***Scientific Report***

Regarding the implementation of the PCE_ PNII-ID-PCE-2011-3-0226 project,

*ACCES TO NEW BIOACTIVE MOLECULES BY DEVELOPING ORIGINAL BIOCATALYSTS FOR CLICK CHEMISTRY REACTIONS*

*Between January - December 2015*

*Phase V*

*The objectives for Phase V of the project were fully achieved. The collaboration between the members involved in the project resulted in published papers or papers written for publication, in which the various project activities are presented.*

*This phase aimed to biologically characterize the compounds obtained in the previous stages by studying the interaction of compounds with nucleic acids and to study the electrochemical properties of the synthesized compounds by making determinations of cyclic voltammetry.*

*The results of the activities included in stage V are further summarized.*

## *5.1. Biological characterization of the synthesized compounds*

### *5.1. 1. The interaction of the compounds with DNA*

*DNA plays an important role in biological processes since it carries the hereditary information codes required for the synthesis of all proteins and enzymes. DNA directly or indirectly controls the structure and function of the cell. Ever since the discovery of the structure of DNA, it has been a prime target for various therapeutically important small molecules that belong to different classes of drugs, ranging from anticancer drugs to antibiotics. Apart from interacting with DNA associated proteins or interacting through DNA–RNA hybrids, small molecules may directly bind to the DNA helix. Such interactions result in diverse downstream processes like interfering with the activity of various important enzymes and proteins involved in maintaining the structure and functions of the cell. The interaction of small molecules with DNA has been studied extensively. These studies provide insights into the development of effective therapeutic drugs that could control gene expression. Newer and more effective DNA-targeted drugs against several diseases can be easily developed. Understanding the mechanism of action of various anti-cancer drugs became possible by studying the drug–DNA interactions(Sayeed Ur Rehman et all, 2015).*

*Spectroscopic studies (absorption and fluorescence) can provide important information on how the nucleic acid binds fluorescent molecules (fluorophores) or drugs (Modukuru N. K. et al., 2006, Muhammad Sirajuddin et al.,2013; Sayeed Ur Rehman et all, 2015).*

*The photophysical behavior of indolizines has been studied and efforts have been made to develop derivatives with improved and exceptional fluorescent properties. Due to their characteristic fluorescence behavior, indolizine derivatives have been used as photochemical sensors, hydrogen bonding sensors and as fluorescent sensitive probes to VOCs (Matthieu Becuwe et al., 2011, Bankim Chandra Ghosh et al., 2011, Mustafa K. Bayazit et al., 2014 ).*

*They have been used for studies of DNA interactions and as photochemical dyes (Dinică et al., 1999, Furdui et al., 2007).*

*The researches on the interaction of nucleic acids with different biologically active molecules synthesized in the earlier stages of the project contribute to the understanding of the mechanism of interaction. The interaction of nucleic acid with organic compounds, differently functionalized (products of cyclization reactions - fluorescent indolizines, figure 1), obtained in the previous stages through biocatalysis, was done by spectral methods (UV-VIS and IR).*

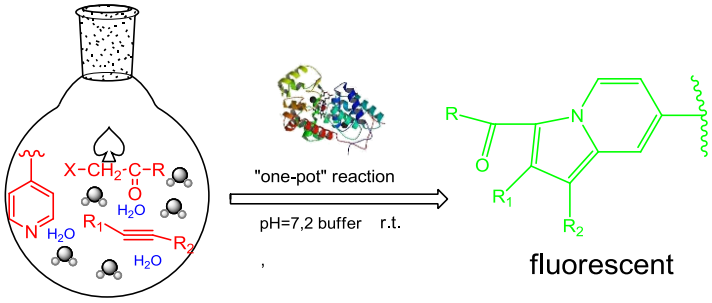
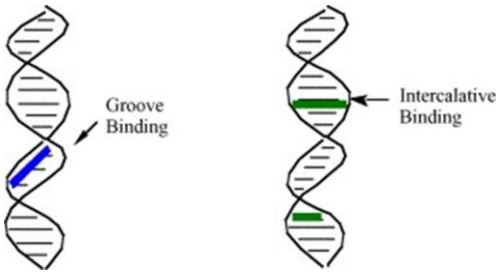



*Figure 1 Indolizine synthesis*

*UV-vis absorption measurement is a simple, but effective method in detecting DNA-compound complex formation. Generally, when a small molecule interacts with DNA to form a new complex, the changes should appear in absorbance and in the absorption maximum position. If the binding type is intercalating, the π∗ orbital of the intercalative molecule can be coupled with the π orbital of DNA base pairs, thus leading to a decrease in energy tranzition π∗ → π resulting in a bathochromic effect. On the other hand, the coupling of the π orbital partially occupied by electrons lead to a reduction of the probability of transition and at the same time resulting in a hypochromic effect. Generally, hiperchromic and hypochromic effects are spectral characteristics of the double helix DNA structure; hiperchromic means breaking the secondary structure of DNA and hypochromic means that the binding molecule to DNA is through electrostatic or interlacing effect that can stabilize duplex DNA, while the redshift indicates the destabilization of the DNA duplex.*




