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Acid-base equilibria in ethaline. An approach providing a strategy for the pH modulation in deep eutectic solvents

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ABSTRACT

The possibility of changing the pH in deep eutectic solvents when they are used as solvent media is fundamental in chemistry, since acid-base equilibria are of critical importance for almost all areas of chemistry, such as synthetic reactions, isolation of specific analytes, chromatographic separations, protein stability and enzyme catalysis. Such a possibility of modulating the pH of DESs is particularly interesting for enhancing the electrocatalytic properties of some electrode surfaces or promoting the stability and in some cases improving the activity of biorecognition systems such as antibodies, enzymes and aptamers that are particularly useful for biosensor applications. The aim of this investigation was the evaluation of the possibility of easily modulating the acid-base properties of the DES ethaline, adopted in our tests as the prototype of DESs because it displays a moderate viscosity at room temperature, by simply adding typical pH buffers such as weak acids or bases or salts consisting of weak acids and strong bases. This approach results to be particularly advantageous compared to that often used of modifying the proton activity of DESs by changing one or both of its precursors (HBA or HBD), since it allows their chemical-physical properties, such as density, viscosity, conductivity and surface tension, to be kept unchanged. With this purpose, acid-base equilibria in the DES ethaline were studied using cyclic voltammetry, pH measurements at glass electrodes, acid-base titrations and spectrophotometric measurements by exploiting suitable electrochemical probes, such as hydroquinone and alizarin: this last being an acid-base indicator. The results obtained with these measurements are all consistent with each other and provide an effective strategy to achieve pH modulation in DES, since the approach taken here in ethaline can be easily transferred to any other DES, provided it is capable of solubilizing the desired buffer species.

1. Introduction

Nowadays, the green trends driving modern society towards economically and environmentally sustainable changes are directing the analytical chemistry research towards alternative and innovative solutions in the development of new analytical approaches [1]. This has led to an ever-increasing demand for inexpensive miniaturized analytical devices that can also be used outside laboratories and capable of performing analyses in short times, thus significantly reducing waste production and consumption of energy and reagents [2,3].

This demand is accompanied by the growing need for the utilization of green solvents with properties and characteristics functional to these emerging changes in terms of eco-compatibility, low cost, safety and easy transportability [4–7]. In this context, deep eutectic solvents (DESs) represent a valid solution since they can be prepared with precursors that can be derived from biodegradable and/or renewable sources and, moreover, they display low volatility and flammability. DESs have found application in several fields ranging from metallurgy and electrodeposition, gas capture, battery technologies, biocatalysis, organic chemistry, nanomaterials synthesis, cosmetics or pharmaceuticals (THEDES) [8,9].

In the analytical field they are particularly suitable for the use as modifiers of stationary phases in chromatographic separations, as media for electrophoretic separations, as extraction solvents and as electrolytes for electroanalytical applications [10–14]. In particular, DESs have proven to be a viable alternative to aqueous and non-aqueous media as they have several advantages, including a low vapor pressure and a high extractive power against a wide range of substances. Their use can be particularly suitable when small volumes are employed, such as in the case of miniaturized cells and paper-based devices where the

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evaporation of the solvent can greatly affect the measurement repeatability [15], or when electroanalytical sensors are adopted for monitoring gas-phases by three-electrode cells with planar configuration where the presence of the electrolytic medium between the electrodes is required for the entire monitoring duration [16].

The most frequently used DESs consist of the hydrogen bond acceptor (HBA) choline chloride (ChCl), mixed with hydrogen bond donors (HBDs) such as alcohols, amides, carboxylic acids or sugars [17–19]. Choline is non-toxic, inexpensive and can be classified as a provitamin in Europe [20]. Among these choline-based DESs, ethaline (choline chloride/ethylene glycol in the molar ratio 1:2) is used in many applications also because it is characterized by low cost, high biodegradability and low toxicity [21]. In addition, it has several advantages as a solvent as it is stable at room temperature and exhibits high electrical conductivity and low viscosity (about 36 mPa s at 25 °C), in comparison with other choline-based DESs such as maline, oxaline, reline and glyceline [20,22–28].

The physicochemical properties of choline-based DESs, such as conductivity, density, surface tension, acidity, and basicity, can be easily modified to meet specific requirements by changing the nature of the HBD, as well as the HBD/choline ratio. Therefore, a series of choline-based DESs have been synthesized using p-toluenesulfonic acid (PTSA), trichloroacetic acid (TCA), monochloroacetic acid (MCA), trifluoromethansulfonic acid, urea and guanidine as HBDs to control their acidity or basicity [29–32]. Unfortunately, the modification of the acid-base properties of choline-based DESs by using these HBDs with strong acid character leads to a notable increase of their viscosity and this entails a negative effect on several aspects related to their use (*e.g.* ChCl-PTSA 1:1, 2:1 and 1:2 at 45 °C have viscosity of 170, 284 and 160 mPa s, respectively) [33]. Thus, for instance, their sampling with micropipettes becomes difficult, the sensitivity for electroanalytical measurements lowered, due to the decrease of the diffusion coefficient and the diffusion of chemical species in extractions processes becomes heavily limited, as well as becomes limited the diffusion of analytes in DESs soaked in porous media, as happens in paper-based devices [34–36].

