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Physicochemical investigation of Psoralen binding to double stranded DNA through electroanalytical and cheminformatic approaches

Douglas Vieira Thomaz [1]*, Matheus Gabriel de Oliveira[1], Edson Silvio Batista Rodrigues[1], Vinicius Barreto da Silva [2]*, Pierre Alexandre dos Santos [1]*

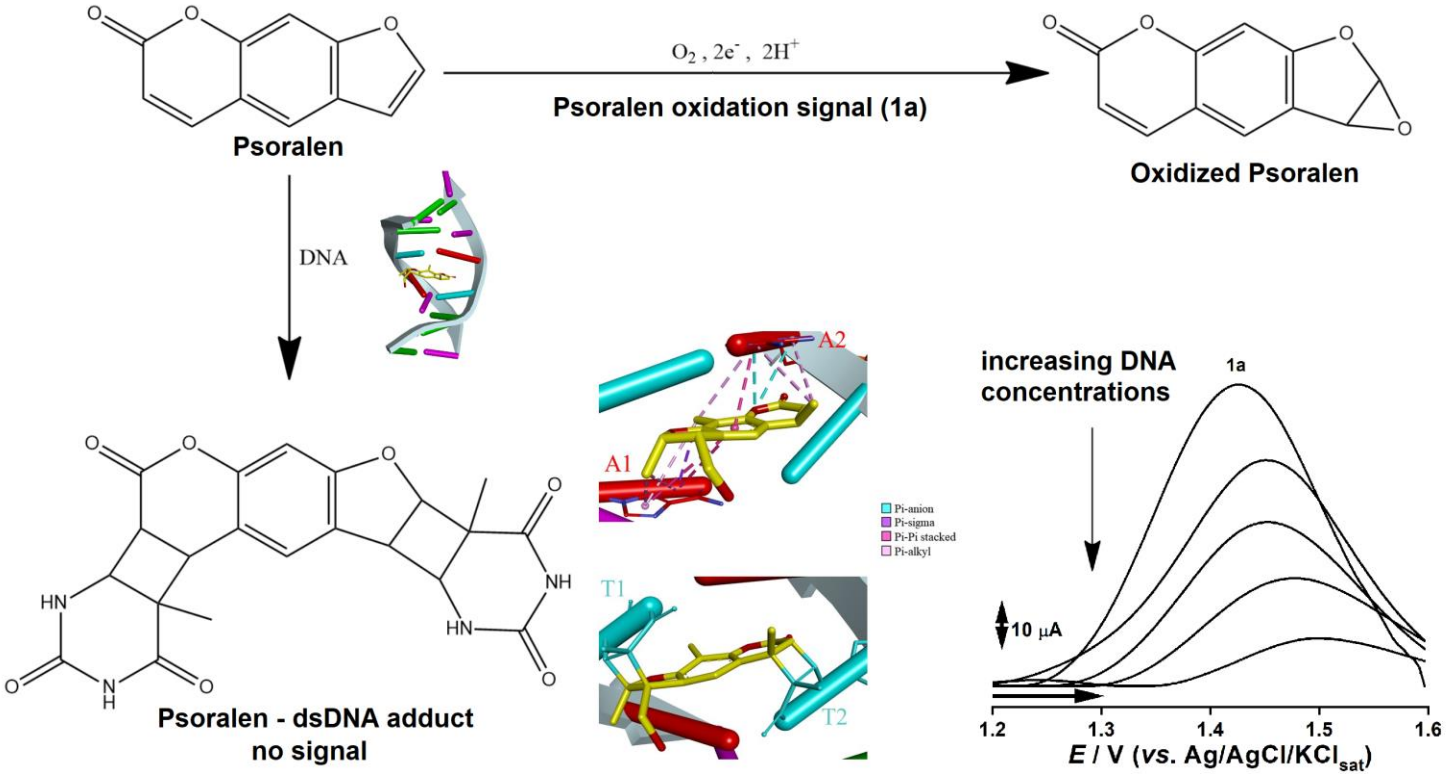
[1] Federal University of Goias;

[2] Pontifical Catholic University of Goias.

* Corresponding author: douglasvthomaz@gmail.com; viniciusbarreto.farmacia@gmail.com; pierre_farma@yahoo.com.br

Physicochemical investigation of Psoralen binding to double stranded DNA through electroanalytical and cheminformatic approaches

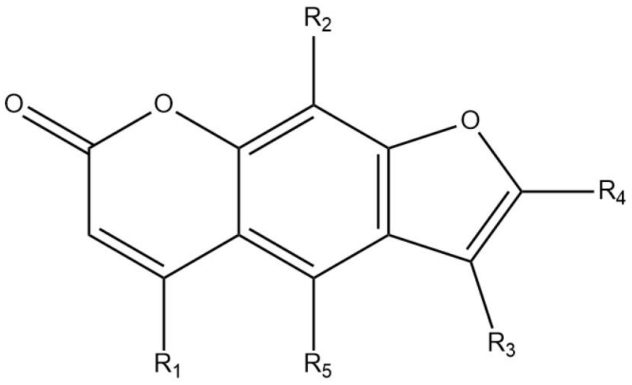
Graphical Abstract



Abstract:
This work showcased the first physicochemical investigation of Psoralen (PSO) binding to double stranded DNA (dsDNA) through electroanalytical methods. Results evidenced that PSO presents one non-reversible anodic peak at E_{pa} ≈ 1.42 V which is associated with its oxidation and the formation of an epoxide derivative, moreover, PSO analytical signal (*i.e.* faradaic current) decreases linearly with the addition of dsDNA while the electric potential associated to PSO oxidation shifts towards more positive values, indicating thence that dsDNA addition hinders PSO oxidation. These findings were corroborated by the chemoinformatic study, which evidenced that PSO intercalates noncovalently between base-pairs of the DNA duplex, and then irreversibly form adducts with both DNA strands, leading up to the formation of a cross-link which bridges DNA helix, what explains the linear dependence between the faradaic current generated by PSO oxidation and the concentration of DNA in the test-solution, as well as the dependence between E_p and the addition of dsDNA solution. Therefore, the findings herein reported evidence the applicability of electroanalytical approaches such as voltammetry in the study of DNA intercalating agents.
Keywords: voltammetry; intercalating agent; furanocoumarin; thermodynamic; kinetics.

