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USING ORTHODONTIC WIRE ELECTRODES IN GEL ELECTROPHORESIS DEVICE

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ABSTRACT

Gel electrophoresis is an essential device in biology laboratories and life science centers for the analysis of macromolecules such as DNA, RNA, and protein. This separation technique mainly depends on using a set of electrode wires to generate an electric field within the gel medium. The most common failure is the electrode wire cut. This fault is due to corrosion or inadvertent cut of the thin platinum wire. Platinum wires are delicate, expensive, and not available in local markets. In this paper, orthodontic nickel-free stainless steel wire (diameter = 0.5 mm), is used as an alternative to the Platinum wire. The effectiveness of the orthodontic stainless steel wire was confirmed under high-level voltages (100, 150, and 250 Volts), and the data produced was reasonable, with good visualization of DNA fragments. However, the Anode wire experienced corrosion after about 11 hours, while the cathode is still effective. The study concluded that while not a long-term solution, nickel-free stainless steel orthodontic wire offers a simple, inexpensive (< $1) alternative to platinum electrodes for gel electrophoresis, especially for students and researchers conducting short-term experiments. However, using stainless steel with nickel is suggested to potentially enhance the lifespan of the electrodes due to nickel's contribution to corrosion resistance. Further research is recommended to explore its wider applications in molecular biology research and improve experimental procedures using this alternative electrode materiale.

KEYWORDS

Corrosion; Gel Electrophoresis; Molecular biology; Nickel-free Stainless steel; Platinum.

1. INTRODUCTION

Advancements in electrode technology are essential as they play a crucial role in optimizing the movement and aggregation of charged particles in various applications (Hashim and Abdulhadi, 2022). Gel electrophoresis device (GED) uses a method for resolving Deoxyribonucleic acid (DNA) fragments based on their molecular weight (Lee et al., 2012; Zavala-Meneses et al., 2024). It consists of a plastic container fitted with platinum wires, which act as electrodes, and high voltage power supply. It is important in graduate laboratories, molecular biology research centers (Green and Sambrook, 2019; Abd and Al-Khafaji, 2024; Hussein et al., 2024; Pandey, Momeni and Pandey, 2024; Abd and Al-Khafaji, 2024), and in hematology laboratories (Munkongdee et al., 2020; Arishi, Alhadrami and Zourob, 2021; Santiago et al., 2023). The approach is based on the use of a charge field to isolated various-sized macromolecule fragments in a porous gel medium. One end of the gel is positively charged (anode), while the other is negatively charged (cathode), due to the electric field (Kumar and Derbigny, 2019). Because macromolecules have a negative electrical charge related to the existence of phosphate groups, molecules prefer to migrate from the gel's negative pole to its positive pole when an electrical current is applied (Lee et al., 2012). Finally, when the materials have been sorted using the GED, bands denoting particles of a variety of sizes can be observed.

Electrodes play a critical role in generating the necessary electric field for gel electrophoresis. Typically, high electrical conductivity materials like platinum or graphite are used to make them. Although these materials have been in widespread use for a long time, they have several drawbacks, such as cost and fragility (Aryal et al., 2019). One of the most failure of the GED device is the platinum wire breakdown. This failure is due to the unintended cut of the platinum wire during experimental settings. The platinum wire is expensive, thin, readily breaks, and it is quite difficult to work with. Various materials were used in earlier studies to create low-cost GEDs. Stainless steel (SS) wire was employed in previous research as both anode and cathode electrodes, with acceptable outcomes (Sepel and Loreto, 2002; Shirazu, Lee and Abd-Elmissih, 2009). Whereas, when employing electrodes made from copper, $Cu(OH)_2$ deposit occurred on the anode electrode (Britos, Goyenola and Oroño, 2004). Therefore, regular cleaning is recommended to maintain better electrical conductivity. Regarding aluminum and nichrome electrodes, a study revealed that the electrophoresis process can be conducted for 6-8 hours without being corroded (Aryal et al., 2019).

