potential sweep scan rate, v). The magnitude of the acidity constant (K_a) of TFA in non-aqueous solvent will determine the degree of reversibility of C_2 chemical reaction. For solvents with high dielectric constant (e.g. FM, $\epsilon = 109$), C_2 will be reversible (Supplementary Material) because K_a has a high value, while in solvents with lower dielectric constant (e.g. ACN, $\epsilon = 37$), K_a has a lower value, and a less reversible CV was observed (Fig. 4).
- There is a solvent effect on the potential for the electro-reduction peaks (principally on E_{cp2}), as it was explained (see subsection 4.1.2) for experiments performed in absence of added acid. This effect also appears on the potential of the electro-reduction peak in the presence of acid. A direct relationship of $\Delta E_{acid} = E_{cp1,acid} - E_{cp1}$ with the Gutmann donor number DN [27] is observed: the higher DN, the smaller ΔE_{acid} . For example $\Delta E_{acid} = 760$ mV for ACN (DN: 14) and 465 mV for FM (DN: 24), with TEAP. Because the solvent effect on E_1/E_2 electrochemical steps was already considered in absence of added acid, the solvent effect now taken in account should be exclusively on C_2 (see Scheme 2). Strong solvation of protons by solvents with high DN produces a decrease in the protonation (and on equilibrium, C_2) rate constant, and therefore a small shift of the electro-reduction peak potentials in the presence of acid is observed. In this case the global reaction trends to be reversible (Fig. 10), while for solvents with lower DN the global electrochemical reaction is less reversible (Fig. 4).
- The supporting electrolyte also influences the anodic potential shifts, but the effect is less predictable due to the involvement of negative and positive charges.

The peak current intensity is also affected by the presence of added TFA. In solvents for which the two electro-reduction processes E_1/E_2 are separately observed (EtOH, DMF, TMU, ACN) the current intensity measured in the presence of acid (two electrons) should be twice that measured in the absence of added acid (one electron). Table 3 shows that the value for the ratio cf_{acid}/cf_{cp1} varies between 0.8 and 4.4 with TEAP as supporting electrolyte. Here, the same considerations with respect to the equilibrium of association (C_{eq} , Scheme 2) described above should



Scheme 4. Structure for the adduct formed by reaction of 1^+Cl^- with KOH.

be taken into account. For FM solvent in which in absence of acid both electro-reduction processes occurs at the same potential, the current measured without or with added acid should be similar (Table 3). For this solvent with high dielectric constant, the ionic association is limited, so that higher current intensity is measured. For the other solvents (EtOH, DMF, TMU, ACN) in which the association (C_{eq} , Scheme 2) is more important, the added acid also causes the shift of this equilibrium to the right side, and the measured ratio cf_{acid}/cf_{cp1} is higher.

4.1.3.2. In presence of base. The main reaction of 1^+Cl^- in the presence of KOH is the formation of the adduct $1-OH$ (Scheme 4) with a typical purple colour. This species, soluble in most common organic solvents, was reported in aqueous NaOH [32].

The neutralization of the positive charge of 1^+ hinders the electro-reduction processes of the dye, and therefore the voltammetric reduction peak was observed at potentials more cathodic than in absence of base in all studied systems (Figs. 4, 5, 7, 8, 10). CV electro-reduction was quasi-reversible (e.g. FM, Fig. 10).

The formation of the stable 1^* radical by chemical reduction of 1^+Cl^- in homogeneous phase in presence of KOH was observed as a secondary reaction in DMF (Fig. 8). The formation of the 1^* radical was evidenced by an anodic sweep carried out from the rest potential of the solution (inset Fig. 8). The presence of the radical in solution was confirmed by ESR measurements in similar conditions (e.g. see Fig. 9 for $1^+Cl^-/DMF/KOH$). The spectrum was simulated using hyperfine parameters similar to those reported by Stanoeva [38], where a similar ESR spectrum was obtained for 1^+Cl^- reduction with potassium in dimethoxyethane solvent.

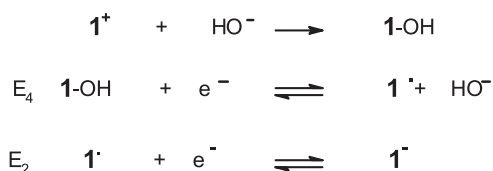
A reaction mechanism to explain the observed 1^+Cl^- electro-reduction behaviour in the presence of KOH is postulated in Scheme 5.

Both electro-reduction processes (E_4 and E_2) occur at the only voltammetric peak observed. The 1^* radical produced at stage E_4 readily accepts another electron at E_2 ($E_2 \geq E_4$) [39]. In the anodic sweep the species 1^- and 1^* are re-oxidized irreversibly and reversibly, respectively (not shown).

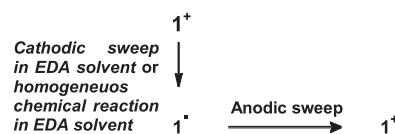
Solvent and supporting electrolyte have an effect on the cf_{base}/cf_{cp1} ratio (Table 3) and on the peak potentials, although the dissociation equilibrium (C_{eq} , Scheme 2) does not exist in the presence of base due to the $1-OH$ adduct formation (Scheme 4).