Introduction

- Psoralen (PSO) is a photosensitizing linear furanocoumarin derivative (Figure 1) whose biologic potential is therapeutically explored to treat cutaneous conditions such as psoriasis and vitiligo, as well as some forms of cancer;
- Although the therapeutic use of furanocoumarins is well reported, the exact mechanisms of action regarding their many biological effects are still unknown;



PSO
R_1 - H; R_2 - H; R_3 - H; R_4 - H;
4'-hydroxymethyl-4,5',8-trimethylPSO
R_1 - CH_3; R_2 - CH_3; R_3 - CH_2OH; R_4 - CH_3;
Bergapten
R_1 - H; R_2 - H; R_3 - H; R_4 - H; R_5 - OCH_3;
Bergaptol
R_1 - H; R_2 - H; R_3 - H; R_4 - H; R_5 - OH;
Xanthotoxin
R_1 - H; R_2 - H; R_3 - OCH_3; R_4 - H; R_5 - OH;
Isopimpinellin
R_1 - H; R_2 - OCH_3; R_3 - OCH_3; R_4 - H; R_5 - H;

Figure 1. Linear furanocoumarins and their structures, namely: Psoralen (PSO); 4'-hydroxymethyl-4,5',8-trimethylpsoralen; Bergapten; Bergaptol; Xanthotoxin; Isopimpinellin.

Introduction

- Several authors investigated PSO-DNA binding through spectrophotometric methods as well as theoretical approaches, resulting thence in the understanding that covalent linking is involved in PSO anchorage on DNA strands through PSO-Thymine adducts (Figure 2);

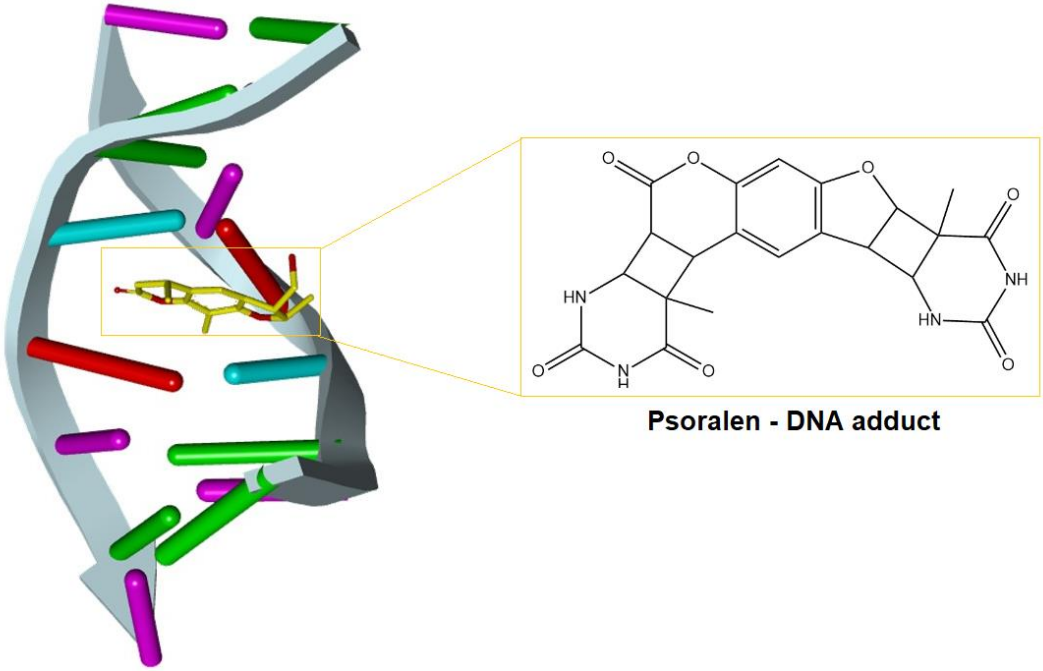
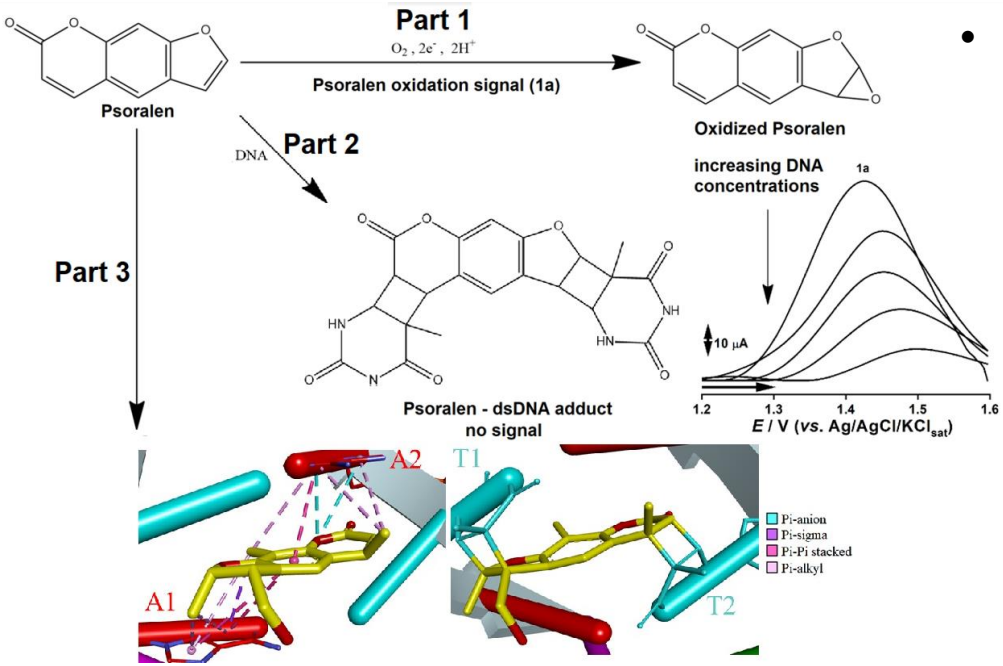


