



Dedicated to Professor Victor-Emanuel Sahini
on the occasion of his 85th anniversary

ELECTROCHEMICAL STUDY OF THE OXIDATION OF USNIC ACID IN DIFFERENT ORGANIC SOLVENTS

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Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) studies of electrochemical behaviour of usnic acid (UA) in different organic solvents (dimethylformamide, DMF, dimethyl sulfoxide, DMSO, and acetonitrile, ACN) in the presence of tetrabutylammonium perchlorate were performed. A unique very well-shaped anodic peak at different anodic peak potentials and no cathodic counterpeak was recorded in each case. A diffusional behaviour of the electroactive species was found. The position of the anodic peak was referred by using ferrocene as internal standard. The product of the symmetry factor of the electrochemical barrier by the number of electrons exchanged in the charge transfer rate determining step, namely $n\beta$, was calculated.

INTRODUCTION

An important source of biologically active compounds is lichens which contain primary and secondary metabolites. First class of metabolites like carbohydrates, proteins, lipids and others are used on metabolism and structure of lichens. The secondary metabolites are produced by the fungus. Among this type of metabolites is usnic acid (UA). Artificial devices are used for repairing or replacing some damaged parts of the body, but their surfaces can be contaminated by bacteria and fungi that can adhere and form biofilm and thus creating local resistance and/or systematic infections. In order to avoid contamination, containing UA-vegetal extracts from lichens, which possesses antimicrobial activity, can be used. Usnic acid [2,6-Diacetyl-7,9-dihydroxy-8,9b-dimethyl-1,3(2H,9bh)-dibenzofurandione] is one of the most studied secondary lichen metabolite.

UA have antibacterial activity against of gram-positive bacteria, including *Staphylococcus aureus*, *Enterococcus faecalis*, and *Enterococcus faecium*,^{1,2} inhibition of viruses effect,^{3,4} anti-tumour activities,⁵ analgesic and antipyretic capacity,⁶ anti-inflammatory activity comparable to ibuprofen,⁷ antifungal, antiprotozoal activity,⁸ antiherbivore and anti-insect effect.⁹

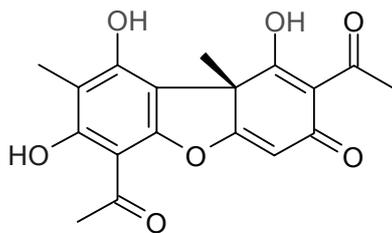
Due to its properties, UA could be used as a preservative or active ingredient in medicinal products for therapeutic purposes, perfumery, toothpaste, mouthwash, creams, sunscreens, deodorants, health food supplements to promote weight loss.^{10,11}

Usnic acid's activity and properties depend upon its structure (tautomeric species are present). In order to understand the UA mechanism of action at a molecular level, establishment of conformational preferences (H-bonding pattern, keto-enol tautomerism in specific medium) or electrochemical behaviour, several techniques were used.

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For determination of UA were used high-performance liquid chromatography (HPLC), micellar electrokinetic chromatography (MEKC), capillary zone electrophoretic (CZE).¹²⁻¹⁴ Physicochemical parameters (redox potential, number of electrons transferred, electrode reaction, rate constant) were determined by electrochemical measurements using voltammetry of microparticles (VMP),¹⁵ abrasive stripping voltammetry (AbrSV),¹⁶ cyclic voltammetry (CV), differential pulse voltammetry (DPV) and square wave voltammetry (SWV).⁴

It has been found that usnic acid possess three redox positions, due to the three -OH groups. These studies were performed using usnic acid mechanically attached to GCE, in different pH values of buffer solutions (Scheme 1). It is interesting that, depending on the pH value and the organic or inorganic type of solvent, the appearance of one or another pick is favoured.



Scheme 1 – The structure of usnic acid.