When choosing an electrode for gel electrophoresis process, several key points should be considered. First and foremost, the material should have excellent corrosion resistance to

withstand galvanic corrosion; second, for the setting purposes, the wires should have high tensile strength, so that the electrodes could withstand bending during fixing into the groove. Last but not least, biocompatibility is important to ensure that no deposit get into the gel during test.

Research on the feasibility of using nickel-free dental stainless steel wire as an alternative to platinum wire in GED is limited. All the above mentioned studies focused on using low cost wires as a substitute to the platinum wire for teaching purposed and they all shared the same issue of anode corrosion. Ni-free dental wires are low cost and affordable in the local markets. This research hypothesized that dental wires should withstand the galvanic corrosion and it could be used in GED.

2. METHODOLOGY

The candidate wire is the orthodontic SS (LEOWIRE® (C0400-05), Ø = 0.5 mm) which is used as an archwire for dental brackets. A 200 mm wire was made straight and then fitted in the tank grooves using silicon glue as shown in Fig.1. Both anode and cathode were replaced. About 30 mm length was twisted around the lid of electrophoresis unit. After confirming wires setup and conductivity, the gel electrophoresis process was performed as illustrated in Fig.2.

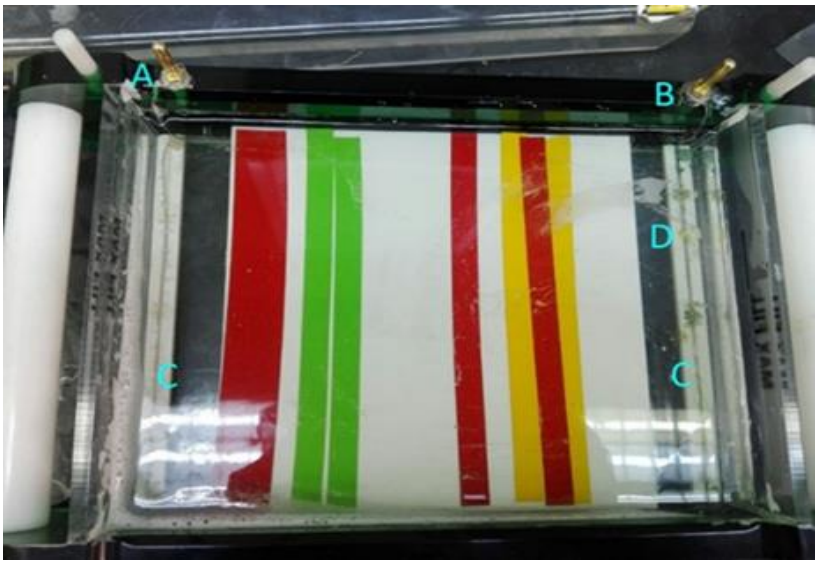

Fig. 1. Stainless steel wire fitted inside the GED tank: A: cathode; B: anode; C: stainless steel wire; and D: silicon glue for wire fitting

In order to facilitate visualization of the sample's passage through the gel, the DNA is separated, preprocessed using Polymerase Chain Reaction (PCR), and then dissolved in a solution containing blue dye. Running buffer has been organized in accordance to conventional laboratory practices. Tris-Borate-Ethylenediaminetetraacetic acid (TBE) Buffer (1X) was used

with 1.5% agarose gel. The DNA specimens were placed into the boreholes of the gel container, which was then completely submerged in the buffer solution.