Figure 2. General structure of PSO-DNA binding through PSO-Thymine adducts.

Introduction

- Although spectrophotometry allows the investigation of physicochemical properties of drug-DNA interactions, alternative approaches such as electroanalysis may provide valuable information regarding the redox processes therein involved;



- Therefore, this work is aimed at the characterization of PSO electrochemical behavior at glassy carbon electrode (**Part 1**) and in the presence of commercial calf-thymus double stranded (ds) DNA (**Part 2**), as well as the exploration of the electroanalytical findings using *in silico* approaches (**Part 3**).

Results and discussion (Part 1)

- At first, PSO electrochemical behavior at glassy carbon electrode was investigated through cyclic and square wave voltammetries. Results are showcased in Figure 3.

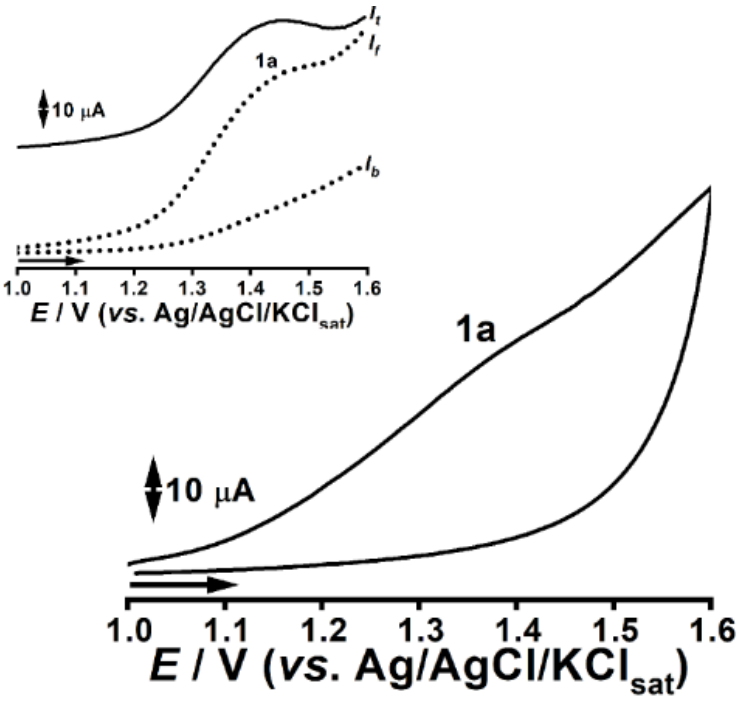
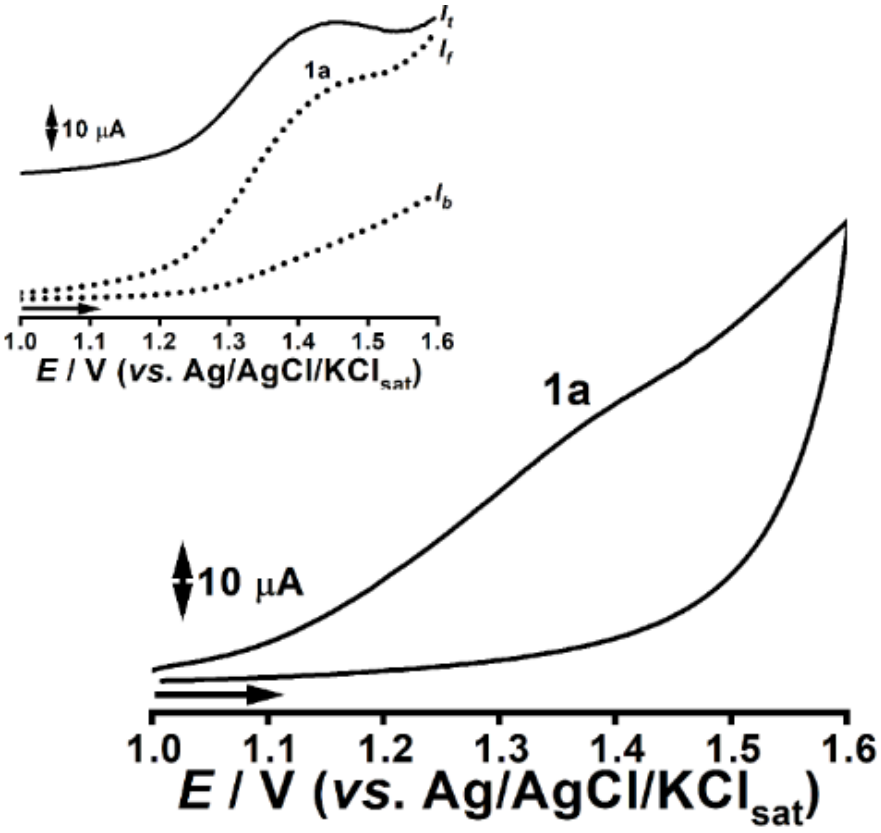


Figure 3. Cyclic and Square Wave voltammograms showcasing the anodic peak (1a), which is associated to Psoralen oxidation. All assays performed in 0.1 mol L^{-1} PBS solution, pH 7.0.