In this study we have chosen to solve the UA, which has a low solubility in water, in different organic solvents (DMSO, DMF, and ACN) in order to investigate, by CV and DPV techniques, its behaviour as an electroactive species in an electrolyte solution. The UA activity is dependent upon its structure which allows a dynamic balance of different tautomeric species and the elucidation of its conformations responsible for the biologic activity is of great importance. For the same reason, the electrochemical study, even if classical CV and DPV tools are unable to observe the rapid tautomers interconversion, is interesting to be done for understanding the behaviour of the UA. In these organic solvents, polar aprotic and able to solvate by the negative head of the dipole the deelectronated species obtained by oxidation. It is possible as the rapid interconversion of the tautomeric forms to be, in some degree, hindered and, as a consequence, the electrochemical response to be simplified in comparison with that in water. The complex behaviour is determined by possible and probable tautomeric equilibria, on

their turn influenced by the polarity, dipole moment, polarizability and hydrogen bonding. It is well known that a protic solvent solvates rather anions via hydrogen bonding and an aprotic solvent solvates rather cations, by the negative head of the dipole, if it has a sufficiently large dipole moment. So it appears as possible to get a supplementary insight in the UA redox behaviour by studying it in these solvents and by using CV and DPV techniques.

EXPERIMENTAL

Equipment. Electrochemical experiments (CV and DPV) were carried out using the potentiostat-galvanostat system AutoLab PGStat 12, controlled by GPES (General Purpose Electrochemical System) electrochemical interface for Windows (version 4.9.007). Three electrodes in one-compartment cell (10 ml) were used in all experiments. Glassy carbon electrode (GCE), provided by Metrohm (3 mm in diameter), served as working electrode (WE). All potentials were measured and referred to Ag wire, used as quasi-reference electrode (QRE). The counter electrode (CE) was a graphite rod.

Reagents. Anhydrous acetonitrile (Aldrich), dimethyl sulfoxide, (Aldrich) and dimethylformamide (Carlo Erba) were used as solvents. Tetrabutylammonium perchlorate, TBAClO₄, indifferent electrolyte, (Aldrich), ferrocene (Fc), internal standard, (Fluka) and usnic acid (Sigma Aldrich) were used as received. The solution containing also the usnic acid and the supporting electrolyte was deaerated with Ar, and low pressure inert gas atmosphere was maintained above the solution during the electrochemical experiments.

Voltammetric parameters. The experimental conditions for cyclic voltammetry were: scan rates of 10, 25, 50, 75 and 100 mV/s, two scans, and equilibration time of 60 seconds. All the CV experiments were performed in the potential range from 0.0 V till 1.8 V in order to have a good comparison. For differential pulse voltammetry a step potential of 10 mV in combination with modulation amplitude of 25 and 50 mV, respectively, were used, on the same potential interval and with the same equilibration time. The both procedures ensured very reproducible results. The differential pulse voltammograms presented were baseline-corrected using automatic application included in GPES version 4.9.007 software. The reason for this mathematical procedure is improving identification and observing peaks over the baseline without introducing any artefact, although the peak intensity might be reduced in some cases, compared to that of the untreated curve.

RESULTS AND DISCUSSION

The electrochemical behaviour of UA at bare GCE was studied in different organic solvents both by CV and DPV techniques but using the same supporting electrolyte. All cyclic voltammograms exhibit only one evident anodic peak not accompanied by a reduction counterpeak. This anodic peak has all the features of an irreversible