The possibility of changing the pH of solvent media without drastically altering their original composition is fundamental in chemistry, since acid-base equilibria are of critical importance to almost all areas of chemistry, such as synthetic reactions, isolation of specific analytes, chromatographic separations, protein stability and enzyme catalysis [37–40].

The pH of water can be easily controlled by dissolving suitable species displaying specific acid-base properties, while in non-aqueous media, such as organic solvents and ionic liquids (ILs), the pH control is more difficult. Consequently, the reactions occurring in these media are frequently investigated without a pH control, even though the use of buffers composed of highly hydrophobic acids and their sodium salts or highly hydrophobic bases and their hydrochlorides has been suggested [41–43]. More recently, ILs with buffer characteristics, defined as ionic liquid buffers (IL-buffers), miscible with either non-aqueous polar solvents such as methanol, dimethylformamide and dichloromethane or ILs like [BMIM][PF_6] and [BMIM][BF_4] have been proposed [44,45].

The aim of this investigation was to evaluate the possibility of easily modulating the acid-base properties of DESs by simply adding weak acids or bases or salts consisting of weak acids and strong bases. For this purpose, the use of both organic and inorganic salts was tested, including those used to make Good's buffers (CAPS and CAPSO, together with their sodium salts) [46,47], in order to also create media potentially suitable for bioanalytical applications. Acid-base equilibria in proton-electron transfer reactions were studied using both spectrophotometric and voltammetric approaches, by exploiting suitable electrochemical probes, such as hydroquinone and alizarin: this last being an acid-base indicator.

All our investigations were conducted in ethaline (choline chloride/ethylene glycol in the molar ratio 1:2), although strictly speaking it is not really a DES but rather an electrolyte, *i.e.* choline chloride (ChCl) dissolved in ethylene glycol (EG). In fact, the true eutectic point was found to be in the ChCl:EG molar ratio of 1:4.85 [48,49]. Despite this, we have used ethaline because it behaves like an ideal binary mixture which still has characteristics very similar to those of a DES.

2. Experimental section

2.1. Chemicals and instrumentation

Choline chloride, ethylene glycol, potassium hexacyanoferrate(II), ferrocene, hydroquinone, alizarin Red S, potassium chloride, sulfuric acid, sodium hydroxide, sodium acetate, acetic acid, sodium hydrogen phosphate, sodium dihydrogen phosphate, sodium phosphate, phosphoric acid, 3-cyclohexylamino-1-propanesulfonic acid (CAPS) and CAPS sodium salt, 3-cyclohexylamino-2-hydroxy-1-propanesulfonic acid (CAPSO) and CAPSO sodium salt, sodium citrate, disodium citrate, trisodium citrate, citric acid, tris(hydroxymethyl)aminomethane (Tris base), tris(hydroxymethyl)aminomethane hydrochloride (Tris HCl) were purchased from Sigma-Aldrich (Sigma-Aldrich, Milan, IT). They were of analytical reagent grade and were employed without further purification. Ultrapure water was obtained from an Elgastat UHQ-PS system (Elga, High Wycombe, UK).

pH buffers in ethaline or in ultrapure water were prepared by dissolving a proper amount of acid and its conjugated base in the solvent. In this regard, it is worth underlining that while the dissolution of CH_3COOH and CH_3COONa in ethaline occurred almost immediately, the dissolution of the phosphate species took a long time owing to the high viscosity of the medium. Therefore, their dissolution in ethaline was carried out under stirring for a whole night inside a desiccator.

Potassium hexacyanoferrate(II), ferrocene, hydroquinone, alizarin Red S (10 mM) solutions were prepared by adding weighed amounts of these species to known volumes of ultrapure water or ethaline containing or not appropriately chosen pH buffers. A conventional cell equipped with a glassy carbon electrode (CHI Instruments, Texas, USA), a platinum counter electrode and a Ag/AgCl, 3 M KCl reference electrode (CHI Instruments, Texas, USA) was used. In order to equip standard CHI aqueous reference electrodes with additional salt bridges, Teflon heat-shrink tubes closed at their terminal end with a porous septum (Princeton Applied Research, Ametek, Photo Analytical SRL, Settala, Milan, Italy) were inserted at their lower end.

Spectrophotometric measurements were performed with a benchtop spectrophotometer (Varian Cary 50 bio, Victoria, AUS).

3. Results and discussion

3.1. Identification of the most suitable reference electrode for measurements in ethaline

While the development of stable reference electrodes in ionic liquids was the subject of several investigations [50–52], this issue was rather neglected in DES. In order to acquire reliable voltammograms during the electroanalytical measurements described below, particular attention was paid to the identification of a reference electrode capable of assuming in ethaline a stable and necessarily reproducible potential over time. The DES ethaline was in fact adopted in all our tests as the prototype of DESs because it displays a moderate viscosity at room temperature [53–55], thus making easier all the electroanalytical measurements reported below. As previously reported, this can be considered in contrast to the fact that ethaline is not in principle a true DES, but it still behaves as an ideal binary mixture which nevertheless displays characteristics coinciding with those of a DES.