Results and discussion (Part 1)



- Results showcased that under CV and SWV, PSO presents one non-reversible anodic peak at E_{pa} ≈ 1.42 V;
- This peak correlates to the oxidation of electroactive moieties in PSOR molecule;

Figure 3. Cyclic and Square Wave voltammograms showcasing the anodic peak (1a), which is associated to Psoralen oxidation. All assays performed in 0.1 mol L^{-1} PBS solution, pH 7.0.

Results and discussion (Part 1)

Thereafter, the electro-oxidation of PSO was investigated towards its kinetics and thermodynamics by varying the scan rate and pH of supporting electrolyte solution. Results are showcased in Figure 4.

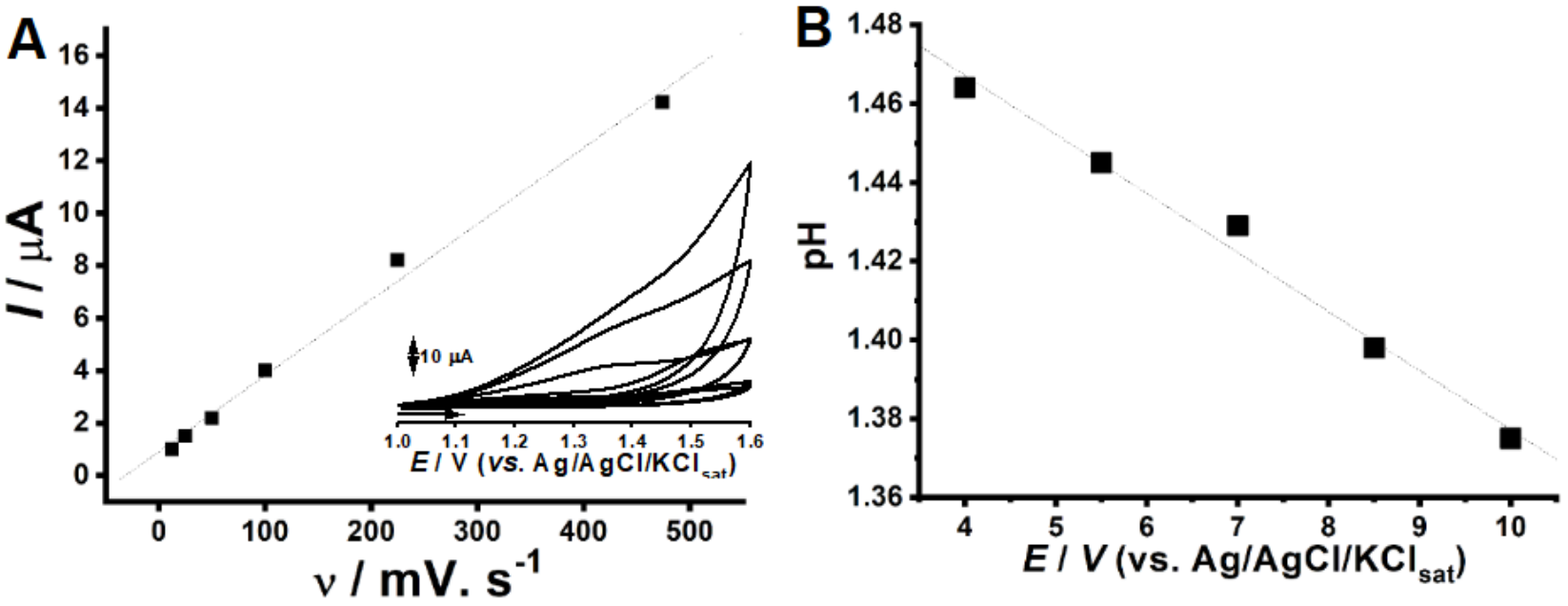
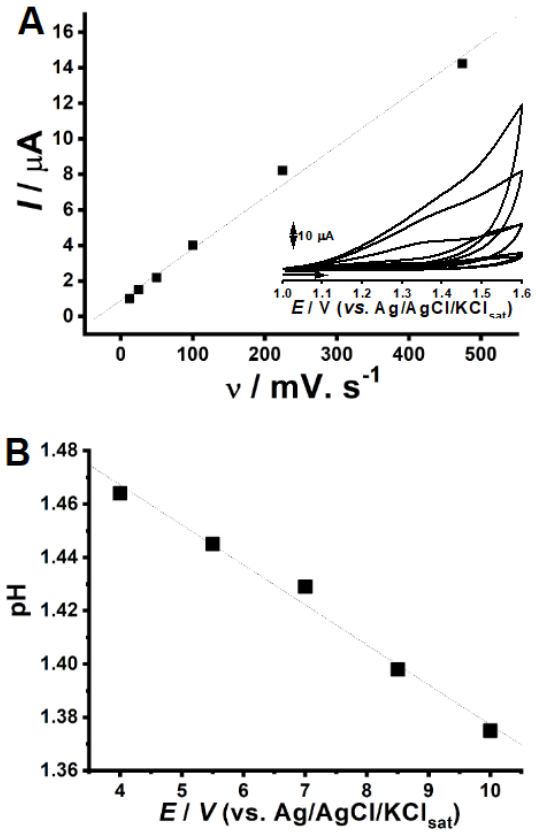