*The mode of binding of a compound is affected by the geometrical, steric and electrostatic characteristics. Thus, binding by intercalation is common for aromatic and heterocyclic plane molecules, while electrostatic interaction may occur in the presence of cationic functions and of the side chain.*

### *Samples and DNA preparation*

*To study the interaction of the fluorophore with the nucleic acids, in a first stage of laboratory investigations the absorption spectra of indolizines were determined. Indolizine stock solutions were prepared by dissolving an appropriate amount of compound in buffer Tris-HCl, pH 7,6. The*

*UV-VIS spectra of the dyes were recorded between 200 and 800 nm, in aqueous buffer solution. Indolizines possess two absorbtion bands in the UV-Vis region .*

*Steady state fluorescence measurements were conducted with a Infinite 200 Pro NanoQuant Microplate Multimode Reade (300 µL / well) and the measurements were performed at 24°C. The dye concentration ranged from 2 to 20 µM depending on the experiment.*

**F***igure 2 Indolizine solutions*

*The recorded fluorescence spectra showed that the donor groups (eg. methoxy) linked to phenyl ring produce a bathochromic movement and an increase in fluorescence intensity, while the electronwithdrawing groups (nitro) although causes a bathochromic shift of the emission maximum they have the effect of "quenching" the fluorescence.*

### *The concentration of calf thymus DNA*

*Deoxyribonucleic acid sodium salt from calf thymus (Sigma Chem. Co., USA) was used without further purification, and its stock solution was prepared by dissolving an appropriate amount of DNA in doubly distilled water and stored at 4 ◦C. The concentration of DNA in stock solution was determined by UV absorption at 260 nm using a molar absorption coefficient $\varepsilon_{260}$ = 5000 L mol$^{-1}$ cm$^{-1}$. Purity of the DNA was checked by monitoring the ratio of the absorbance at 260 nm to that at 280 nm. The solution gave a ratio of >1.8 at $A_{260}/A_{280}$, which indicates that the DNA was sufficiently protein-free. For measurements, the DNA was dissolved in 10 mM Tris-HCl, pH 7 buffer and was used for all the spectroscopic binding analysis.*
*The concentration of nucleic acids was determined with the same microplate reader which has a special quartz plate suitable for 16 samples which guarantees high performance and a high rate of reproducibility for the quantification of nucleic acids. The plate is compatible with an eight-channel pipette to easily distribute samples for analysis. 2 µl of each DNA dilution were transferred on the special NanoQuant plate which subsequently was read. All measurements were performed in triplicate.*

### ***Effect of DNA on UV-VIS spectra***

*The spectra were recorded by progressive addition of DNA solution to indolizines. Absorption titrations were carried out by keeping the concentration of the solution sample constant, while adding concentrated solution of DNA in progressively increasing amounts in physiological conditions, until the saturation in hypochromism was observed.*

*The absorption spectra of DNA-indolizine solutions are shown in the figure below. DNA binding to indolizines leads to the red shift of the absorption band of the dye, visible at high DNA concentration.*

*The red shift (or blue shift), hyperchromic (or hypochromic) effects, and the isochromatic point are spectral properties of DNA-drug interaction, which are closely related with the double helix structure. The hypochromicity at the maximum absorption of DNA (260 nm) indicates the compaction of DNA due to the electrostatic interaction. Intercalation induces the hyperchromicity at this wavelength (Reza Hajian et al., 2013).*

*With the increasing of indolizine concentration, the spectrum shows the decrease of the absorbance intensity. Since the indolizine nucleus contains aromatic rings which may facilitate the intercalation, a conventional intercalative interaction is possible.*

### *Fluorimetric titration. Quenching of fluorescence by DNA*

*Fluorescent quenching techniques involve a variety of molecular interactions such as excited state reactions, formation of ground-state complex, molecular rearrangements, energy transfer, and collision. In this context, fluorescence quenching experiments were undertaken to investigate the interaction of synthesized compounds, indolizines, with DNA. The binding of compounds with DNA, by maintaining the concentration of compounds constant and varying the concentration of DNA was studied by fluorescence spectroscopy*
*The fluorescence spectra was recorded at different molar ratios DNA:indolizine, with excitation of the samples at the wavelength of the lowest energy maximum absorption band.*

*The absorption and emission spectra have stressed the strong interactions between DNA and studied fluorophores, which is manifested by fluorescence quenching with increasing molar ratio DNA: fluorophore. Titration curves (fluorescence intensity to molar ratio of DNA: indolizine) show exponential decrease in fluorescence intensity with increasing concentration of the indolizine.*

*The data from the photometric titrations were used to determine the particular ratio of bound ligand molecules to DNA, plotting the relative fluorescence intensity against the molar ratio of DNA : indolizine, in order to establish the molar ratio of maximum interaction. Upon addition of DNA, the fluorescence intensity of the indolizine decreased.*

