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Suggestion of Cyclic Voltammetry based Electrochemical DNA analysis of DNA-Petrol interaction-novel approach in DNA damage analysis

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Abstract

In our current research work,we investigated the DNA interaction with Petrol,to obtain a better understanding of the DNA response when subjected to Cyclic Voltammetry in gasoline medium.

We believe,the current work provides a basis to develop this methodology further,to explain the DNA damage in humans,when exposed to petroleum products. This is a preliminary step in this direction and one of the pioneering efforts to study the electro-chemical response of the DNA-Gasoline analyte, using CV technique. Since the CV technique is well established in the academia and in the industry,we experimented with this idea and expect that there will be further development in this aspect.

Keywords: Cyclic Voltammetry (CV)/DNA/Gasoline/ Electrochemical properties of DNA/DNA damage.

I. Introduction

"Currently, it is known that several chemical agents used or generated by the oil industry are classified as mutagens and/or carcinogens. Among these we have gasoline, diesel, butane gas, styrene, benzene, chloroform, and others. Studies have verified that these chemicals have effects in fertility (abortions, sterility); produce various upheavals, such as dizziness, nausea, muscular pain; and produce chromosomal damage at the DNA level, which in the long or medium run, can develop into cancer and leukemia. The genetic damage in exposed individuals was measured by means of the comet test, chromosomal alterations test, and the study of the CYP 1A1 and MSH2 genes. These methods were applied to determine the genotoxicity of hydrocarbons and their residue in human beings. When conducting these tests on the blood samples of individuals exposed to hydrocarbons (workers of oil companies) and of a control population of the area of study and Quito, it was found that, in effect, the exposed individuals presented a greater amount of damage at the DNA level as well as at the chromosomal level than the individuals from the control populations ($P < 0.001$). Thus, it can be determined that populations that are exposed to hydrocarbons are susceptible to developing genetic damage. Therefore, risk groups can be determined in certain zones where the oil impact has been greater".[1]

"DNA is recognized as a nano-biomaterial, not as a pure biological material, in the research field of nanotechnology. In the past scientific works, the characteristics of DNA including facile synthesis by the solid-phase method, self-assembly, electro-conductivity, information elements, amplification, switching, molecular recognition, and catalytic functions, were appropriately applied. Multiple functions of DNA could be used simultaneously, and activated independently, by molecular switching. Therefore, the combinations of functional sequences of DNA can produce unique materials. It is obvious that the DNA molecule is one of the most promising functional nano-materials.

However, the application of DNA molecules is still under study because of the big gap that exists between theory and practice. We eagerly anticipate a 'coming out' of DNA due to breakthroughs in nano-biotechnology.In our current paper we are not going into the details of DNA as there is plenty of literature available already".[2]

"Gasoline is a complex mixture of over 500 hydrocarbons that may have between 5 to 12 carbons. Smaller amounts of alkane cyclic and aromatic compounds are present. Virtually no alkenes or alkynes are present in gasoline. Gasoline is most often produced by the fractional distillation of crude oil. The crude oil is separated into fractions according to different boiling points of hydrocarbons of varying chain lengths. This fractional distillation process yields approximately 25% of straight-run gasoline from each barrel of

crude oil"[4-11].

Cyclic Voltammetry (CV) is an electrochemical technique which measures the current that develops in an electrochemical cell under conditions where voltage is in excess of that predicted by the Nernst equation. CV is performed by cycling the potential of a working electrode, and measuring the resulting current. A CV system consists of an electrolysis cell, a potentiostat, a current-to-voltage converter, and a data acquisition system. The electrolysis cell consists of a working electrode, counter electrode, reference electrode, and electrolytic solution. The working electrode's potential is varied linearly with time, while the reference electrode maintains a constant potential. The counter electrode conducts electricity from the signal source to the working electrode.[3-5,7]