Figure 4.A. Plot of anodic peak 1a faradaic current (*I*) vs scan rate (ν), linearity (r^2 = 0.9932).**B.** Plot of pH vs electric potential associated to peak 1a (*E*), linearity (r^2 = 0.9874). All assays performed in 0.1 mol L^{-1} PBS solution.

Results and discussion (Part 1)



- Results showcased a linearity between electric current and scan rate (Figure 4.A), what thence suggests an adsorption-controlled electrochemical reaction;
- The linearity between electric potential and pH suggests equivalence of protons and electrons in PSO electro-oxidation (Figure 4.B).

Figure 4.A. Plot of anodic peak 1a faradaic current (*I*) vs scan rate (ν), linearity (r^2 = 0.9932).**B.** Plot of pH vs electric potential associated to peak 1a (*E*), linearity (r^2 = 0.9874). All assays performed in 0.1 mol L^{-1} PBS solution, pH 7.0

Results and discussion (Part 1)

Considering the electrochemical behavior of PSO, as well as literature regarding its oxidative degradation, the following electro-oxidation reaction was proposed (Figure 5).

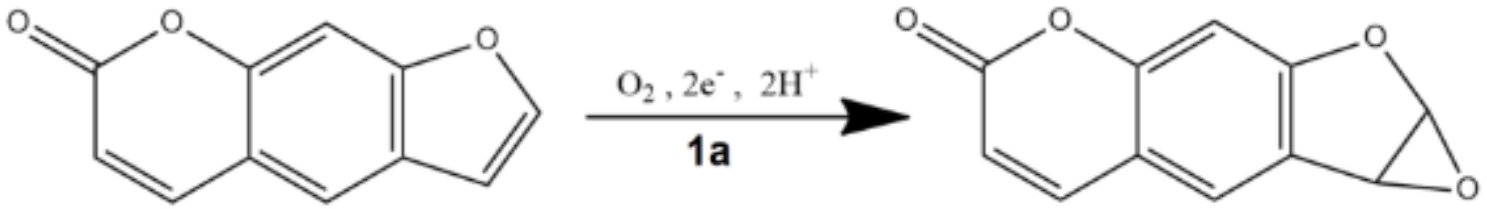


Figure 5. Proposed electro-oxidation reaction to PSO at glassy carbon electrode.

Results and discussion (Part 2)

In order to investigate the physicochemical changes associated to PSO binding to dsDNA, several DPVs were conducted at 1 mmol L^{-1} PSO solutions under increasing proportion of 0.75 µg L^{-1} dsDNA solution, which ranged from 25 µL to 425 µL. The final volume of all solutions was of 1 mL. Results are depicted in Figure 6.

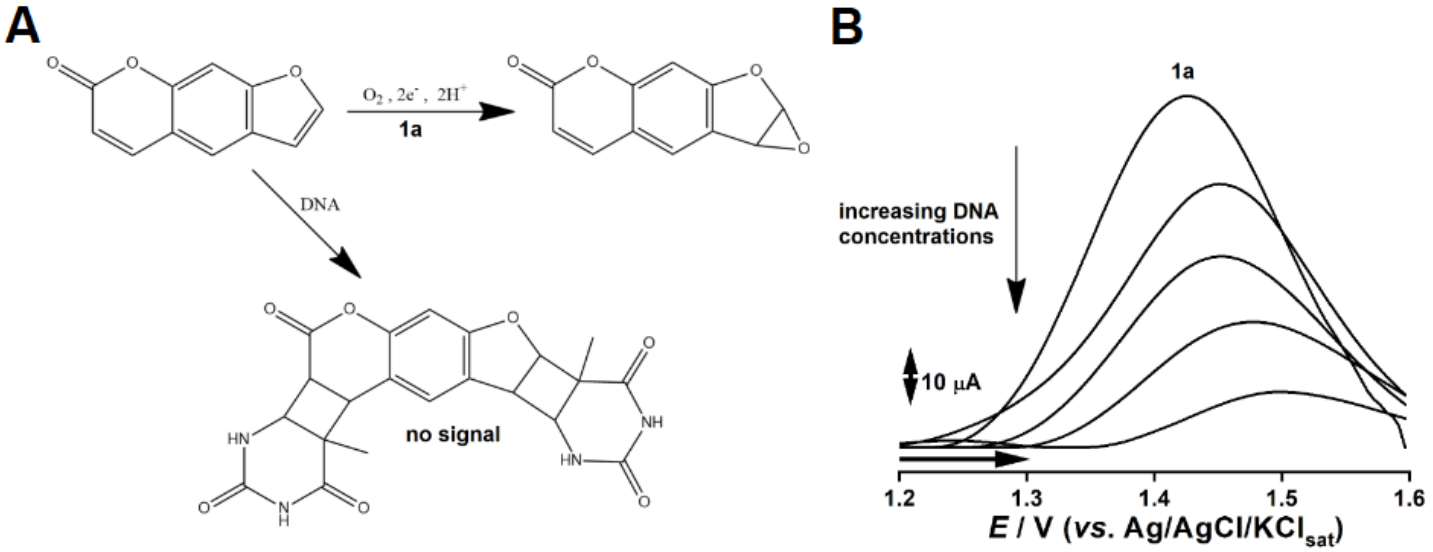
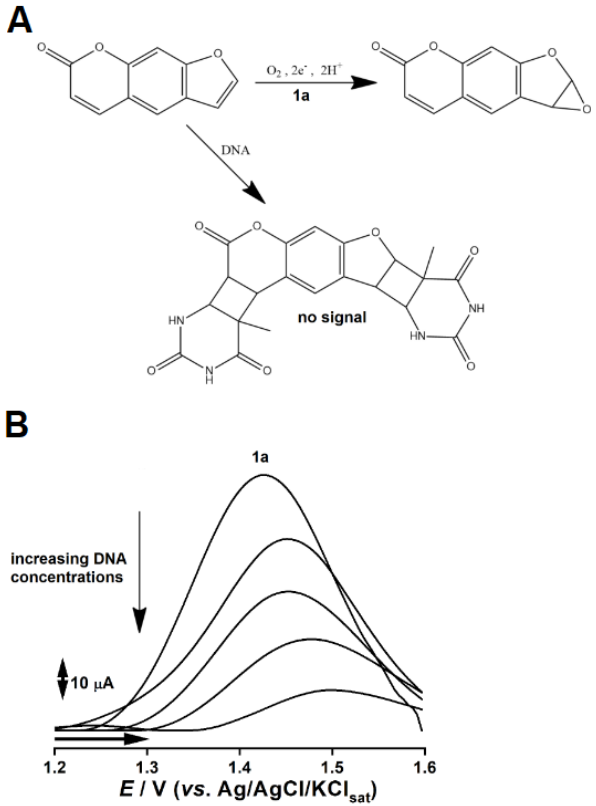