All our tests conducted with this purpose were performed in solutions of potassium hexacyanoferrate(II) dissolved in chloride containing media, used as a typical model compound undergoing a reversible oxidation process.

We first tried to use as the reference electrode a simple silver wire covered with a AgCl layer directly dipped in ethaline, exploiting the spontaneous presence of chloride ions in ethaline. Unfortunately, this reference electrode was not stable over time due to the progressive solubilization of the AgCl layer in ethaline, which was already noted previously by other authors [56]. Consequently, this electrode displayed a constant potential only for short times (about 20 min), while for longer times the potential varied to a large extent.

Therefore, we opted for the use of a standard CHI aqueous reference electrode consisting of Ag/AgCl, 3 M KCl equipped with a porous septum. At the base of this electrode, a small Teflon tube was inserted, in turn equipped with a porous septum, inside which a salt bridge was originated by alternately introducing a 3 M KCl solution in water or ethaline, so as to prevent the diffusion of the solutions from one cell (analyzed sample) to another (reference electrode). When this reference electrode, equipped with an ethaline salt bridge, was dipped in ethaline solutions containing 10 mM potassium hexacyanoferrate(II), an anodic-cathodic reversible system located at $E_{1/2}$ of about −5 mV was recorded at the glassy carbon working electrode. It is shown in Fig. 1 (blue line), where it is compared with the anodic-cathodic reversible system recorded with the same reference electrode for an aqueous 1 mM hexacyanoferrate(II) solution at the same glassy carbon working electrode (black line). The different concentration of the hexacyanoferrate(II) ion in the two media was necessary because peak currents are proportional to the square root of the diffusion coefficient which is in turn inversely proportional to the solvent viscosity. Consequently, an increase of the solvent viscosity caused a marked decrease of peak currents. The potential difference of about 189 mV found for the same reversible system in the two media (see Fig. 1) must obviously be attributed to the change in free energy for the transfer of one mol of hexacyanoferrate(II) from its standard state in water to that in ethaline [57].

When voltammograms were recorded on 10 mM hexacyanoferrate (II) solutions in ethaline by using the same reference electrode, but equipped with an aqueous 3 M KCl salt bridge, its anodic-cathodic reversible response shifted to more anodic potentials by about 18 mV (red line in Fig. 1). This small shift conceivably occurred as a consequence of the different value of the liquid junction potential taking place when two solutions of different composition and concentrations are in contact with each other and it is generated by the different mobility of the ions in the two solutions.

Peak currents recorded in all these voltammetric measurements conducted in ethaline showed optimal repeatability (RSD ≤ 1.8 %) and no electrode passivation (fouling) was observed even when voltammetric scans were performed repeatedly. Given the excellent performance provided by the reference electrode consisting of Ag/AgCl, 3 M KCl equipped with an ethaline salt bridge, it was used in all the voltammetric measurements reported below.

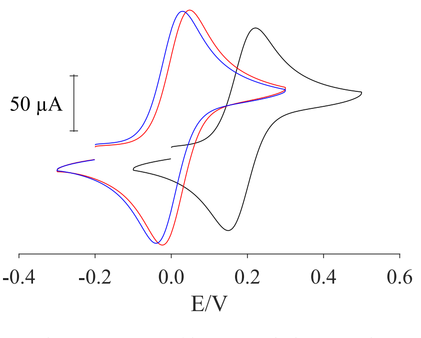

Fig. 1. Cyclic voltammetric profiles recorded at a glassy carbon working electrode for a 10 mM hexacyanoferrate(II) dissolved in pure ethaline *vs* a Ag/AgCl 3 M KCl reference electrode equipped with an ethaline salt bridge (blue line) compared with that recorded at the same working electrode for a 10 mM hexacyanoferrate(II) dissolved in pure ethaline *vs* a Ag/AgCl 3 M KCl reference electrode equipped with an aqueous 3 M KCl salt bridge (red line). The black line is the cyclic voltammetric profile recorded at the same glassy carbon working electrode for a 1 mM hexacyanoferrate(II) dissolved in a 0.1 M KCl aqueous solution *vs* a Ag/AgCl 3 M KCl reference electrode not equipped with any salt bridge. Scan rate 50 mVs^{-1}. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.2. *Voltammograms recorded in ethaline added with pH buffers*

Preliminary tests were performed to verify whether the addition of buffer species to ethaline was able to cause any unexpected effect on voltammograms recorded at a glassy carbon electrode for 10 mM solution of potassium hexacyanoferrate(II), which does not participate to any acid-base equilibrium. With this purpose, the following buffers were assayed: 50 mM acetic acid+50 mM sodium acetate; 50 mM phosphoric acid+50 mM sodium dihydrogen phosphate; 50 mM sodium dihydrogen phosphate+50 mM sodium monohydrogen phosphate.