one, the diagnostic test of a slow (irreversible) electrode reaction being fulfilled. Sometimes one can notice the appearance of one or even two others anodic peaks of very low current intensity. These can be observed during the recording of the cyclic voltammograms, disappearing then in the background current because the oxidation current of the solvent is very large at the end of the potential range investigated. The oxidation is determined by the hydroxyl groups attached to aromatic rings. Depending on the solvent, it is possible as this peak to be generated by oxidation of the hydroxyl group. Two different criteria were used for determining, by interpreting the cyclic voltammograms, the product of the symmetry factor of the electrochemical barrier. This product, namely $n\beta$, represents the number of electrons exchanged times the symmetry factor of the electrochemical barrier in the charge transfer rate determining step. Firstly, the difference $E_{pa,10v} - E_{pa,v} = 30/n\beta$ mV at 25°C (*i.e.*, for a tenfold increase of the scan rate from a scan rate of v mV/s to a scan rate of $10v$ mV/s). Secondly, the product $n\beta$, was calculated from the difference $E_{pa} - E_{pa/2} = 48/n\beta$ mV, at 25°C (*i.e.*, the difference in potential between the anodic peak potential and the potential at the half height of the anodic peak on its rising part, for a slow (irreversible) electrode reaction). The above-mentioned product $n\beta$ could offer rough information about the shape of the electrochemical barrier.

Also the DPV technique was applied in order to confirm the main data obtained by CV. Using DPV were followed both the value of the anodic peak potential of the evident anodic peak and also the appearance of the another anodic peak when the experiment is in progress.

To refer the anodic peak potentials in different solvents, ferrocene (0.3 mM) was used as internal standard and measured against the quasi or pseudo reference electrode used in this study, namely Ag wire. The anodic peak potential of UA and the half wave potential, peak-to-peak separation potential, and the peak-to-half-height-peak potential

separation of ferrocenium/ferrocene redox couple are presented in Table 1.

As expected, the Fc^+/Fc redox couple give rise to a very fast (reversible) electrode reaction, all the diagnostic tests being fulfilled.

Fig. 1 presents the cyclic voltammetry behaviour of UA in DMF as solvent at different scan rates. A unique well-shaped anodic peak appears and it shifts anodically with increasing scan rate from 1.455 V at 10 mV/s till 1.501 V at 100 mV/s. So that $E_{pa,10v} - E_{pa,v} = 46$ mV. No reverse peak being obtained in the range of the scan rate investigated.

This behaviour is in accordance with a slow (irreversible) electrode reaction. The $\Delta E_{pa} = E_{pa} - E_{pa/2}$ values are almost constant around approximately 72 mV.

Table 2 contains the $n\beta$ values, 0.65 and 0.67, respectively, calculated from the two criteria invoked above. The two values are very close to each other. Irrespective of the number of electron involved in this charge transfer reaction, one or two, the electrochemical barrier is obviously asymmetric.

The evident anodic peak mentioned above is visible as an irreversible one and its position and its height seem to not be affected in the presence of the internal standard. In the less anodic potential region, comparing the first two cases of cyclic voltammograms, one can affirm the existence of a very small couple of peaks having anodic peak at around 0.488 V in CV and at 0.383 V in DPV, respectively.

Fig. 2 exposes the cyclic voltammograms and Fig. 3 exposes the DPV traces obtained for the background solution (DMF as solvent and 0.1 M TBAClO₄ as supporting electrolyte), for 1 mM UA in background solution, and for 1 mM UA and internal standard Fc in background solution. This couple of peaks practically does not appear for the background solution and is practically overlapped and hidden in the presence of Fc due to their very close positions. It could be assigned to UA itself.

Table 1
Anodic peak potentials of UA and half wave potentials and peak-to-peak separation potential for ferricenium/ferrocene (all in V) redox couple in the three solvents used (75 mV/s).

| Solvent | $E_{pa,UA}$ | $E_{1/2,Fc^+/Fc}$ (V) | $\Delta E_{p,Fc^+/Fc}$ (V) | $\Delta E_{pa,Fc}$ (V) | $\Delta E_{pc,Fc^+}$ (V) |
|---------|-------------|-----------------------|----------------------------|------------------------|--------------------------|
| DMF | 1.499 | 0.631 | 0.057 | 0.048 | -0.052 |
| DMSO | 1.287 | 0.558 | 0.057 | 0.047 | -0.051 |
| ACN | 1.650 | 0.555 | 0.052 | 0.053 | -0.054 |