Figure 6. **.A.** Depiction of PSO electro-oxidation and its binding to dsDNA. **B.** Differential pulse voltammogram showcasing the effect of DNA addition on the anodic peak (1a) which is associated to Psoralen oxidation. All assays performed in 0.1 mol L^{-1} PBS solution, pH 7.0.

Results and discussion (Part 2)



- Results showcased that the anodic signal 1a, which is associated to PSO oxidation, is minimized by the addition of dsDNA solution, what suggests that the binding of PSO to ds-DNA leads to a non-electroactive product in the assayed conditions;
- Results were similar to spectrophotometric investigations of DNA-intercalating agents.

Figure 6. **.A.** Depiction of PSO electro-oxidation and its binding to dsDNA. **B.** Differential pulse voltammogram showcasing the effect of DNA addition on the anodic peak (1a) which is associated to Psoralen oxidation. All assays performed in 0.1 mol L^{-1} PBS solution, pH 7.0.

Results and discussion (Part 2)

Moreover, the linearity of PSO`s electro-oxidation signal (1a) *versus* the addition of ds-DNA solution, and the linearity between 1a and the pH of the support electrolyte solution were investigated. Results are depicted in Figure 7.

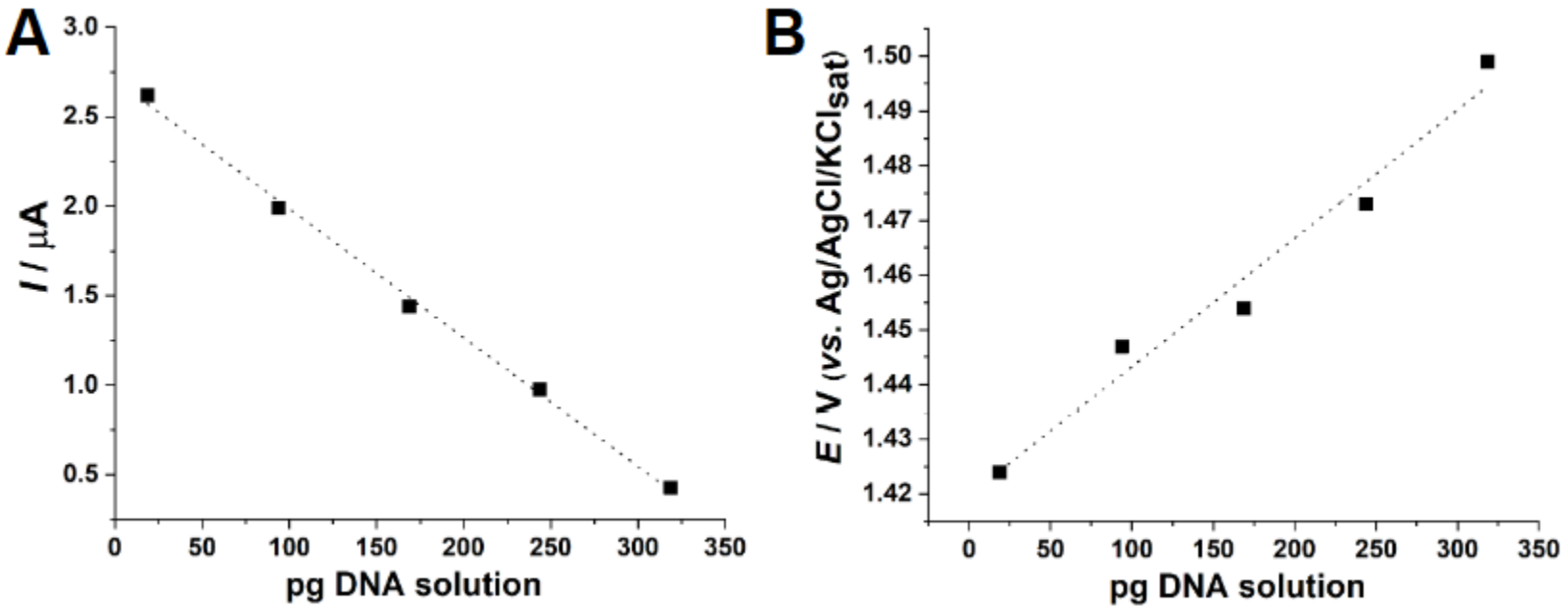
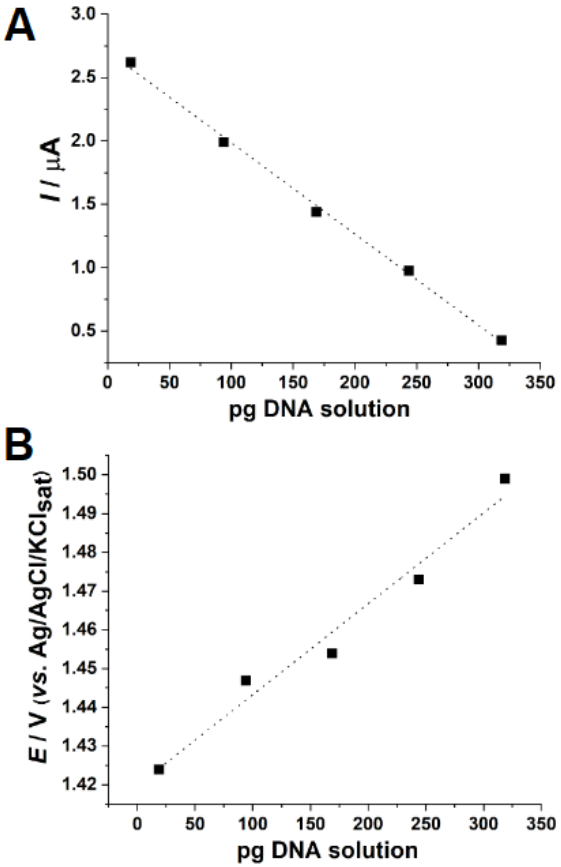