### *The effect of ionic strength*

*Study of ionic strength on the interaction of the DNA molecule is an important method to analyze the binding type between small molecules and DNA. Strong electrolytes, such as NaCl, are used for this purpose. Adding NaCl to the free ligand in the absence of DNA should have a*

*low or no effect on the fluorescence yield. However, in the presence of DNA, $Na^+$ partially neutralizes the negative phosphate groups of the DNA strand resulting in reduction in the electrostatic repulsion between them.*

*Electrostatic attraction between a small molecule and DNA surface is weakened by the addition of $Na^+$ ions. When the molecule binds on the DNA surface, an electrostatic interaction occurs out of the groove and the fluorescence intensity is quenched upon interaction with DNA. A small molecule located in the groove of the DNA helix is more exposed to the ionic strength in the surrounding solvent than a intercalated molecule. The addition of NaCl weakens the interaction and leads to the release of the compound from DNA surface resulting in increased of fluorescence intensity. [S.U. Rehman et al., 2015].*

*The effect of salt concentration was studied by recording fluorescence spectra in the presence of different NaCl concentrations in order to establish the nature (intercalative or electrostatic) of dye-DNA interaction.*

*KI quenching experiments Studies of potassium iodide fluorescence quenching are typically used to determine how DNA binds the drugs that are fluorescent. KI is a small compound which posseses fluorescence quenching properties and can be used to determine the binding type of drugs to DNA.*

*Quenching experiments indicate where molecules bind, either outside or inside the DNA helix. Iodine ions are negatively charged and can quench small molecule fluorescence efficiently in a saturated solution, in an aqueous medium.*

*The negative iodide ions are repelled by the negatively charged phosphate groups of the DNA strand. Any small molecule intercalated in the DNA helix is well protected from being quenched by the saturated anionic solution the fluorophore being restricted. However, electrostatic molecules are exposed to the solvent medium and are not well protected by the saturated anions, even in the presence of DNA.*

*The studies performed have lead to the conclusion that the salt concentration doesn't influence significantly the fluorescence intensity in the presence of DNA, so the presence of electrostatic interaction can be excluded.*

## The interaction of molecules with nucleic acids by FTIR spectroscopy studies

*Fourier transform infrared spectroscopy (FT-IR) has numerous applications in biological sciences. This provides an easy way to identify functional groups present in the molecule, and the identity of the pure compound can be determined. Infrared radiation (IR) is passed through a sample and some of it is absorbed while other is transmitted. Data results are shown as a range that acts as a molecular fingerprint of the compound or molecule. Drug-DNA interaction study highlights the changes in intensity and spectral shifts of the bands. Pure DNA molecular fingerprint is in the spectral region of 1800-700 $cm^{-1}$ due to plane vibration of nitrogenous bases, stretching vibrations (asymmetric and symmetric) of phosphate groups and stretching vibration of deoxyribose. The binding of the small molecules of pentose phosphate backbone of DNA is studied by monitoring the change in the bands at 1228 and 1087 cm-1 which are caused by asymmetric and symmetric vibrations of the phosphate groups [H. Arakawa, et al.,2000].*

*The transition conformation of the DNA double helix to form B to form A or to form B to form Z can be detected readily by binding of drugs to DNA. [T. Zhao, 2014, S.T. Saito, 2012, D.K. Jangir, 2011, H. Arakawa, 2000].*

*Linking the analyzed molecules to DNA bases are demonstrated by evaluating spectral bands at 1741 cm-1 which is mainly attributed to stretching plan vibration of guanine (G), the spectral bands at 1668 $cm^{-1}$ caused by basic vibrations of thymine (T), and the spectral bands at 1581 and 1496 cm $cm^{-1}$ due to the stretching vibration of adenine (A) and cytosine (C).*

## 5.2. Studies of electrochemical properties

### 5.2. 1. Studies of cyclic voltammetry

*New heterocyclic pyridinium compounds as N,N' di-(p-bromophenacyl)-4,4'-bipyridinium dibromide (Lr) and N,N' di-(p-bromophenacyl)-1,2-bis(4-pyridinium)-ethane dibromide (Lm), synthesized in the previous stages of the project, were investigated applying cyclic voltammetry to evaluate their electrochemical behaviour. The stability of the new heterocyclic pyridinium compounds in aq. media depends on pH and the dependence was correlated with spectrophotochemical data. Ethylenic group from Lm induces changes on the stability and on the electrochemical performances of the ligand. The quasireversible process on electron transfer between functional groups depends on pH and also on the scan rates of the potential applied. The alkaline pH of aq. media is more favorable than acidic pH for the ligands stability and the electron transfer process on platinum electrode. The study of the redox potential (cyclic voltametry ) of the two ligands indicatse the role as mediator candidate in reduction mechanism. We believe that the present work will stimulate the investigations of the chemical features of ligands and their role in biological and medicinal chemistry.*