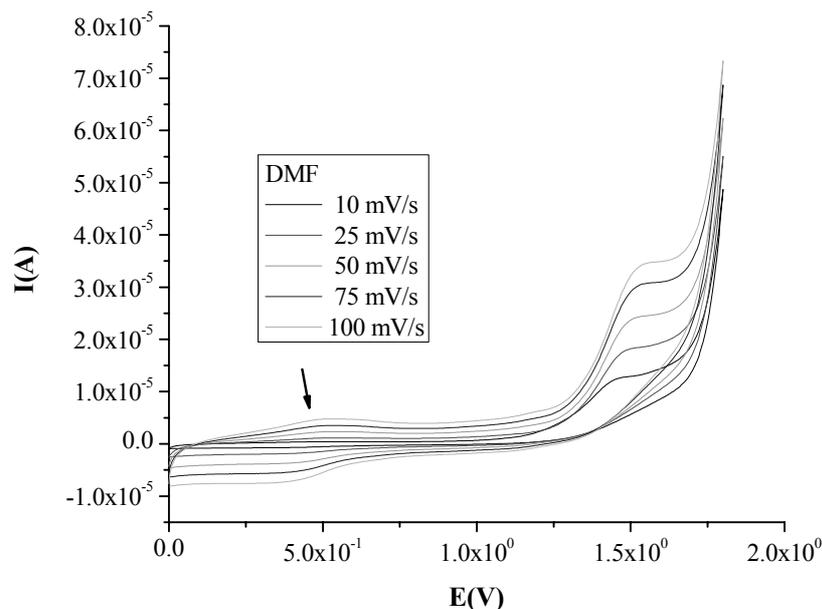


Fig. 1 – Cyclic voltammetric behaviour of UA (1 mM) in DMF as solvent and 0.1 M TBAClO_4 as supporting electrolyte. Scan rates of 10, 25, 50, 75, 100 mV/s, potential range from 0.0 V till 1.8 V, GCE as WE, Ag wire as QRE, and graphite rod as CE.

Table 2

Main CV data (in V, excepting dipole moment values) obtained for UA behaviour in the three solvents used together with their dipole moments

| Solvent | $E_{\text{pa},10\text{v}} - E_{\text{pa},\text{v}}$ | $E_{\text{pa}} - E_{\text{pa}/2}$ | $n\beta$ from | | μ (D) |
|---------|-----------------------------------------------------|-----------------------------------|-----------------------------------------------------|-----------------------------------|--------------|
| | $= 30/\beta n$ | $= 48/\beta n$ (mV) | $E_{\text{pa},10\text{v}} - E_{\text{pa},\text{v}}$ | $E_{\text{pa}} - E_{\text{pa}/2}$ | |
| DMSO | 0.041 | 0.063 | 0.73 | 0.74 | 3.96 |
| DMF | 0.046 | 0.072 | 0.65 | 0.67 | 3.82 |
| ACN | 0.042 | 0.067 | 0.71 | 0.72 | 3.92 |