Figure 7. A. Plot of anodic peak 1a faradaic current (*I*) vs the amount of added DNA (r^2 = 0.9973). **B.** Plot of electric potential associated to peak 1a (*E*) vs the amount of added DNA (r^2 = 0.9712). All assays performed in 0.1 mol L^{-1} PBS solution, pH 7.0.

Results and discussion (Part 2)



- Results suggests that Psoralen-DNA binding is concentration-dependent, which is a known behavior of DNA-intercalating agents;
- Moreover, the linearity between the electric potential and dsDNA addition suggests that the presence of DNA reduces the proneness of Psoralen oxidation at electrode surface, therefore hinting that PSO-DNA adduct is thermodynamically stable under the assayed conditions.

Figure 7. A. Plot of anodic peak 1a faradaic current (*I*) vs the amount of added DNA (r^2 = 0.9973). **B.** Plot of electric potential associated to peak 1a (*E*) vs the amount of added DNA (r^2 = 0.9712). All assays performed in 0.1 mol L^{-1} PBS solution, pH 7.0.

Results and discussion (Part 3)

In order to further investigate the results, previously published nuclear magnetic resonance models were retrieved from Protein Databank (PDB entry: 204D) and compared to voltammetric findings. Results are displayed in Figure 8.

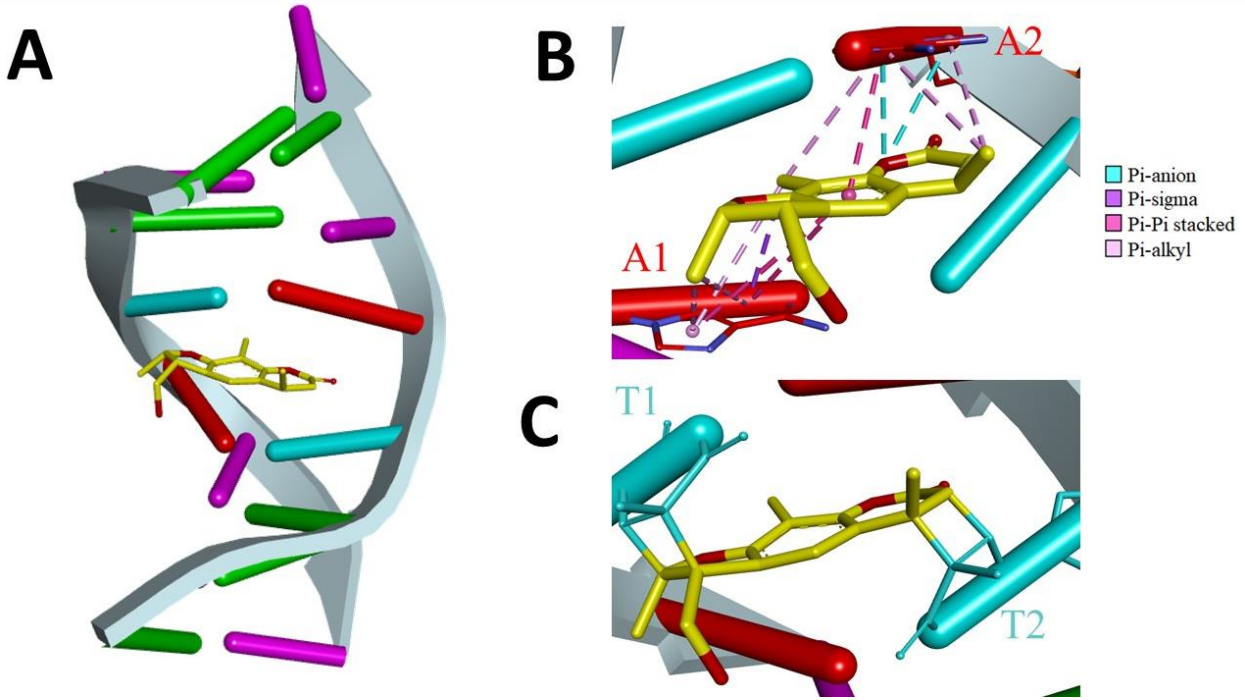
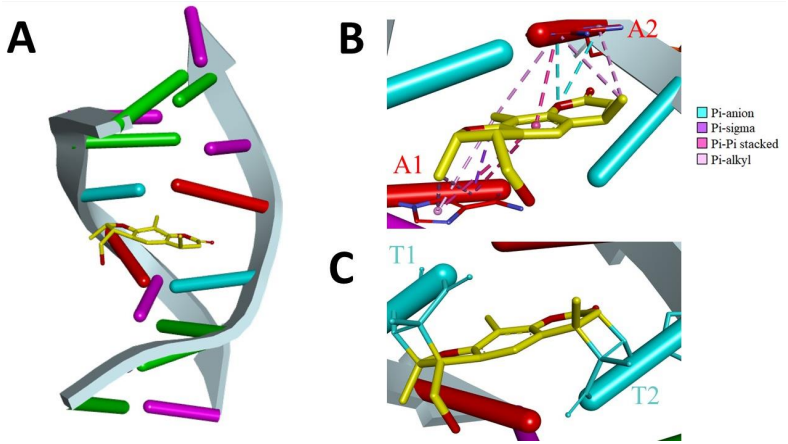