The purpose of the electrolytic solution is to provide ions to the electrodes during oxidation and reduction. A potentiostat is an electronic device which uses a dc power source to produce a potential which can be maintained and accurately determined, while allowing small currents to be drawn into the system without changing the voltage. The current-to-voltage converter measures the resulting current, and the data acquisition system produces the resulting voltammogram.[16-18] Cyclic Voltammetry can be used to study qualitative information about electrochemical processes under various conditions, such as the presence of intermediates in oxidation-reduction reactions, the reversibility of a reaction. CV can also be used to determine the electron stoichiometry of a system, the diffusion coefficient of an analyte, and the formal reduction potential, which can be used as an identification tool. In addition, because concentration is proportional to current in a reversible, nernstian system, concentration of an unknown solution can be determined by generating a calibration curve of current vs. concentration.[7]

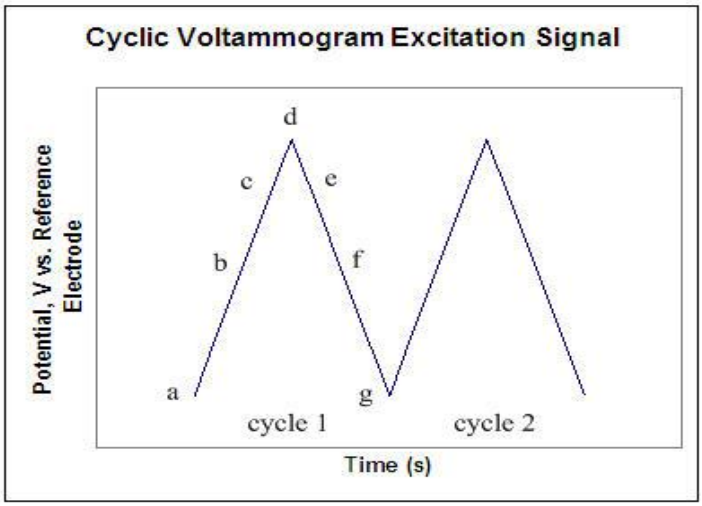

Figure 1: typical excitation signal of a CV instrument

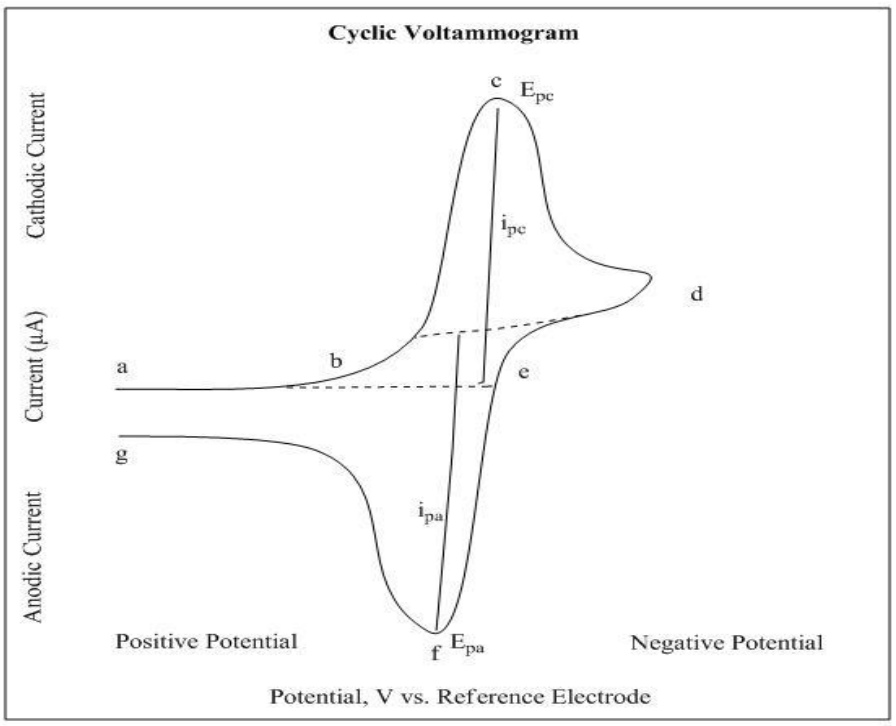

Figure 2: typical cyclic voltammogam for comparision with our DNA electro-chemical response.