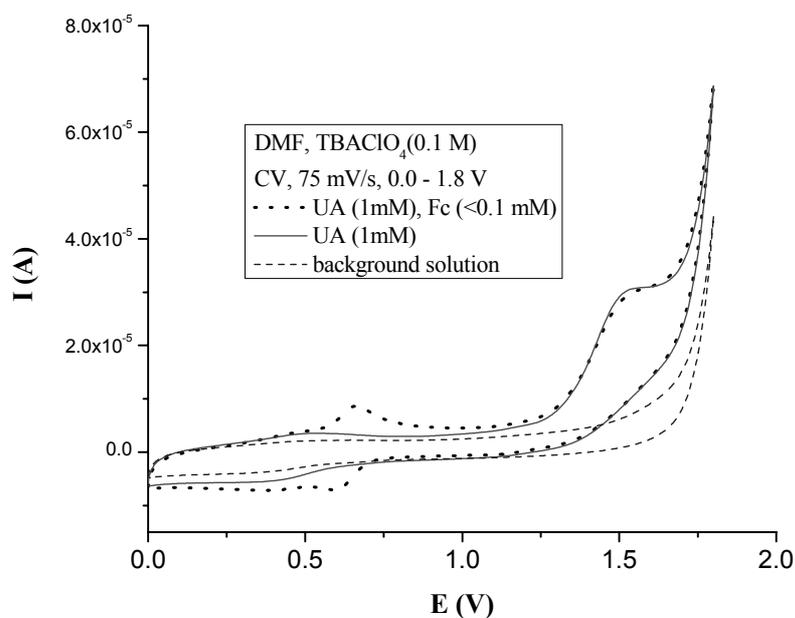


Fig. 2 – Cyclic voltammograms of (a) background solution + UA (1 mM) and Fc (<0.1 mM), (b) background solution + UA (1 mM), and (c) background solution (DMF as solvent and 0.1 M TBAClO_4 as supporting electrolyte). CV-parameter $\nu=75$ mV/s. Potential range from 0.0 V till 1.8 V, GCE as WE, Ag wire as QRE, and graphite rod as CE.

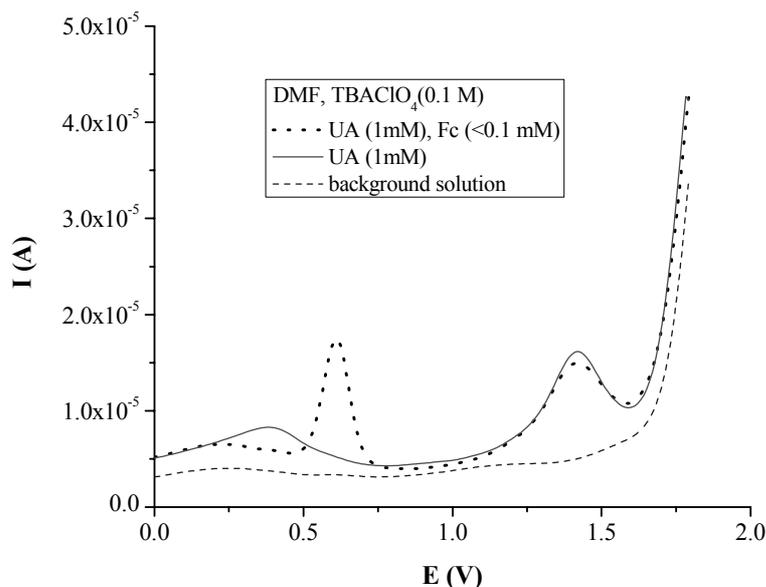


Fig. 3 – Differential pulse voltammetry traces of (a) background solution + UA (1 mM) and Fc (0.3 mM), (b) background solution + UA (1 mM), and (c) background solution (DMF as solvent and 0.1 M TBAClO_4 as supporting electrolyte). DPV-parameters $\text{SP}=10$ mV and $\text{MA}=50$ mV. Potential range from 0.0 V till 1.8 V, GCE as WE, Ag wire as QRE, and graphite rod as CE.

To confirm the CV data obtained, DPV experiments were performed, using as operational parameters $\text{SP}=10$ mV and $\text{MA}=25$ mV or 50 mV; from experiments done with $\text{SP}=10$ mV and $\text{MA}=50$ mV, it results an anodic peak at 1.410 V (see Fig. 3 and Table 3). The main DPV data are collected in Table 2 for UA and ferrocene. Using ferrocene as internal standard electroactive probe, one finds that the anodic peak of UA is 786 mV in DPV ($\text{SP}=10$ mV, which is equivalent to 20 mV/s, and $\text{MA}=50$ mV) and 801 mV in CV (25 mV/s) more anodic than the anodic peak of ferrocene. The plot $\ln I_{\text{pa}}$ vs. $\ln v$ is also linear with a slope of 0.45 for UA in DMF ($R^2 = 0.9974$, $\text{SD} = 0.024$), the electroactive species being a diffusional one. From I_{pa} vs. $v^{1/2}$ ($R^2 = 0.9977$, $\text{SD} = 3.65 \cdot 10^{-7}$) one obtain the following equation $I_{\text{pa}} = 1.48 \cdot 10^{-6} + 7.59 \cdot 10^{-5} v^{1/2}$, and finally the diffusion coefficient $D_{\text{UA,DMF}} = 1.92 \cdot 10^{-5} \text{ cm}^2/\text{s}$.