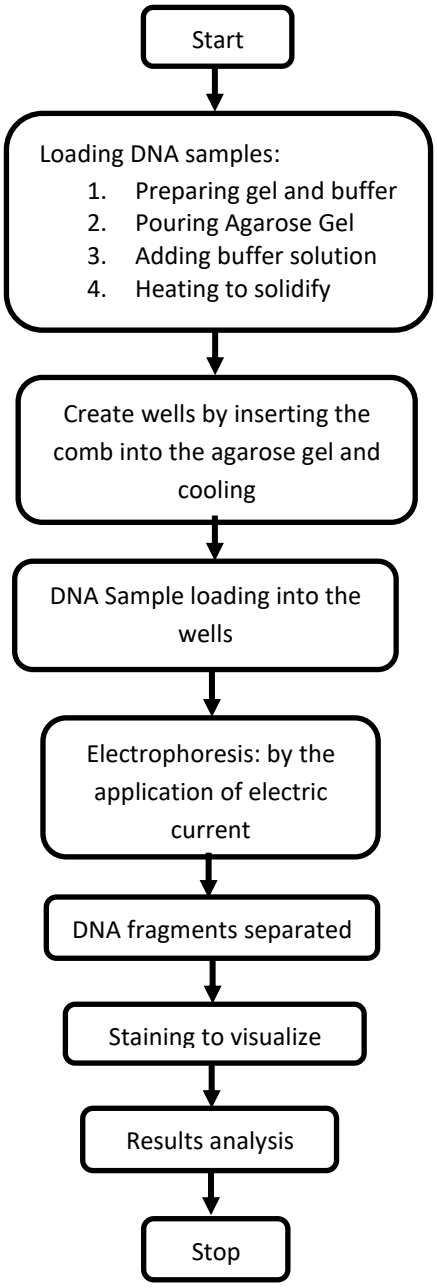

Fig. 2. Gel electrophoresis process

In order to facilitate visualization of the sample's passage through the gel, the DNA is separated, preprocessed using Polymerase Chain Reaction (PCR), and then dissolved in a solution containing blue dye. Running buffer has been organized in accordance to conventional laboratory practices. Tris-Borate-Ethylenediaminetetraacetic acid (TBE) Buffer (1X) was used with 1.5% agarose gel. The DNA specimens were placed into the boreholes of the gel container, which was then completely submerged in the buffer solution.

The gel container was totally submerged in the buffer solution after the DNA samples were inserted into the boreholes. The circuit was then completed by closing the cover of the electrophoresis tray and applying a stable voltage using a DC power supply (shown in Fig. 3). The boreholes on the gel had to be on the cathode side in order for DNA to flow towards the anode when an electric field was applied. The test runs were done with a steady voltage for about one hour. The gel was removed once the time was up and the DNA bands were seen under Ultraviolet light (UV).

Fig. 3. DC power supply connected to the electrodes of GED with potential difference of 100 V

There is a trade-off between applied voltage and the analysis resolution. While higher voltage can speed up the separation process, the clarity of DNA bands is reduces and produce false results. Further, there are several problems that can arise from excessive heating resulted from high voltages: DNA denaturation; Gel melting; Buffer degradation and uneven migration across the gel (smiling effect). At lower voltage, DNA fragments need more time to segregate according to size differences. This usually improves the resolution between bands, particularly for organisms of similar length. In order to achieve the best results, it is important to balance the run time against resolution requirements, taking into account the particular nature of DNA samples and the goals of experiments.

As electrochemical reactions and corrosion accelerated with high power, high voltages were applied for testing the electrode performance. Four tests were conducted successfully for around one hour each. Table 1 shows the electrical power applied through the electrodes. The method was conducted at Molecular Biology Laboratory, Medical Research Unit, College of medicine, Al-Nahrain University, Iraq. After that, 6 experimental tests (about 1 hour run/test) were performed before wire failure.

Table 1. The power applied during gel electrophoresis tests

Tests	Run [kVh]	Applied voltage [V]	Current [mA]	Power [W]
1	5.80	100	130	13
2	5.75	157	236	37
3	5.72	149	249	37
4	5.71	250	130	32

3. RESULTS AND DISCUSSION

The first promising impression about the electrode work, while the circuit is operating, is the bubbles that formed mostly on the anode as shown in Fig. 4.