Figure 8.A. Model of the cross-linkage between PSO and DNA. **B.** Adenine bases stacked coplanar with PSO. **C.** PSO covalently attached to thymines through a cyclobutane ring. The figure was generated with Discovery Studio v.2019.

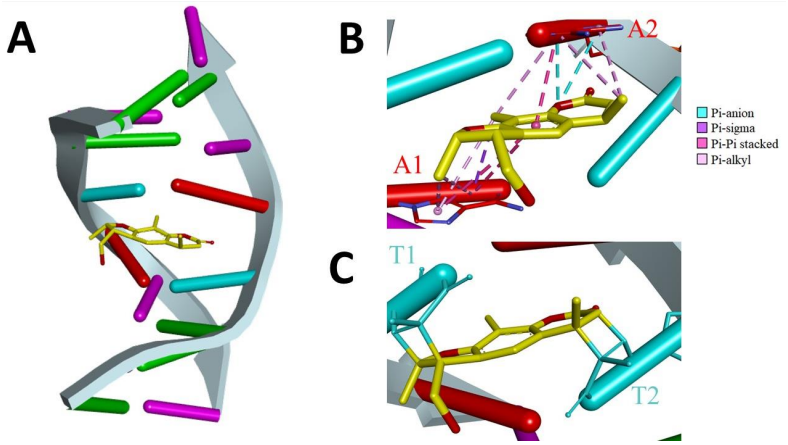
Results and discussion (Part 3)



- The model for the cross-linkage between PSO and dsDNA showed that the 2-fold symmetry of the DNA duplex is broken by PSO adduct formation (Figure 8.A);
- PSO initially intercalates noncovalently between base-pairs of the DNA duplex, what may stabilize its structure due to charge delocalization as well as lead to steric hindrance, thence hindering electro-oxidation and increasing the electric potential associated to peak 1a.

Figure 8.A. Model of the cross-linkage between PSO and DNA. **B.** Adenine bases stacked coplanar with PSO. **C.** PSO covalently attached to thymines through a cyclobutane ring. The figure was generated with Discovery Studio v.2019.

Results and discussion (Part 3)



- The adenine bases are stacked coplanar with PSO, and this arrangement allows electrostatic (Pi-anion) and hydrophobics (Pi-sigma, Pi-Pi-stacked and Pi-alkyl) interactions (Figure 8.B);
- The covalent structure of the cross-link fixes the orientation and position of thymines and bridges the helix. This bond is irreversible in the assayed conditions , what therefore explains the linear dependence of the faradaic current generated by PSO oxidation, and the concentration of dsDNA in the test-solution.

Figure 8.A. Model of the cross-linkage between PSO and DNA. **B.** Adenine bases stacked coplanar with PSO. **C.** PSO covalently attached to thymines through a cyclobutane ring. The figure was generated with Discovery Studio v.2019.

Conclusions

This work showcased the first physicochemical investigation of PSO linking to dsDNA through electroanalytical methods. Results evidenced that PSO presents one non-reversible anodic peak which is associated to its oxidation and the formation of an epoxide derivative, moreover, PSO analytical signal (*i.e.* faradaic current) decreases linearly with the addition of dsDNA while the electric potential associated to PSO oxidation shifts towards more positive values, indicating thence that dsDNA addition hinders PSO oxidation. These findings were corroborated by the chemoinformatic study, which evidenced that PSO binding leads to the formation of a cross-link which bridges DNA helix and stabilize the adduct against electro-oxidation, what explains the linear dependence between the faradaic current generated by PSO oxidation and the concentration of DNA in the test-solution, as well as the dependence between E_p and the addition of dsDNA solution.

Acknowledgments

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