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The effect of tobacco concentration on the DNA blood samples using the AC electrical impedance spectroscopy.

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Abstract: The electrical impedance of DNA of female and male healthy blood samples was recorded in vitro. The Nyquist Plots for female and male samples were of the same feature. The peak of each Nyquist Plot was increased and shifted to higher real electrical impedance values as tobacco concentration increased from 1µl to 4µl. The Bode module plots confirm these results for female and male DNA blood samples. The start values of the A.C electrical resistance and electrical capacitance were 95.98KΩ and 2.36µF for female DNA and were 183.6KΩ and 5.47µF for male DNA, respectively. These values were changed to be 82.65KΩ and 3.18 µF for female DNA and 177.35KΩ and 5.30µF for male DNA, respectively, under the effect of 1µl tobacco concentration for each of them. The increase of tobacco concentration gradually more than 1µl and up to 4µl, these values reach 139.63KΩ and 5.81µF for female DNA, and 219.42KΩ and 9.00µF for male DNA, respectively. These results were attributed to the increase of covered area in the lungs alveolus by the smoke in female and male DNA. Finally, these results illustrate that the covered areas of the lung alveolus by the smoke were wider in the case of female DNA than that of male DNA.

Keywords: tobacco concentration, DNA blood samples, the AC electrical impedance spectroscopy

1. Introduction

DNA is an important biopolymer that holds the genetic hereditary information of living organisms (Tan 2022; Devine and Jheeta 2020; Mukhamedshina et al 2020). The continued

existence of biological species requires its genetic material to be chemically stable. The biological process of making two identical duplicates of DNA from a single original DNA molecule is known as DNA replication (Brazda et al 2020).In eukaryotes such as animals and plants, DNA is stored inside the cell nucleus, while in prokaryotes such as bacteria and archaea, the DNA is stored in the cell's cytoplasm. DNA, unlike enzymes, does not operate directly on other molecules; instead, enzymes act on DNA and replicate its information into more DNA (DNA replication) or transcribe it into protein (protein synthesis). Histones, for example, are involved in the packing of DNA as well as the repair of DNA damage that produces mutations (Wein 2022, Esiobu et al 2022). The two strands of DNA are called polynucleotides because they are made up of simpler monomeric units, nucleotides (Schmidt 2022). Each nucleotide is made up of one of four nitrogenous bases (cytosine [C], guanine [G], adenine [A], and thymine [T]), a sugar called deoxyribose, and a phosphate group. Covalent bonds (also known as phosphor-diester bonds) are formed between the sugar of one nucleotide and the phosphate of the next, forming an alternating sugar-phosphate backbone. When two single strands of DNA unite through hydrogen bonds of complementary base pairs, the nitrogenous bases of the DNA double helix are produced (Holmlin et al 1997).

Additional nitrogenous bases are divided into two groups: pyrimidines and purines. In DNA, the pyrimidines are thymine and cytosine; the purines are adenine and guanine (Berg et al 2002, Carr2016). DNA stores biological information. The DNA backbone is resistant to cleavage and both strands of the double-stranded structure store the same biological information. This information is reproduced when two strands are severed (Casella 1978). The two DNA strands run in opposite directions and are therefore antiparallel. Each sugar has one of four types of nitrogenous bases (informal bases) attached. The genetic information is encoded by the sequence

of these four nitrogenous bases along the spine. In a process known as transcription, RNA strands are generated using DNA strands as a template. These RNA strands are translated according to the genetic code to determine the amino acid sequence of proteins in a process known as translation (Bailey et al 2022). At present, the extensively used method for direct measurement of DNA electrical conductivity is the measurement of I-V curves. In such an experiment, a single DNA molecule or a number of parallel DNA molecules is first deposited between two electrodes, and then the electron transport is measured under dry conditions by applying a voltage over the electrodes and measuring the responses. Here "dry condition" means "out of solution." The I-V characteristics of a solution containing DNA do not reveal any information on DNA electrical conductance but only tell Faradaic redox processes at the electrode interfaces and ionic movement inside the solution. Although DNA is normally prepared and stored inside aqueous buffer solutions, it is very stable in the air and its structure and properties could not be changed if it is dried in air without heating Zhuravel (Zhuravel et al 2020). **It**is not convenient to measure the I-V curves under these conditions. As a relatively simple method, an electrochemical a.c. impedance spectroscopy measurement may probably distinguish the electrical current from the ionic one. Electrical impedance spectroscopy (EIS), a powerful and sensitive technique for studying the kinetics and electrode interface, has received growing attention because of its non-invasive and label-free nature.