The results obtained in these tests indicated that the presence of buffer species in ethaline did not cause the modification of both peak potentials and peak currents of the anodic-cathodic system exhibited by the hexacyanoferrate(II) ion, which remained that shown in Fig. 1. The same absence of effects due to the presence of the aforementioned buffer species was also found when the probe consisting of hexacyanoferrate (II) was replaced by ferrocene, which is also a typical model compound capable of undergoing a reversible oxidation process totally independent of pH, benefiting from its excellent solubility in ethaline.

After these preliminary checks, voltammograms were recorded at a glassy carbon electrode in solutions of hydroquinone and alizarin Red S in ethaline. These two compounds are two model species whose electrochemical behavior is instead influenced by acid-base equilibria. When these two compounds were simply dissolved in ethaline without the addition of buffer species, they exhibited the anodic-cathodic systems shown in Fig. 2, which correspond to the oxidation and associated backward reduction of the bisphenol unit present in them. As for alizarin Red S, at more negative cathodic potentials (< -0.2 V) it showed a further cathodic peak, not shown in Fig. 2, which corresponded to the reduction of the quinone unit present in its molecule.

Subsequently, voltammograms were repeated for each of these two species when they were dissolved in ethaline containing the pH buffers that could be prepared with the species listed in the Experimental Section. For each of these buffers, equal concentrations (50 mM) of acid and conjugated base were used in order to exploit their maximum buffering capacity. The buffers used are listed in Table 1, together with the values of their proper acid-base constants in aqueous medium (first two columns).

Assuming that the pKa for these acid-base couples in ethaline were coincident with those known in water, the pH range thus explored was approximately between 3 and 10. As expected, the anodic-cathodic system exhibited by both hydroquinone and alizarin shifted towards more negative cathodic potentials as the pH decreased, as shown in Fig. 3A and B where square-wave voltammograms recorded in the presence of some of the employed buffers are reported for each of the two analytes.

However, when the anodic peak potentials recorded by square wave voltammetry for both hydroquinone and alizarin Red S were plotted against the pH values inferred for these buffers on the basis of their pKa values in water, they did not show a linear trend with a slope of approximately 60 mV per pH unit. This result leaves no doubt that the pH values reported are incorrect as they are deduced on the basis of the pKa values displayed in water by the buffers employed, which instead turn out to be significantly different when these acid-base couples are dissolved in ethaline.

3.3. *pH measurements in ethaline with a glass electrode*

The ability of a glass electrode to detect pH is due to the fact that on

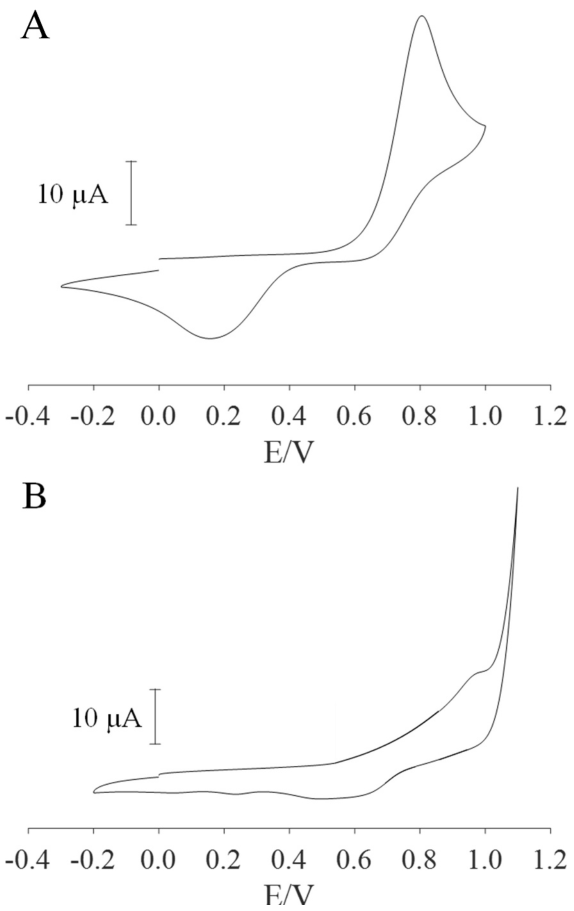

Fig. 2. Cyclic voltammetric profiles recorded at a glassy carbon working electrode for 10 mM unbuffered ethaline solutions of hydroquinone (A) and alizarin Red S (B) *vs* a Ag/AgCl 3 M KCl reference electrode equipped with an ethaline salt bridge. Scan rate 50 mVs^{-1}. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1

. Acid-base buffers added to ethaline (50 mM of acid and conjugated base), together with the pKa in water of the acids present in these buffers and the pHs recorded in ethaline by a glass electrode. [*]