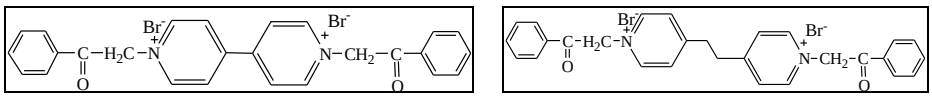


***Figure 12.*** *Structure of bipyridinium salts Lr and Lm*

*The elucidation of the electro-changes of the synthesized compounds is useful for the electrochemical investigation [R. Palin et al.,, 2002]. Compounds can be used as biological redox indicators, electrochemical sensors, electronic transporters and precursors for compounds such as indolizines, biologically active compounds which can serve as potential markers and fluorescent ligands for estrogen receptors [M. Eda et al., 2008, D. H. Evans et al., 2001]. This research also helps us to understand the mechanism of cycloadditon of these ligands with dipolarophils for obtaining indolizine compounds [Dinica et al., 2013]. The behaviour of the compounds bearing aryl substituents on the quaternarized nitrogen atoms has been examined showing that the spectroscopic and electrochemical properties are fine tuned by the nature of the nitrogen ring. These quaternisation reactions will be done in aq. media. The redox potentials of the compounds are interesting for determining the donor - acceptor properties of the radicals [F. Tepl et al.,, 2012]. Another aspect that we will study in the future would be understanding reactions of cycloaddition and substitutions, realized in aq. media [Dinica et al., 2013]. Viologen compounds present a significant interest due to their changing properties and colour in*

*connection with the reaction medium. In this work, the electrochemical behaviour of two pyridinium heterocyclic compounds has been recorded. The compounds were studied for the first time by cyclic voltammetry in aq. media.*

### *Stability of ligands*

*The synthesized compounds were N,N' di-(p-bromophenacyl)-4,4'-bipyridinium dibromide (named rigid Ligand-Lr) and N,N' di-(p-bromophenacyl)-1,2-bis(4-pyridinium)-ethane dibromide (named mobile Ligand-Lm) [3]. The stability of ligands derived from 4,4'-bipyridil (Lr) and 1,2-bis(4-pyridil)-ethane (Lm) respectively was performed by spectrophotometric analysis and open circuit potential (OCP) measurements. The aq. compounds (0.1 mM) were prepared and the analysis was conducted by adjusting the pH in range 3-11. Solutions shown variable colours depending on pH, beginning from light yellow to violet (N,N' di-(p-bromofenacyl)-4,4'-bipyridinium dibromide (Lr) and orange (N,N' di-(p-bromofenacyl)-1,2-bis(4-pyridinium)-ethane dibromide (Lm). A discoloration of the solution was observed when the ligand concentration was lower than $10^{-5}$ M.*

*The UV/VIS spectra of aq. solutions were analyzed at 264 nm (λmax) [Dinica et al., 2012] with the purpose to study the initial stability and after 24 hours (Figure 13). The highest absorbance are registered for Lr on pH 3 (weak yellow colour) and pH around 7 (weak violet colour) when protonated species are formed. Fresh aq. Lr solution presents most stable form in lower alkaline medium (pH around 8). On the other hand, Lm is more stable in lower acidic medium (pH around 6) and the dication form is unstable in alkaline media. This behaviour suggests the presence of the anionic species AH- at the addition of base excess and transition from AH2 to AH-.*

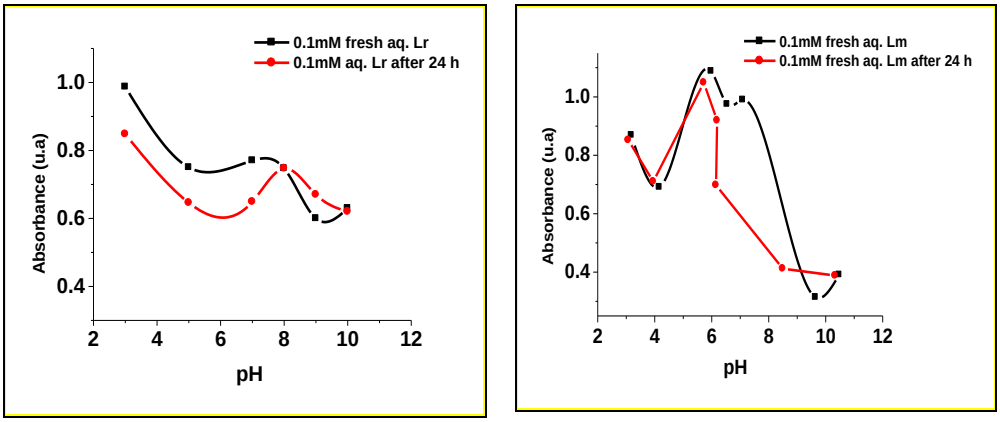



*Figure13. Overview of absorbance vs pH of aq. Lr and Lm*

*The ligands solutions kept at room temperature shown after 24 hours a slightly absorbance change; for Lr it decreases with about 2 units. The electro-oxidation process is more active due to favourable dynamic acid-basic equilibrium of the electrons transfer reaction from the dication to produce radical cation ($AH.^{+}$), and then neutral species. At the same time Lm indicates stability in acidic medium (until pH 6), but an evident decrease of absorbance values in neutral and alkaline medium. This behaviour could be explained by the ethylenic group from Lm, that is more favourable for some cycloarrangement additions in alkaline than in acidic media.*