Generally, a representative electrochemical interface may be expressed as an electrical circuit, in which the electron-transfer resistance(R_{ct}) characterizes theelectrode's interfacial traits, as the semi-circle diameter at higher frequencies of the Nyquist plot gained by electrical impedance spectroscopy(EIS) (Hauff et al 2022). This technique has gained wide applications (Ranjan et al

2022; Mohanraj et al 2020; Guo et al 2021; Yazdanparast et al 2020). This a.c. impedance spectroscopy has been introduced into the present work to directly measure the electrical impedance of double-stranded DNA (Henley et al 2020; Guezguez et al 2021; Stepanov et al 2020; Stanfill et al 2020; Mondol et al 2021; Azzopardi et al 2021; Chen et al 2021; Mohammed and Bourawy 2020). Toxicants are present in all tobacco products, and smokeless tobacco products contain cancer-causing compounds (Hernández et al 2020; Zawawi et al 2020; Herrera ; Vafaiee et al 2021; Uwaya et al 2022; Ulusoy-Ghobadi et al 2008; Ugalde-Reyes et al 2022).

The aim of this article is to study the effect of tobacco in different concentrations on the female and male DNA samples. This will be carried out using electrical impedance spectroscopy (EIS).

2. Experimental technique

2.1 DNA preparation

The DNA solution was prepared in vitro by the separation of the red and white blood cells by adding 9 milliliters from cold extraction buffer to only one milliliter of the given blood sample using the rate of 12,000 rpm centrifuge for 10 minutesto get rid of pure red blood cells. The same steps were repeated until the white blood cells were precipitated on the bottom of the falcon tube. The falcon tube containing white blood cells was poured into Eppendorf of lysis buffer and kept at 37 °C for 24 hours. Then 200 µl of NaCl was added to the Eppendorf content and centrifuged at 12,000 rpm within 10 minutes to get rid of the protein. 600 µl of Isopropanol was added to the remaining in the Eppendorf(the upper liquid) and centrifuged again to get the DNA. The obtained DNA was washed using ethyl alcoholat 70 % concentration and centrifuged at8000 rpm for 5

minutes Then 50 µl of Tris EDTA (TE) buffer was added to the DNA and kept under healthy conditions.

1- The electrical impedance spectroscopy (EIS) and Dielectric constant measurements:

A.C electric potential of the value of 10mv was applied across the two tungsten electrodes dipped in their sample cell parallel to each other. These two electrodes were adjusted to be of the same area. The ratio of the electrode area and the electrode separation distance adjusted to be unity. The results of the electrical impedance and the electric capacitance (Dielectric constant) of the given sample, were curried in vitro at room temperature (23°c) and in the frequency range of 100 kHz to 10 mHz.

2.2 Tobacco treatment:

The unit mass of tobacco was spread out in 30 millimeters of distilled water, boiled within 10min, and then soaked overnight. The required electric parameters were determined in a cumulative manner as tobacco concentration increased from 0µL to 4µL.

3. Results and discussion

Fig(1a) represents the Nyquist diagram for pure and impure DNA samples. The addition of tobacco to DNA stepwise keeps the Nyquist shape as it is all over the experiment with some changes. These changes were noticed as the peak intensity and the peak half-width were increased. Also, the peak maximum shift to higher values of the electrical impedance may mean that the increase in tobacco concentration increases the electric resistance of the female DNA sample. This may lead to DNA oxygen starving. Fig(1b) represents the Bode module plots for the electrical

impedance of the same female sample. These results show that the electrical impedance goes in the same sense of the results for fig (1a), specially at higher frequency values. This means that the two results confirm each other qualitatively.

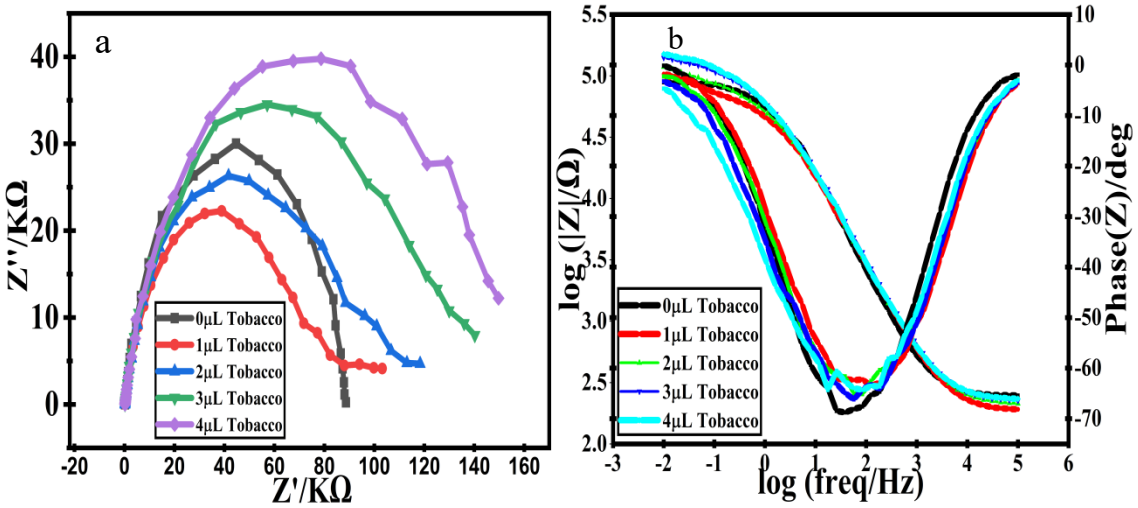

Fig. (**1**$_{a,b}$) a) Nyquist and b) Bode-module plots for the electrical impedance measurements for female DNA sample