Acid-base buffer	pKa in water	pH measured in ethaline by a glass electrode(*)
$H_3PO_4 + NaH_2PO_4$	2.1	3.0
Citric acid + sodium citrate	3.1	4.1
Sodium citrate + disodium citrate	4.7	5.5
Acetic acid + sodium acetate	4.8	6.2
Tris HCl + Tris base	7.7	8.2
$NaH_2PO_4 + Na_2HPO_4$	7.2	8.4
CAPSO + CAPSO sodium salt	9.6	8.9
CAPS + CAPS sodium salt	10.3	9.8

[*] Since these detected pH values refer to ethaline solutions in which conjugated acid and base were added in equal concentrations, they are coincident with the pKa of the corresponding weak acids used.

both the glass surface contacted by the internal aqueous solution (usually containing NaCl or KCl) and the surface contacted by the analyzed sample a very thin gel layer is formed due to the dipolar interactions created between the polar sites of the glass silicates and the internal and external polar medium. In both of these two layers, an adsorption/desorption equilibrium is therefore established between the protons and alkaline ions that occupy the lattice positions of the silicate skeleton that constitutes the membrane, thus determining a potential difference between each face of the membrane and the contacted solutions. The potential difference between the face of the membrane and the internal solution is always constant, while the potential difference between the other side of the membrane and the solution under test depends on its proton activity. Consequently, it has to be expected that glass electrodes are able to detect the pH even in polar media other than water, provided that sufficient amounts of alkaline ions are dissolved in them, even deriving from the addition of saline components of the buffers. On the other hand, pH measurements in DESs by glass electrodes were frequently reported in recent years [58–61].

Of course, this ability to detect the pH in polar solvents other than water will be possible only after the electrode has been conditioned in the analyzed sample for a sufficient time to allow both the formation of the aforementioned gel layers and the establishment within such layers of the equilibrium between protons and alkaline ions. In this regard, it should be underlined that the establishment of this equilibrium within the gel layers is expected to require longer times in the case of the use of samples involving relatively high viscosity polar solvents such as ethaline.

On the basis of these considerations, we performed a series of pH measurements in ethaline added with the buffers listed in Table 1 (50 mM of acid and conjugated base) using a glass electrode which was preliminarily stored for at least 1 h in the tested solution. Under these conditions, stable and repeatable over time pH values could be achieved. These measurements were carried out on five different samples of each buffered ethaline solution, thus achieving values that did not differ from each other by more than one tenth of pH unit. The values thus acquired are reported in Table 1, third column.

Since these pH values refer to ethaline solutions in which equal concentrations of weak acids and conjugated bases have been introduced, they turned out to be coincident with the pKa value in ethaline of the corresponding weak acids used, as expected on the basis of the Henderson-Hasselbach equation ($pH = pKa + \log[B]/[A]$, where [B] and [A] are the concentrations of the conjugated base and acid, respectively). These results represent a significant confirmation of the fact that the pKa value of the acid-base couples considered is significantly different in ethaline from the pKa values that characterize these couples in water, as mentioned at the end of the previous section.

These pKa in ethaline appear to be, except for the values detected for CAPSO and CAPS, significantly higher than those in water, thus suggesting that ethaline has a lower basicity than that of water, it being less inclined to bind the protons released in the dissociation of the tested acids.

Note, however, that CAPSO and CAPS exceptions are not uncommon. In fact, in studies aimed at comparing the strength of weak acids in different organic solvents with that in water it was found that the variation in the pKa value observed when passing from one solvent to another is not always the same for all the acids considered. In fact, for some of them of a particular nature, minimal differences are found. Thus, for example, for the acidity of protonated nitrogen bases belonging to the aniline compounds (they being just the bases conjugated with CAPSO and CAPS which are in fact substituted anilines), relatively modest differences have been reported [62] between the pKa values in water and those in DMSO, THF and CH_3CN.

It has been suggested that this apparent anomaly is attributable to the solvation energy of the base formed as a consequence of the acid deprotonation [63,64]. This effect is due to the fact that bulky bases with delocalized charge are poorly solvated in water because it disturbs its hydrogen bonded network, causing in such a way a loss of energy to produce the “holes” in the solvent to fit the solute. At the same time, such bases do not act as hydrogen bond acceptors to any appreciable extent. Consequently, these bases are probably better solvated in ethaline, thus contributing to making the strength of the conjugated acids more accentuated.