To compare the values of the cf_{base}/cf_{cp1} ratio it should be considered that besides the absence of the homogeneous equilibrium C_{eq} in the presence of the base, the diffusion coefficients of the involved species ($1-OH$ and 1^+Cl^-) could be different.

Other authors have proposed the formation of species resulting from the lost of one $-N(CH_3)_2$ and one methyl group in aqueous



Scheme 5. General electrochemical scheme proposed for electro-reduction of 1^+Cl^- in non-aqueous solvent solution in the presence of KOH.



Scheme 6. Chemical and electrochemical reactions of 1^+Cl^- in EDA solution.

solution in the presence of NaOH [40]. However, our results obtained in experiments performed with 1^+Cl^- in solution of EtOH and ACN solvents in the presence of KOH, indicate that 1^+Cl^- produces reversibly only the species $1-OH$, because the neutralization (or acidification) of a solution of 1^+Cl^- in the presence of base (we proposed $1-OH$) with TFA leads to the original colour and CV, that it to say, CV measured in absence of acid (or in acid media).

4.1.4. EDA solutions

The basic and reductor solvent EDA [41] reacts with 1^+Cl^- in homogeneous phase to yield 1^* (Scheme 6). This radical was stable at room temperature for about a week, and then its decomposition started to be significant. The formation of 1^* was observed by CV (Supplementary Material) in cathodic and anodic sweeps measured from the rest potential of the solution, at different reaction times.

The ESR spectrum of a solution of 1^+Cl^- in EDA measured 25 min after the preparation of the solution is shown in Fig. 11, together with the fit. The resulting values of the hyperfine parameters are included in the figure caption. Hyperfine interactions are seen with all magnetic nuclei, indicating that the unpaired spin is delocalized within the entire system, with the highest hyperfine coupling constant (hfc) corresponding to the ^{14}N nucleus in the heterocycle.

4.2. Anodic behaviour

The supporting electrolyte effect on the anodic peak potentials and cf s for 1^+Cl^- in ACN solution is shown in Supplementary Material. The increase in cf when the CVs were registered in the presence of $NaClO_4$ followed the same trend as the cathodic response. This behaviour confirms the hypothesis of the change of current intensities due to the homogeneous equilibrium C_{eq} (Scheme 2). The anodic process observed has been attributed to chloride oxidation in aqueous solution [18]. The products of this oxidation react with 1^+Cl^- molecule because in the subsequent cathodic sweep new peaks at -0.07 and -0.44 V are observed. Also in this cathodic sweep a decrease in the current intensity for the first step of electro-reduction of 1^+Cl^- (compared with I_{cp1} in an initial cathodic sweep), and the almost disappearance of the peak corresponding to the second stage is observed. The incorporation of one or two chlorine atoms to the molecule is the most probable reaction, as observed in aqueous solution [14].

5. CONCLUSIONS

The knowledge of the effect of the experimental conditions on the electrochemical behaviour of 1^+Cl^- in solution of non-aqueous solvents is particularly important if the best conditions to perform large scale electrolysis to transform the 1^+Cl^- molecule must be chosen. In this way it will be possible to produce neutral or charged intermediates which can react in homogeneous phase to produce new molecules. The fact that commonly the successive stages of electron transfer are separated in non-aqueous solvents would make possible to design and perform different ways of reaction (e.g. to use 1^* as reagent for other electrochemical or chemical reaction), and hence to obtain derivatives of the parent molecule which synthesis in aqueous media will be difficult.

While this paper discusses mainly the cathodic behaviour of 1^+Cl^- , it is possible to carry out a more detailed study of the anodic response of this dye.

- a 1^+Cl^- in neutral non-aqueous media is electro-reduced and electro-oxidised. Electro-reduction of 1^+Cl^- takes place by two successive one-electron charge transfer processes. The anodic process has been attributed to chloride oxidation. The reversibility, the current function and the potential peak separation of the electrochemical processes depend on the solvent/supporting electrolyte system and the concentration of the dye. In the first step, the dye becomes a paramagnetic and stable radical and in the second step the radical becomes an anion. This electrochemical behaviour is different from that observed in aqueous medium.
- b Effects of the experimental variables on the peak potentials and the peak current functions for the two reduction steps are reported, but quantitative relationships are not established because various effects occur simultaneously.
- c Acid or base added to the electrolytic medium originates a drastic change in the electrochemical response. In both cases it was established the mechanistical origin of the observed changes.
- d In some basic medium (KOH/DMF or EDA solutions), the chemical formation of the same stable radical generated by electro-reduction of the dye was detected and it was characterized by EPR spectroscopy. In other solvents (KOH/EtOH or ACN) a new species is reversibly formed.
- e Dye dissociation equilibrium preceding the first charge transfer step in non-aqueous solvent solution is proposed. The shift of this equilibrium markedly influences the electrochemical behaviour of the dye.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.electacta.2015.01.196>.

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