The cyclic voltammograms obtained for UA in DMSO and ACN, respectively, at different scan rates, are not represented here. Instead, Fig. 3 compares the cyclic voltammograms recorded at 75 mV/s, on the entire investigated domain of potential, for 1 mM UA in the all three solvents used. In the less positive potential domain exists, in each case, a couple of peaks of very low intensities. In DMSO: the anodic peak is at 477 mV in CV and 383 mV in DPV. In ACN this peak appear at 0.636 V using CV and 0.655 V using

DPV, respectively. In the presence of Fc as internal standard, this couple of peaks is practically hidden by the very well-defined redox response of Fc^+/Fc redox couple.

DPV experiments were performed to confirm the results obtained using CV for the evident anodic peak, situated at more positive potentials. The operational parameters for DPV were $\text{SP}=10$ mV and $\text{MA}=25$ mV or 50 mV. From experiments done with $\text{SP}=10$ mV and $\text{MA}=50$ mV, it results an anodic peak at 1.249 V in DMSO and 1.551 V in ACN (see Table 3). The main DPV data are also collected in Table 2 for UA and ferrocene in DMSO and ACN, respectively. Using ferrocene as internal standard electroactive probe, one finds that the anodic peak of UA is 654 mV in DPV ($\text{SP}=10$ mV, which is equivalent to 20 mV/s, and $\text{MA}=50$ mV) and 683 mV in CV (25 mV/s) more anodic than the anodic peak of ferrocene in the DMSO case as solvent. As concern the ACN as solvent, using ferrocene as internal standard electroactive probe, one finds that the anodic peak of UA is 1108 mV in DPV ($\text{SP}=10$ mV, which is equivalent to 20 mV/s, and $\text{MA}=50$ mV) and 1048 mV in CV (25 mV/s) more anodic than the anodic peak of ferrocene. The main electrochemical data, concerning the anodic peak potentials of UA, ferrocene and the $n\beta$ values are also included in Tables 1, 2, and 3. In both cases only one very well-shaped anodic peak (with no counterpeak) appears which shifts in the anodic direction with

increasing scan rate as it happens in the case of a slow (irreversible) electrode reaction. The plot $\ln I_{pa}$ vs. $\ln v$ is also linear with a slope of 0.45 for UA in DMSO ($R^2=0.9933$, $SD=0.039$) and of 0.46 for UA in ACN ($R^2=0.9995$, $SD=0.011$). In both cases the UA acts mainly as a diffusional species in the electrode reaction.

From I_{pa} vs. $v^{1/2}$ ($R^2=0.9966$, $SD=4.68 \cdot 10^{-7}$ for DMSO and $R^2=0.9989$, $SD=3.89 \cdot 10^{-7}$ for ACN) one obtain the following equations $I_{pa} = 1.23 \cdot 10^{-6} + 7.96 \cdot 10^{-5} v^{1/2}$ for DMSO and $I_{pa} = 2.26 \cdot 10^{-6} + 1.19 \cdot 10^{-4} v^{1/2}$ for ACN, respectively. The diffusion coefficients are $D_{UA,DMSO} = 1.92 \cdot 10^{-5} \text{ cm}^2/\text{s}$ for DMSO and $D_{UA,ACN} = 4.45 \cdot 10^{-5} \text{ cm}^2/\text{s}$ for ACN.