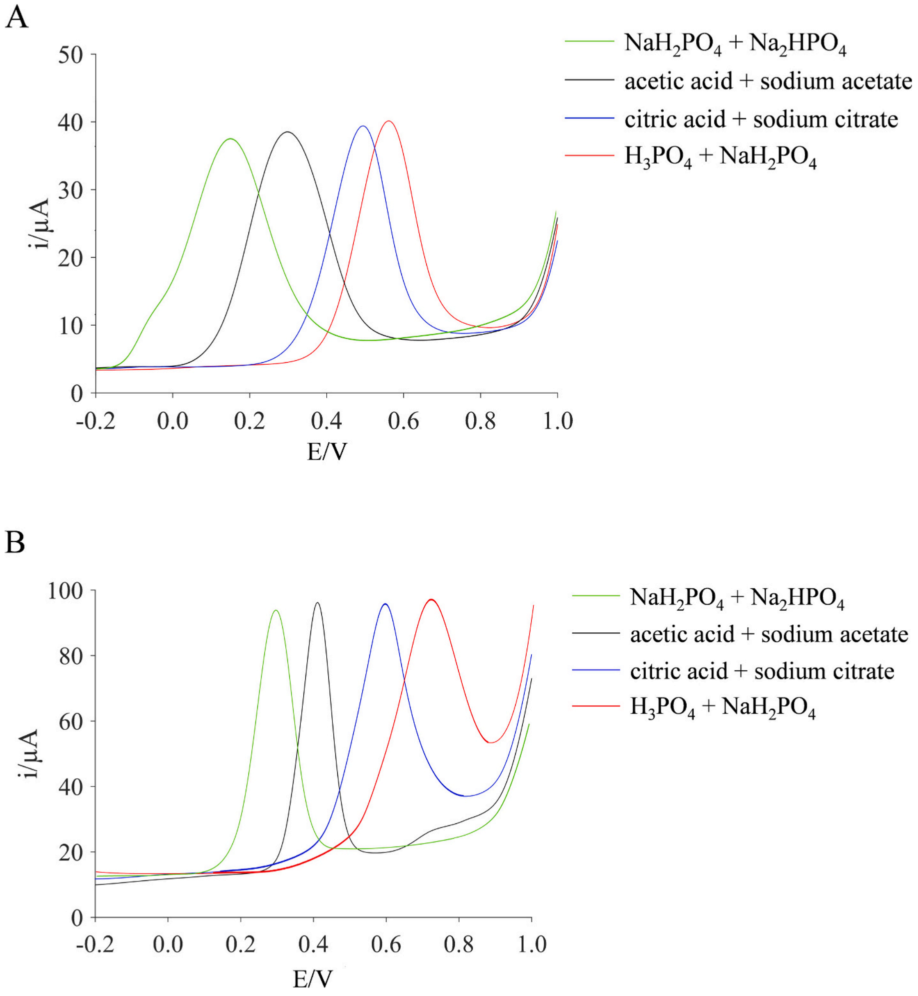

Fig. 3. Square wave voltammograms recorded at a glassy carbon working electrode for 10 mM buffered ethaline solutions of hydroquinone (A) and alizarin Red S (B) *vs* a Ag/AgCl 3 M KCl reference electrode equipped with an ethaline salt bridge. The concentration of all buffering introduced species (reported in the Figure) is 50 mM. Scan rate 50 mVs^{-1}. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The most relevant result provided by these pH measurements performed by a glass electrode is represented by the fact that they allow us to unequivocally confirm the correctness of the pKa values deduced in ethaline and reported in Table 1 (third column). In fact, when the anodic peak potentials recorded by square wave voltammetry for both hydroquinone and alizarin (see previous section) were plotted against the pH values consistent with these pKa a very satisfactory linear trend could be achieved for each of these species, as shown in Fig. 4A and inset for hydroquinone and alizarin Red S, respectively. A slope of approximately 80 mV per pH unit was found, in agreement with their quasi-reversible behavior displayed in voltammetric experiments (see Fig. 2). The pH values reported in Fig. 4 were obtained by mixing in ethaline the acids and conjugated bases reported in Table 1 in different ratios, however in any case between 3:1 and 1:3 in order to achieve in all cases a satisfactory buffering capacity.

3.4. Acid-base titrations in ethaline

In order to acquire further confirmation of the pKa values in ethaline determined by using glass electrode measurements, we carried out the acid-base titration of some of the weak acids involved in the aforementioned buffers. With this purpose, both acetic and phosphoric acid were dissolved in ethaline at a concentration of 5 mM and they were then titrated in triplicate with 100 mM NaOH, in turn dissolved in ethaline, by using a glass electrode as indicator. The concentration of titrant much higher than that of the titrated acids was intentionally adopted to minimize the dilution effects.

The corresponding titration curves thus obtained are shown in Fig. 5. As expected, a single equivalent point was found for the titration of acetic acid (Fig. 5A), while two equivalent points were identified for the titration of phosphoric acid (Fig. 5B). In fact, the third dissociation constant for this acid is conceivably so low (as occurs also in water) to make detectable the vertical region near the corresponding equivalence point.

These titration curves were used to identify the pH when each weak acid is exactly half neutralized, in view of the fact that the pH at 50 % neutralization becomes coincident with its pKa. In this way, pKa values in ethaline were identified which are strictly coincident with those reported in the third column of Table 1, thus supporting our previous findings.

3.5. Spectrophotometric measurements in ethaline

Alizarin Red S (sodium salt of 1,2-dihydroxy-anthraquinone-3-sulfoacid) bears two hydroxyl groups in position 1 and 2 and a sulfonic group in position 3 which ensures its high solubility in polar solvents. In water the color of this compound changes with pH, thus making it an acid-base indicator. As long as the acidity of the solution is such that it does not allow the dissociation of the two hydroxyl groups ($pH \leq$ of about 3.5) alizarin Red S exhibits a yellow color (λ_{max} = about 420 nm).