The quantitative result can be drawn from a table (1) as follow. The start value of pure DNA electrical impedance is 95.99KΩ The addition of 1µl of tobacco reduces this value to 82.65KΩ. The increase of the tobacco concentration over this value and up to 4µl increase the value of the a.c electrical impedance gradually to be 139.63KΩ. This value is near twice the start value. This means that the smoke increases DNA electric resistance which may lead to some type of oxygen starving within, the alveolus of the lungs of the female sample. Also, from table (1) the start value of the electriccapacity of the DNA was 2.36µF. The addition of 2µl of tobacco reduces this value to be 2.7µF The gradual increase of the tobacco concentration over the value of 2µl and upto 4uL increases the DNA electric capacity to be 5.81µF. This may be due to the increase of the DNA-covered areas by the smoke. Accordingly, this may increase the DNA oxygen starving

as a result of the leakage of the gases exchanged in the lungs. The increase of the DNA electric capacity may decrease the female DNA effacing.

Table (1)

Tobacco cocentration (µl)	**R_{ct}(KΩ)**	**C (µF)**
0	95.99	2.36
1	82.65	3.18
2	90.03	2.70
3	123.19	3.05
4	139.63	5.81

Fig (2a) represents the Nyquist plot for male DNA. It is clear that the diagram features are still the same as in fig(1a), i.e. the peak intensities and the half-width increases. Also, the peak maximum shifts to a high erelectrical impedance value as the tobacco concentration increases.

The quantitative study of table(2) shows that the start value of a.c electric resistance is 183.6KΩ. This value decreased to 177.35KΩ due to the addition of 1µl of tobacco. As the tobacco concentration increased over this value and up to 4µl gradually the a.c electric resistance value increased to be 219.42KΩ. It is clear that the effect of tobacco concentration on the male DNA is moderate. This means that the oxygen starving on the male DNA is also moderate.

On the other hand, the start value of the male DNA electric capacity is 5.74µF. This value decreased to 7.00µFdue to the addition of 1µFof tobacco. The increase of the tobacco concentration over the value of 1µl and gradually up to the value 4µl increases the DNA male electric capacity to be 9.00µF. This means the affected area of the alveoli in the lungs in the case of the male sample is narrow than in the case of the female sample. i.e. the oxygen starving in the case of the male sample is not severe.

The results of fig (2b) confirm the points of view of the male DNA electric resistance and the male DNA electric capacity at low and high frequencies.