*The absorbance from UV/VIS spectra are well correlated with OCP measurements. Bispyridinium compounds are organic oxidants and electrochemical techniques are useful to allow the characterization of the electro-oxidation behaviour of electroactive species, to establish the redox potential and to predict the reaction mechanism. The electrochemical investigations of each aq. ligand (0.1 mM) began with monitoring of OCP from fresh solutions (acidic pH). Measurements at neutral and alkaline pH were also performed by adjusting pH using buffer solutions. OCP measurements were performed again after 24 hours, 120 hours and respectively 168 hours (7 days) in order to evaluate the electrochemical stability of the acidic - basic equilibrium and electro-oxidation process of compounds kept in closed tubes in the refrigerator (4°C).*

*Although, initially the aq. ligands presented a difference about 0.5 pH units, no significant differences between OCP values (± 2 mV) were found. OCP was around +72 mV vs. SCE for fresh aq. ligands. The open potential was also measured for the synthesized salts precursors (0.1 mM) by dissolving them in aq. media. The precursors 4,4-bipyridil (for Lr) presents a EWE = +69 mV vs SCE and 1,2 - bis (pyridil) ethane (for Lm) have a potential of EWE = +75 mV vs SCE which does not indicate essential modifications compared with the new ligands obtained.*

*After 24 hours, a difference of about of 30 mV between the acid aq. ligands was determined for the OCP values; Lr indicates +45 mV vs SCE and Lm indicate + 63 mV vs SCE as a result of their structures. The fresh Lm solutions show larger variations of OCP values for the entire pH range because it is more unstable due to the position of ethylenic group in base excess. Meanwhile Lr which does not have the ethylene group in its structure is more stable in the same conditions.*

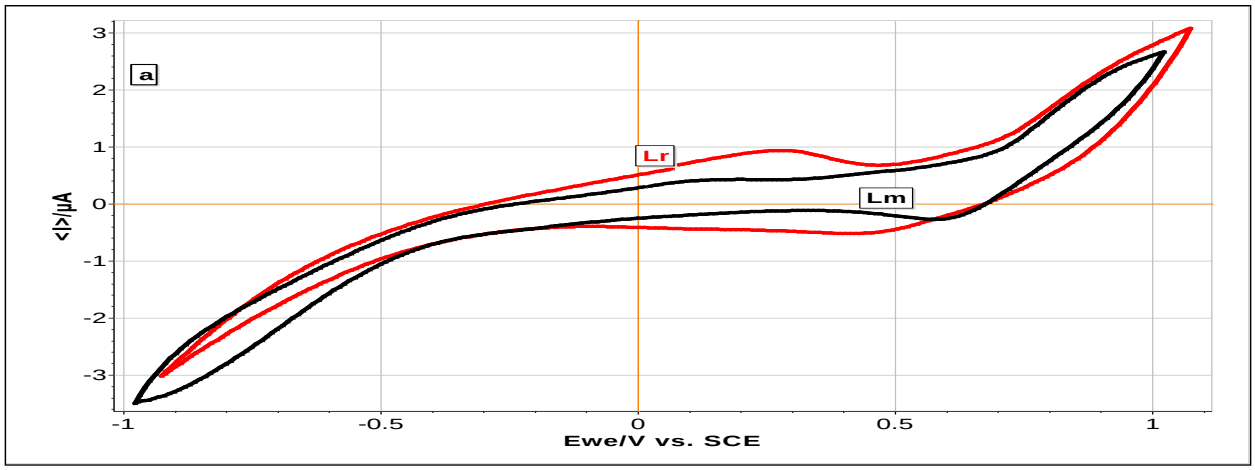
*In time, the OCP data for Lr showed lower variations. Aq. Lr indicates a more positive electro-oxidation potential for the fresh aqueous solutions of pH 6, similar with the solutions kept for 7 days having neutral or alkaline pH. Thus Lr is a more powerful oxidant with greater stability in time and its property is also kept at neutral and alkaline pH. A significant difference was observed for the fresh aq. Lm (pH 5.50) in time, a decrease with 10 mV. Anyway, at the neutral and alkaline pH, OCP values for Lm indicated larger variations than those at acidic pH.*

*Thus, OCP values sustain the ligands structure shown in Figure 12 and explain their stability as confirmed by the spectrophotochemical measurements. The data results are helpful to provide an explanation for the proposed electro-oxidation mechanism of the ligands but are insufficient data for the reaction kinetics in aq. solutions.*

### *Cyclic voltammograms measurements*

*The electrochemical measurements give information on the electrochemical state of the organic compounds on the working electrode in an active or passive state. CV provides information about the redox couples and this remains the most attractive gain of this technique. CVs data are useful for the presence of the protonated/deprotonated structures and supply information about the kinetics of reactions. A series of CVs measurements were obtained in aq. media of the ligands at variable pH, from acidic to alkaline, and different scan rates of the potential applied.*