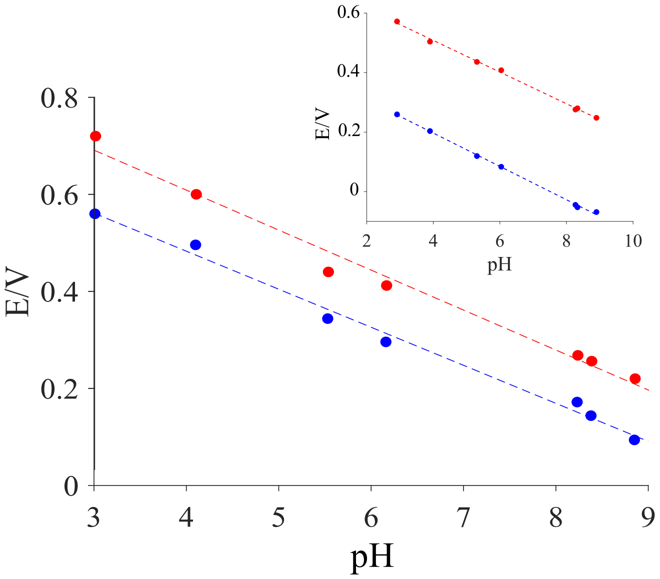

Fig. 4. Dependence on pH of peak potentials for anodic peaks recorded by square wave voltammetry for hydroquinone (blue line; $E_p(V) = -0.0784pH + 0.7966$; $R^2 = 0.9915$) and alizarin Red S (red line; $E_p(V) = -0.0824pH + 0.9378$; $R^2 = 0.9845$) in buffered ethaline and in buffered water (hydroquinone, blue line; $E_p(V) = -0.056pH + 0.4211$; $R^2 = 0.998$ and alizarin Red, red line; $E_p(V) = -0.0532pH + 0.7211$; $R^2 = 0.9975$) (inset). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

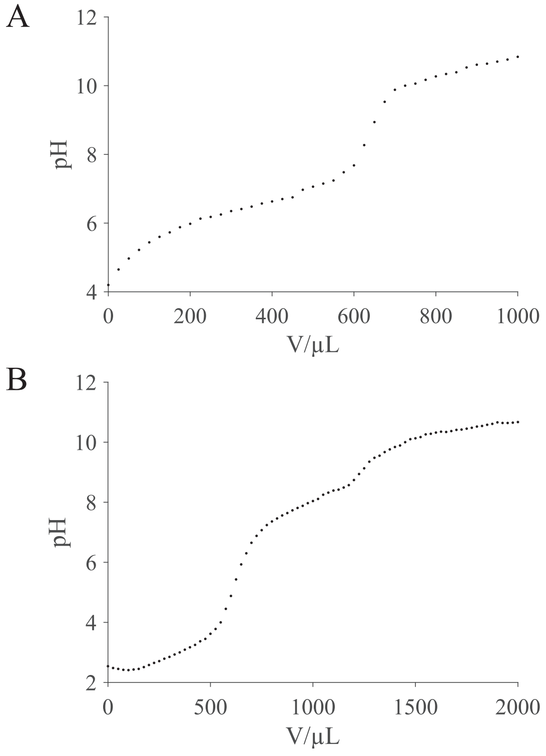

Fig. 5. Titration curves of 5 mM acetic acid (A) and of 5 mM phosphoric acid (B) dissolved in ethaline with 100 mM NaOH, in turn dissolved in ethaline, recorded by using a glass electrode as indicator.

As the pH of the aqueous solution is increased, its color changes from yellow to red, due to the dissociation of one of the two protons of the hydroxyl groups (pH about 6.0, λ_{max} about 520 nm). Finally, at pH > of about 10 this indicator takes on a purple color (λ_{max} about 600 nm), as a consequence of the dissociation of the second hydroxyl group as well.

The first and second dissociation constants of the hydroxyl groups in water reported in the literature were found to be pK_1 = about 5.4 and pK_2 = about 10.7, even if these values vary slightly with the ionic strength of the solutions [65,66].

We verified that this behavior as an acid-base indicator was displayed by alizarin Red S also when it was dissolved in ethaline. With the aim of evaluating the pKa values that constrain the color change of this indicator in ethaline, we recorded a series of spectra in the 350–700 nm range on solutions of alizarin Red S in ethaline (200 μM) to which the pH buffers prepared with the species reported in the Experimental Section and listed in Table 1 were added. Also in this case, for each of these buffers the acids and conjugated bases reported in Table 1 were mixed in different ratios included in the range from 3:1 to 1:3 (total concentration 100 mM) in order to exploit a good buffering capacity. The pH range explored was between 1.5 and 12.0, also in view of the fact that this indicator is known to be unstable in highly alkaline media in which it decomposes slowly [67].

The most significant aspects of these spectra are shown in Fig. 6 that summarizes the transition of the indicator color from yellow to purple, on passing through a red color, which occurred when the pH is progressively increased.