The anodic peak is better defined in DMSO than in ACN and this than in DMF. Its position varies in the anodic direction in the following order

$E_{pa,DMSO} < E_{pa,DMF} < E_{pa,ACN}$ but the potential separation between two neighbouring peaks is enough large, $E_{pa,UA,DMF} - E_{pa,UA,DMSO}$ is around 0.212 V (0.161 V) and $E_{pa,ACN} - E_{pa,DMF}$ is around 0.151 V (0.141 V) in CV (DPV) experiments at 75 mV/s (SP=10 mV, MA=50mV). On one hand, considering this large difference, it is hard to believe that the anodic peak is given by the same anodic electrode reaction and that the shift is determined only by the nature of the solvent (the indifferent electrolyte being the same in all three cases studied) which have more or less very close dipole moment (3.96 for DMSO, 3.82 for DMF, and 3.92 for ACN), and all of them being polar aprotic solvents. On the other hand, the symmetry factor of the charge transfer reaction is more or less the same in each solvent used. Comparing the $n\beta$ values order with the dipole moment order (see Table 2) one can observe a parallel decrease from DMSO to ACN and then to DMF.

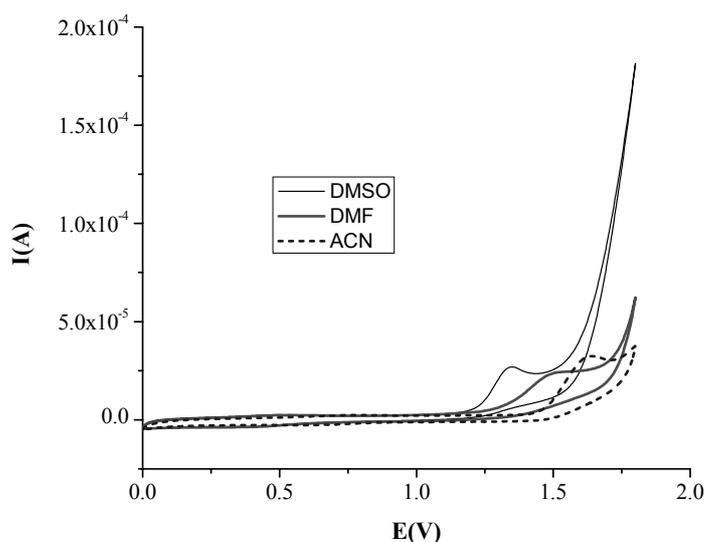


Fig. 4 – The superposition of the three cyclic voltammograms of UA (1 mM) in different solvents: DMSO (black), DMF (red) and ACN (blue), and 0.1 M TBAClO₄ as supporting electrolyte. Scan rate of 50 mV/s, potential range from 0.0 V to 1.8 V. GCE as WE, Ag wire as QRE, and graphite rod as CE.

Table 3

Main DPV (SP=10 mV, MA=50 mV) data (all in V) obtained for UA and ferrocene behaviour in the three solvents used

| Solvent | $E_{pa,UA}$ no Fc | $E_{pa,UA,Fc}$ with Fc | $E_{pa,Fc}$ | $\Delta E_{pa,UA-Fc} =$ $E_{pa,UA,Fc} - E_{pa,Fc}$ |
|---------|----------------------|---------------------------|-------------|-------------------------------------------------------|
| DMF | 1.410 | 1.410 | 0.614 | 0.786 |
| DMSO | 1.249 | 1.188 | 0.534 | 0.654 |
| ACN | 1.551 | 1.601 | 0.493 | 1.108 |

CONCLUSIONS

CV and DPV studies performed using UA dissolved in one of the following three different solvent, DMSO, DMF, and ACN, at a usual GCE demonstrate that: (1) UA participate in an irreversible electrode reaction (only one anodic peak well-shaped, no counterpeak) as a diffusional species; (2) it is easier to oxidize the UA in DMSO than in DMF and than in ACN; (3) both electrochemical techniques, CV and DPV, lead to electrochemical data which are in good agreement; (4) it is not easy to decide whether the anodic peak recorded in each solvent is the result of the occurrence of the same electrode reaction.

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