*Effect of the pH. The pH has an important role for organic compounds as a result of the proton concentration from aq. media. Changes in CVs are the result of the different ligand structures in aq. solutions with variable pH (Figure 14 and 15). The shapes can explain the influence of functional groups from the ligand molecule in aq. media correlated with their stability in time, depending on pH.*



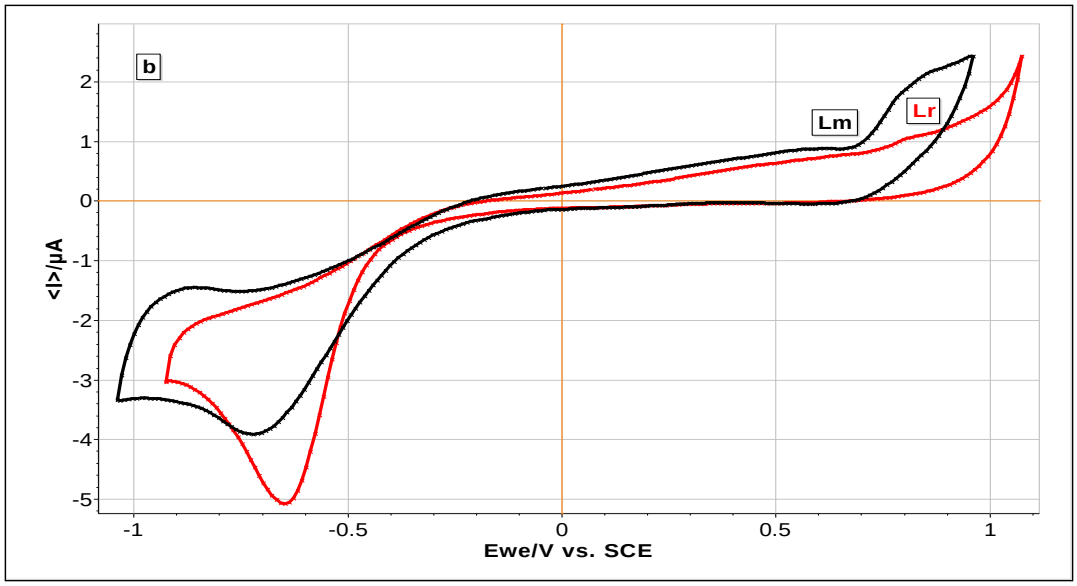



*Figure 14. Cyclic voltammograms in aq. Lr and Lm from fresh solutions with initial pH (a) and at pH 9.0 (b); E = ± 1.0 V vs. SCE, from a negative direction, scan rate 100 mV s-1(a), scan rate 50 mV s-1(b).*

*A similar behaviour appears in the redox process on platinum electrode in both compounds mainly for the anodic current peak. The peak corresponding to the initiation of the anodic oxidation indicates that at least a prototropic couple (AH2-/AH-) of the organic compounds is present. The anodic current peak (ia) around of + 300 mV vs. SCE is more evident for Lr and it appears diminished for Lm, with a difference of 0.5 µA and shifted to a less positive potential.*

*The voltamogramms sustain the electrons transfer between functional groups of ligands and the prototropic couple, depending on the pH of the solution. If the pH solutions are changed, the typical voltammograms will be observed. Figure 14b shows the voltammograms of aq. ligands at a pH of 9.0. The electron transfer is indicated by a characteristic cathodic peak on Pt electrode which is similar in both salts under the same potential conditions and scan rates applied (E = ± 1 V; 50 mV s-1). Lm indicates a slight modification to more negative potential as a result of a favourable rearrangement of ethylenic group present in the structure. An anodic peak can be observed related to the oxidation of Lr to Lr product (radical anion), as an irreversible process due to its higher reactivity. The compounds described by CVs measurements could be more complex and show more than a single redox transaction. Water as proton acceptor suggests that in this case, the proton charge is delocalized over the primary shell of water molecules firmly formed around the pyridinium ligand. Lr indicates an evident reduction process in the alkaline domain (around pH 9), having quite a different behaviour, compared to the acidic*

*and neutral domains (Figure 15a). The scan rate also remains an important condition in these electron transfers (Figure 15b). In alkaline pH, the current peak is shifted to more positive potential around +800 mV while the cathodic reduction is an intensive active process. At the same time Lm does not indicate an obvious electro-oxidation transformation (Figure 16).*

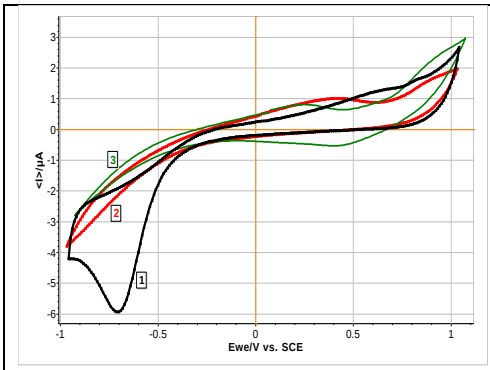



*Figure 15a. Cyclic voltammograms measured in aq. Lr ranging pH: 9.0 (1); 7.0 (2) and 6.0 (3); E = ± 1 V, scan rate 100 mV s-1.*