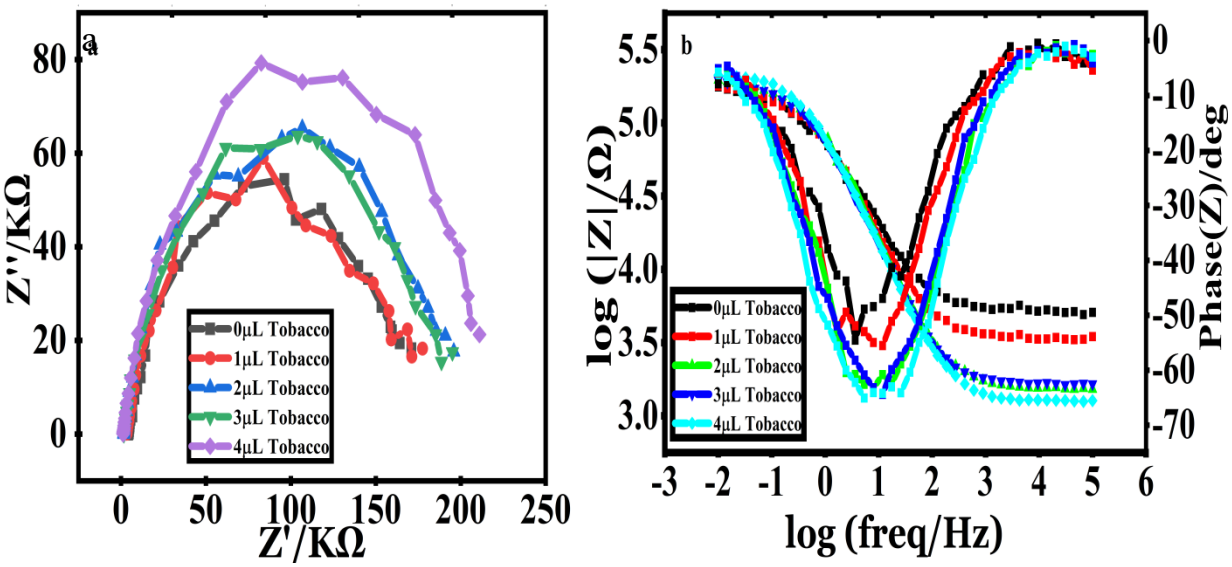

Fig .($2_{a,b}$) a) Nyquist and b)Bode-module plots for male DNA.

Table (2)

Tobacco concentration (µl)	**R_{ct}(KΩ)**	**C (µF)**
0	183.60	5.74
1	177.35	5.30
2	196.79	7.08
3	219.09	7.27
4	219.42	9.0

The obtained results of non-smoke DNA samples prove that the lung electrical resistance and the lung electrical capacitance have been increased due to the increase of the covered areas of the lung alveolus with tobacco smoke. These results in the case of the male DNA samples, the electric resistance, and the electric capacity were increased moderately. This means that the female DNA efficiency becomes less than that of the male DNA efficiency in the given tobacco

concentration range. Accordingly, tobacco severely attacks the female DNA more than the male DNA.

These results were repeated by measuring the DNA electrical impedance of the smoke male sample and the results are illustrated in Fig. (3a,b) and table (3). The qualitative and quantitative results of Fig.(3a,b) and table (3) confirm the previous point of view of the non-smoke male DNA.

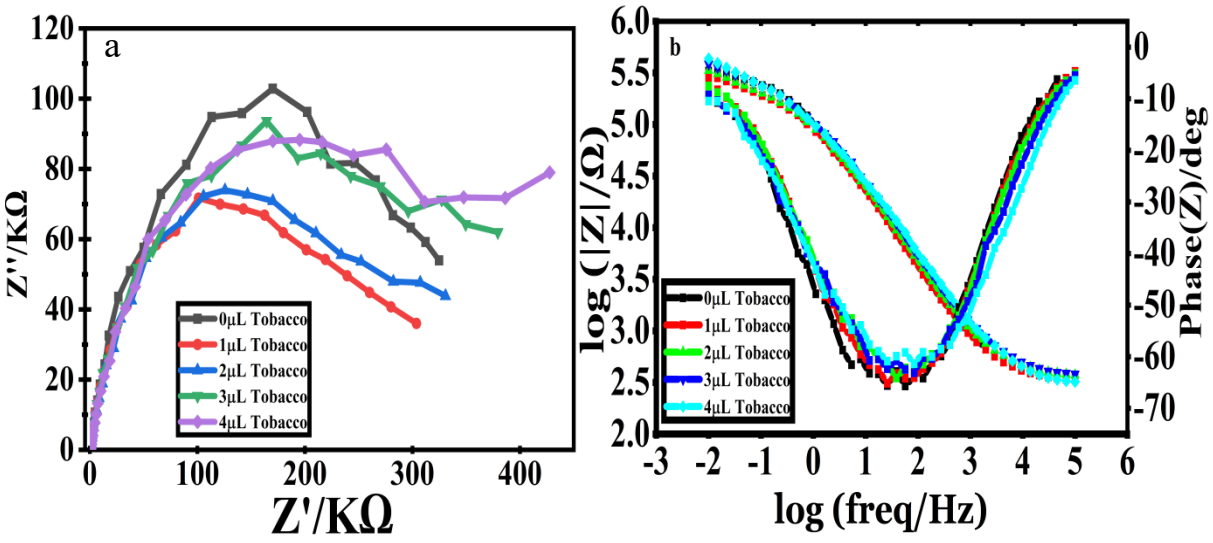

Fig. ($3_{a,b}$) a) Nyquist plots and b) Bode-module plots.

Table **(3)**

Tobacco concentration (µl)	Rct(KΩ)	C (µF)
0	75.60	9.22
1	73.32	10.38
2	77.06	6.72
3	107.09	11.13
4	134.07	12.89

4. Conclusions

The investigation of the electrical impedance and electric capacity of the DNA samples shows that:

1- The electrical resistance and the electrical capacity for non-smoker female DNA were increased as the tobacco concentration increased within the given range.

2- The electrical resistance and electric capacity of the male DNA sample wereincreased moderately.

3- The covered area by the smoke of the lung alveolus in the case of female DNA is wider than the covered area of the lung alveolus in the case of male DNA. This result is more pronouncedin the case of smokedmale DNA.

4- Theattack of tobacco smoke on the female DNA maybeoccurin severe mannerthan in the case of male DNA.

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

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