The color changes of this indicator from yellow to red and from red to purple do not occur also in this case at the same pH values found in water and this once again indicates unequivocally that the dissociation constants of alizarin Red S in ethaline are different, even if not by much, from those in water.

This is better highlighted in Fig. 7 where the absorbance dependence on the pH of each of the three forms of alizarin Red S is reported. This Figure is constructed by reporting the absorbance at each pH recorded at the absorption maxima displayed by the singly (420 nm), doubly (520 nm) and triply (600 nm) charged forms of alizarin Red S, respectively.

From the examination of this Figure, a value of approximately 6.2 can be roughly estimated for pK_1 of alizarin Red S in ethaline, while no concrete deduction concerning pK_2 can be made. This is because the wavelengths at which the mono-deprotonated form of alizarin ($\lambda = 520$ nm) and its bi-deprotonated form ($\lambda = 600$ nm) displayed their maximum absorption are too close to each other and that the absorptivity ε of the mono-protonated form is much higher than that of the bi-deprotonated form (see spectra in Fig. 6).

4. Conclusions

This article proposes a strategy to modulate the pH of choline-based DES by adopting a simple procedure which is in effect the same as that

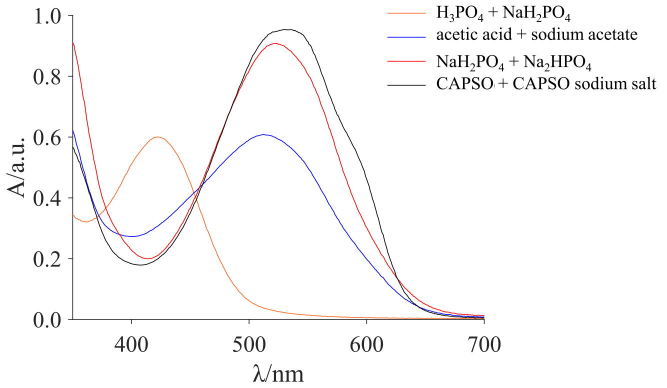

Fig. 6. Absorption spectra of 200 μM alizarin Red S dissolved in ethaline to which the reported buffers were added. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

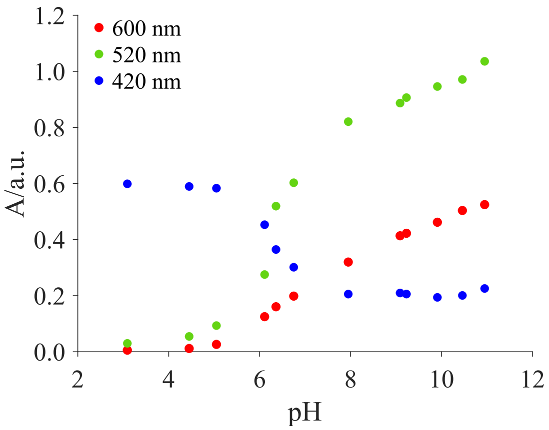

Fig. 7. pH dependence of the absorbance displayed at their absorption maxima by the singly (420 nm), doubly (520 nm) and triply (600 nm) charged forms of alizarin Red S. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

adopted to regulate the acidity or basicity of aqueous solutions. It consists in fact in the simple addition of weak acids or bases or salts consisting of weak acids and strong bases to the aforementioned DES, thus creating real pH buffers. This strategy avoids the need to modify the acidity of DES by resorting to replacement of the hydrogen bond donor (HBD) or modification of the HBD/choline ratio. In fact, all these processes involve notable variations of their density, conductivity, surface tension and, in particular, of their viscosity.

Obviously, the added buffer species must be soluble in the DES and the pH values determined by their presence must be known, remembering that the proton donor and proton acceptor activity of any species always depends on the nature of the medium into which it is introduced. This article also suggests the most effective procedures for evaluating the pH in DES determined by the main species adopted as pH buffers. These methods are based on a combination of simple electrochemical and spectrophotometric measurements which also allow to verify the correctness of the determined value.

The adopted procedure was tested in ethaline, an eutectic mixture composed of choline chloride and ethylene glycol in a 1:2 M ratio, in consideration of its easy preparation and its chemical and physical properties, in particular its low viscosity compared to that of other DESs, which is a critical property influencing fluid flow, mass transfer and heat transfer. These properties are particularly important for various applications, including the dissolution of biopolymers, metal electrodeposition, extraction and separation, as well as for electrochemical studies.

The developed approach can be of considerable use in numerous contexts where chemicals subject to acid-base equilibria are employed. Thus, for example, to optimize the activity and stability of biological macromolecules heavily influenced by pH or when reactions significantly sensitive to proton activity are carried out.

Finally, it must be underlined that this approach taken here in ethaline can be easily transferred to any other DES, provided it is capable of solubilizing the desired buffer species.

CRediT authorship contribution statement

Michele Abate: Investigation, Formal analysis, Data curation, Conceptualization. **Gino Bontempelli:** Writing – review & editing. **Nicolò Dossi:** Writing – review & editing, Supervision, Resources, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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