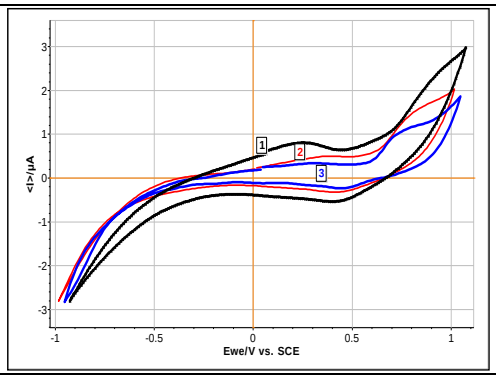



*Figure 15b. Cyclic voltammograms measured in aq. fresh Lr (pH 6.0) at different scan rate: 100 mV s-1(1); 50 mV s-1(2) and 20 mV s-1(3), E=± 1 V*

*It is evident that in the cathodic process on Pt electrode and at alkaline pH the deprototropic conjugate couple (from AH- to AH) changes slower in comparison with acidic and neutral pH. A blockage of the electron transfer by adsorption of the reduced pyridinium ligands on the Pt substrate can suggest this behaviour.*

*Effect of the potential scan rate CVs on platinum electrode have been registered for both aq. ligands (0.1 mM) at different scan rates (100 – 20 mV s-1) for E = ± 1 V (Figures 3b and 4a). No significant shifts were observed in the redox potential when the scan rate was increased from 20 to 100 mV·s-1in CVs of fresh aq. Lr at 6.00 pH (Figure 3b). Moreover, an increase of the anodic current was obtained when the scan rate was higher as an effect of faster prototropic conjugate in the form of AH2/AH-·or AH.-/AH2 from ylides stage that immediately formed when a potential is applied [Dinica, 2012].*

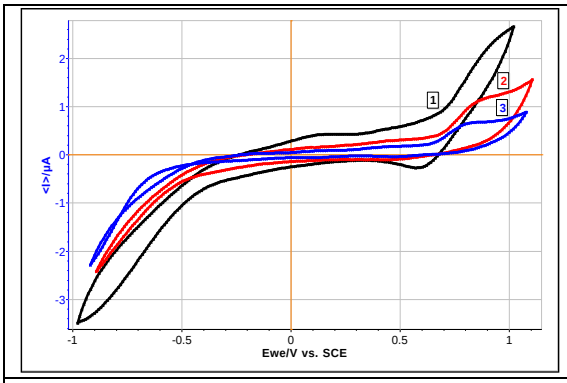



*Figure 16a. Cyclic voltammograms in aq. fresh Lm (pH 5.50), at different scan rate: 100 mV s-1(1); 50 mV s-1(2) and 20 mV s-1(3), E = ± 1.0 V*

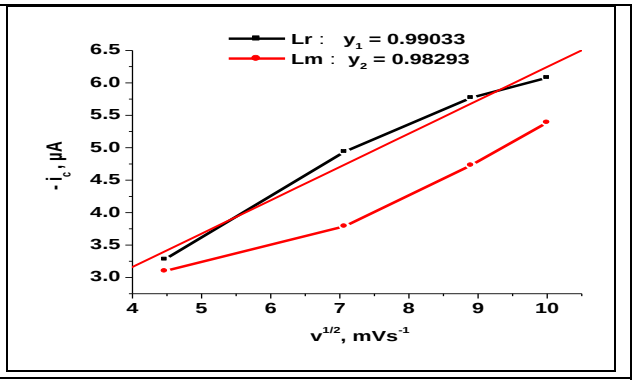



*Figure 16b. Plot of cathodic current vs. square root of scan rates*

*The cathodic peak position obtained at 100 mV s-1 corresponds to the redox changes from potential (Epc) of + 350 mV for Lr to the potential of + 600 mV for Lm (Figure 16a). A comparative study of aq. alkaline pH shows the different behaviour between the pyridinium heterocyclic ligands. The electrochemical parameters are presented in Table 2.*

*Table 2. Electrochemical data vs. scan rate of the potential applied for aq. pyridinium ligands at pH 9.00*

| *scan rate (mV·s-1)* | *- Epc (mV vs SCE)* | | *- ic (µA)* | | *Epa (mV vs SCE)* | | *ia (µA)* | |
|---|---|---|---|---|---|---|---|---|
| | *Lr* | *m* | *r* | *m* | *r* | *Lm* | *r* | *m* |
| *100* | *670* | *01* | *.08* | *.39* | *10* | *876* | *.85* | *.35* |
| *80* | *652* | *86* | *.65* | *.73* | *80* | *780* | *.54* | *.27* |
| *50* | *647* | *74* | *.94* | *.79* | *90* | *760* | *.66* | *.15* |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *20* | *613* | *18* | *.28* | *.10* | *90* | *660* | *.45* | *.87* |