II. Experimentation Methodology and Materials

The materials needed for this experiment are DNA(just general purpose no specific sequencing) and Petrol.DNA and Petrol are added in 1:1 ratio and gently stirred to mix them properly.Here in this current experiment,dsDNA(double strand DNA) was used,then the interaction of DNA with petrol was studied using Cyclic Voltammetry technique to probe the response of DNA in Petrol.

Glassy carbon electrodes (3.0 mm dia.) purchased from Bioanalytical Systems (BAS, West Lafayette, USA) were cleaned mechanically by polishing with wet 0.3 and 0.05 µm alumina slurry (Alpha and Gamma Micropolish; Buehler, Lake Bluff, USA) on microcloth pad (BAS) and by sonicating in water (30 sec.).

Then the electrodes were dipped in 0.5 M sulphuric acid solution and sweeping the potential between –300 mV and +1400 mV (versus a Ag/AgCl reference electrode) with scan rate of 100 mV/s.[14-19]

* **Please make a note:** We are not explaining the CV technique/s because we assume most of the readers are very familiar with this technique. Those who are not familiar are advised to visit one of the references explaining the basics and simple laboratory practices.

The nucleic acids bases are electroactive species. Chemical changes in these bases as well as conformational changes of DNA are expected to modify its electrochemical behavior. Adenine (A) and cytosine (C) sites, which are involved in Watson-Crick hydrogen bonding, are reduced on mercury electrodes. The oxidation site in Guanine (G), which is oxidized on graphite electrodes, is not part of the hydrogen bonds. In addition, nucleic acids are surface-active macro-molecules and this property of DNA molecule makes it suitable for adsorptive preconcentration, e.g. concentration on an active surface. This process enhances its electrochemical behavior which can thus be used as a tool for analyzing its conformational properties and its chemical modification.[5-19]

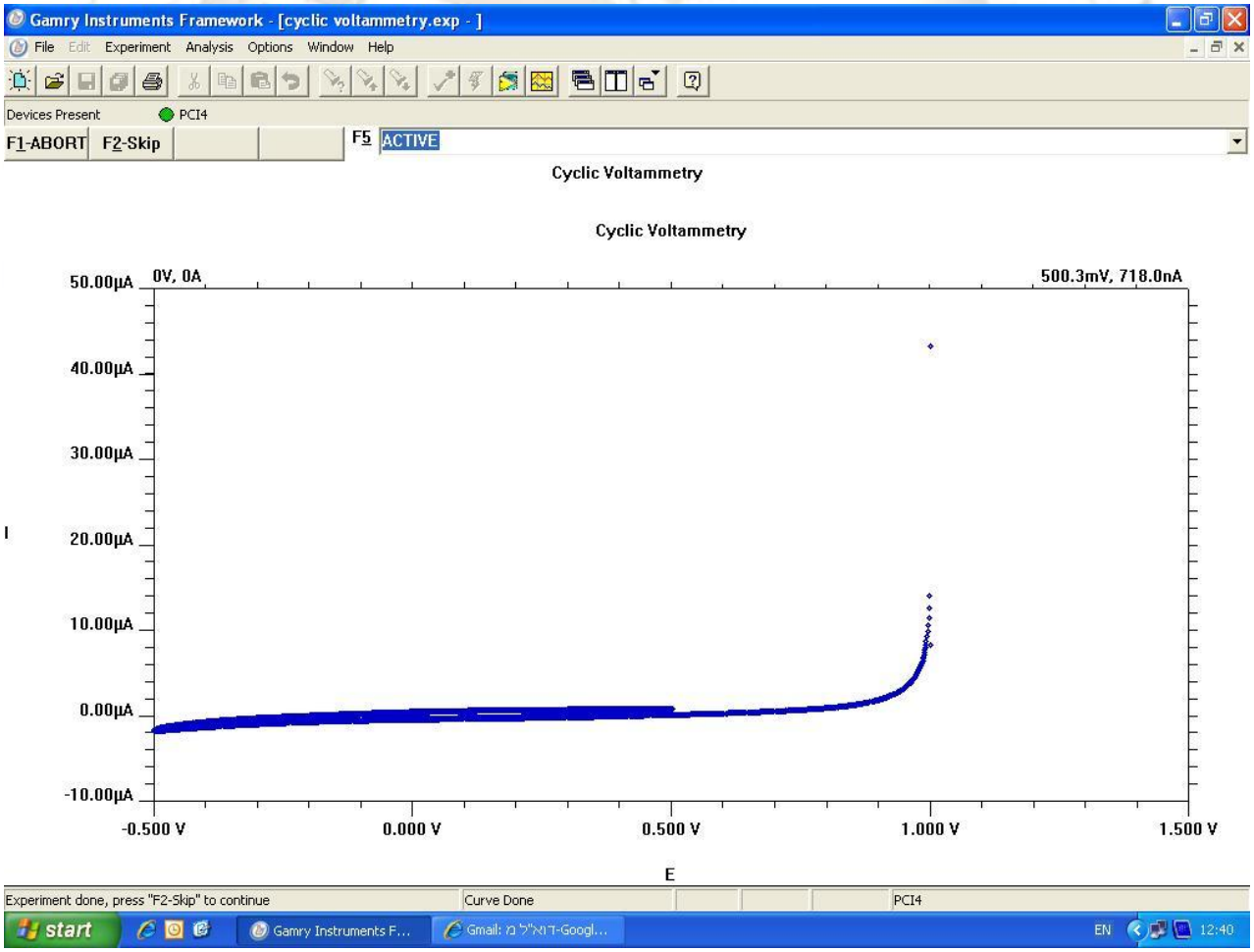

Figure 1: CV Voltammogram of DNA-Fuel mixture -First Run.

Figure 1 explains the electrochemical response of the DNA-gasoline solution
DNA interacts with gasoline and the distortion in its electrochemical response is clearly observed.
To confirm further we experimented further and covered second and third runs using our Gamry CV instrument and also include figures depicting second and third runs. However this investigation is still in the preliminary stages of research and development and need additional in-depth experimentation to evolve a theory to understand the DNA damage analysis in gasoline as the medium.We believe it is possible and feasible to extend the current CV based analysis of DNA damage to say Crude Oil etc