*The reduction peak of ligands was significantly shifted towards a more negative potential, whereas a small shift in the oxidation peak was obtained at aq. Lr. Lm presents a different potential between the anodic peak and the cathodic peak that is considered an effect of quasireversible redox process of electronic transfer, due to the presence of the ethylenic group. Both ligands show characteristic peaks as a result of electron transfer between the functional groups, more evident on cathodic range in alkaline solutions. The cathodic peak at very lower potentials cannot be clearly assigned to reduction (Figure 16b).*

*Assuming that the electron transfer rate is faster, the current Ia is measured while the potential decreases, directly related to the diffusion rate of oxidized species on the Pt surface, by the flux governed by Fick law [A. M. O. Brett et al., 2003]. Thus, the oxido-reduction process involves a transfer of hydrogen proton and electron transfer of pyrididium ligands functioning as mediators in the aq. media. The peak is characterized by Ep, Ia and there is a peak shift while the pH increases.*

*The pyridinium ligands generate some current waves in cyclic voltammogram which are described as two typical one-electron transfer steps. The first step is reduction of AH2 (AH2/AH-) and the second step is the role of electron carrier of pyridinium ligand (AH-/AH.-). To explain this behaviour, a mechanism can be proposed when a catalytic reduction of the H+ ion takes place via a neutral (alkaline) intermediate of $^{1}H$ adsorbed on the Pt electrode in aq. media. The addition of proton donor produces changes, not only to the electroactive species, but also to the overall mechanism of reaction, making it concerted. Aq. alkaline ligands indicate an irreversible electron transfer, confirmed also by the cathodic peak on electrode, which is more evident on Lr than on Lm; ligands exhibit different mechanisms depending on aq. pH.*

*Spectrophotochemical absorbance registered in different solutions, fresh and kept at constant temperature indicates that pH 6-7 is better for stability of aq. ligands. The open circuit potential is a useful tool to characterize the ligands stability in aqueous media on Pt electrode. CV has been applied to characterize the electron transfer of aq. pyridinium ligands and the possibility to become a useful mediator. Electrochemical measurements offer the possibility to study the redox potential and the influence of pH and scan rates applied.*

*A good correlation was found between the electro-oxidation potential and the stability in aq. media of the ligands at acidic and neutral pH. Moreover, at alkaline pH, the pyridinium ligands indicate a difference taking into account the acidic-basic and electrochemical properties*

*which might result from the most favourable arrangement. The presence of the ethylenic group diminishes this effect as a result of more cyclofavourable addition in alkaline pH. We believe that the present work will stimulate further investigations; CV can be used to characterize the reduction ability and electrochemical behaviour of the compounds as pyridinium ligands and their role in biological chemistry. This study helps us better understand the ligands mechanism in aq. media and our purpose is to use them in cycloaddition to obtain indolizines in catalytic systems and for the synthesis of new Ln complexes.*

***Conclusions*** *For the first time spectroscopical and electrochemical studies were performed on the synthetised compounds obtained in previous phases. Following studies, remarkable biological and electrochemical properties were proved for some compounds synthesized by biocatalytic reactions. The interaction with DNA was made by UV-Vis, IR and fluorescence spectroscopy to study the nature of DNA-fluorophore binding. We found that there is a strong DNA-indolizine interaction, indolizine fluorescence is quenched at the increase of the molar ratio DNA:fluorophore. The possible interaction type of indolizines with DNA was determined to be groove binding. The electrochemical properties ware evaluated by cyclic voltametry. We believe that the cyclic voltametry studies will stimulate further investigations; CV can be used to characterize the reduction ability and electrochemical behaviour of the compounds as pyridinium ligands and their role in biological chemistry. This study helps us better understand the ligands mechanism in aq. media and our purpose is to use them in cycloaddition to obtain indolizines in catalytic systems and for the synthesis of new Ln complexes.*

*The objectives of this phase have been fully performed. The collaboration between the partners involved in the project had as a result the development of papers published or submitted for publication, papers containing results of the project interdisciplinary research. Indicators provided were made, some of the results were published in peer reviewed papers as STUDIA UBB CHEMIA, and JOURNAL OF BIOTECHNOLOGY or communicated to international conferences (EuroBiotech 2015, May 2015, Bucharest, Romania, BIOTRANS 2015, July 2015, Vienna, Austria, EuroAliment Symposium 2015, September 2015, Galati, Romania, Trans Mediterranean Colloquium on Heterocyclic Chemistry, TRAMECH VIII, November 2015, Antalya, Turkey).*

*Also, a book chapter, Perspective-Breakthroughs in indole and indolizine chemistry - new synthetic pathways, new applications, was published in Scope of Novel Heterocycles from Organic and Pharmaceutical, edited by InTech-open acces. Other results are submitted for publication or are in progress.*

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