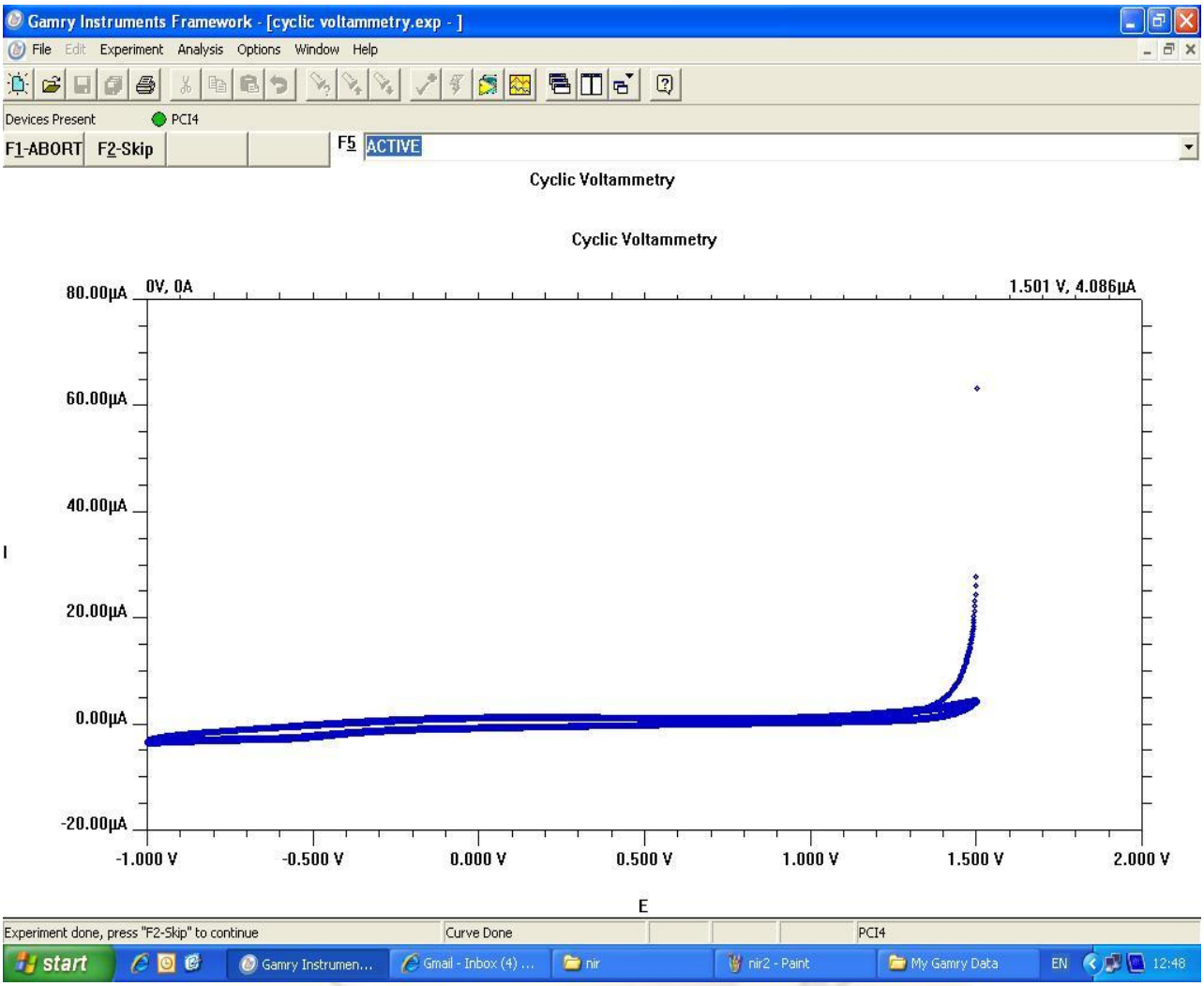

Figure 2 : CV Voltammogram of DNA-Fuel interaction -Second Run

Figure 2. explains the DNA-Gasoline interaction

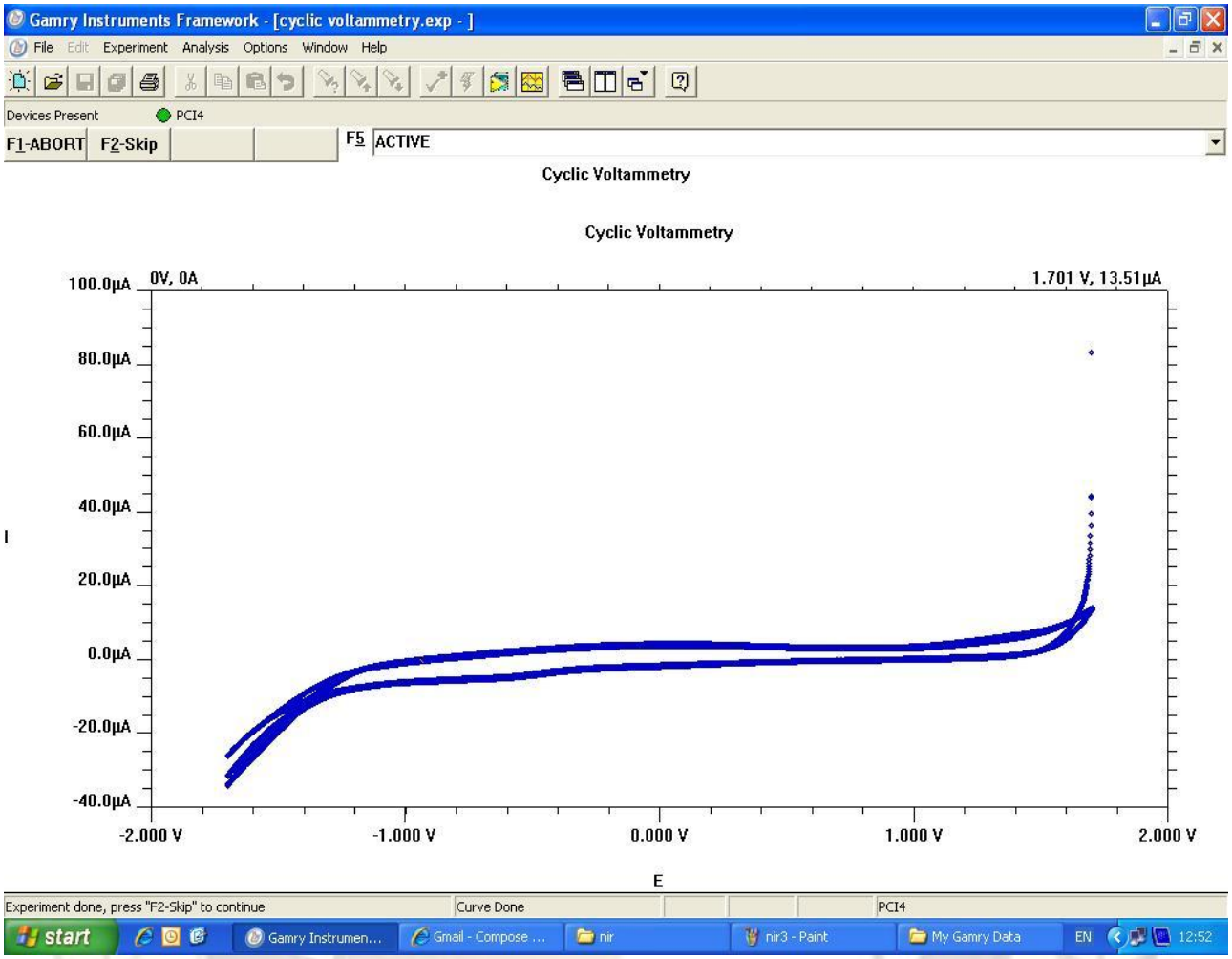

Figure 3: CV Voltammogram of DNA-Fuel interaction -Third Run

III. Conclusion and Future perspectives

To conclude the findings,we experimented here with DNA-Gasoline mixture and subjected the mixture to CV analysis and recorded the DNA responses as Voltammograms in the above mentioned figures. This technique is very promising to perform DNA damage analysis in the presence of petroleum products. Petroleum products are carcinogenic to DNA and damaging in nature.It is worth expanding the research further.

The Electro-chemical responses of the DNA under current/voltage conditions were depicted.
The voltammogram/s responses are comparable,to the already published electro-chemical properties of the pure DNA available in the scientific literature. The difference in the DNA response when mixed with gasoline is thus clearly observable. Also this provides a scope or methodology to develop DNA based electro-chemical sensors,to detect impurities in the petroleum products.

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Jiangnan University,China, for their research support and for providing conducive research environment. We finally thank the authors,of some of the published papers,who willingly gave us permissions to reproduce some materials,from their research papers and also encouraged us to write this paper. We declare further that,we have no competing financial interests in the method presented in this paper. All correspondence regarding this short communication should be addressed to :

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