Fig. 5 shows the separation of a DNA sample ladder on the gel. Each representing a different size DNA fragment of the ladder. Further, the alignment of DNA bands under UV, indicates that the current runs evenly among running wells. After approximately 11 hours run, the anode electrode experience corrosion as shown in Fig. 6.

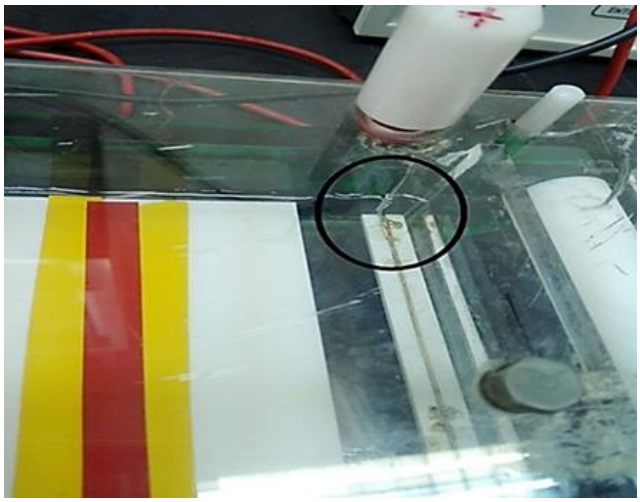

Fig. 4. Bubble formation at the anode electrode

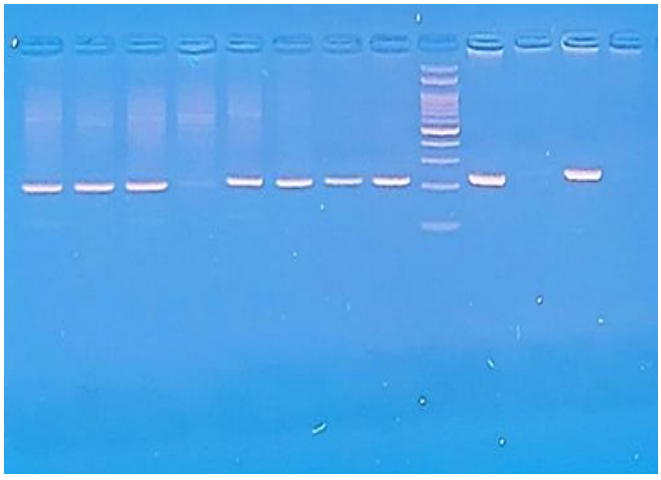

Fig. 5. DNA bands from a 1.5% agarose gel with TBE buffer run for 1 hour at 100 V by the use of dental SS wire electrodes

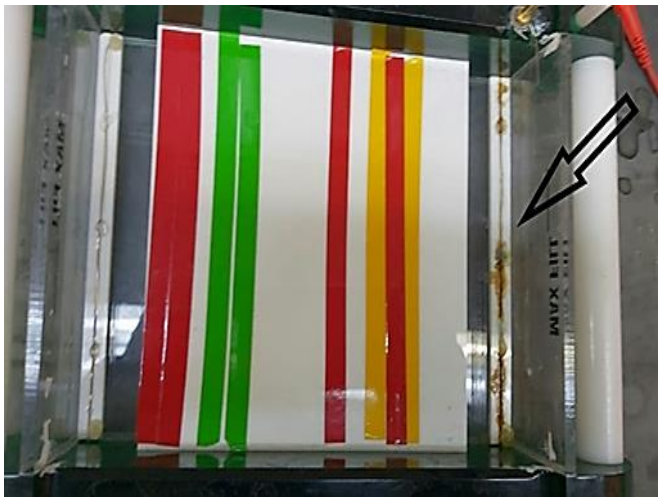

Fig. 6. The anode (referred by the arrow) experience corrosion after about 11 hr run

There are many reasons behind selecting the nickel-free SS orthodontic wire for GED electrodes. The high chromium concentration that makes it withstand the corrosive environment of the laboratory settings (Ekaterina et al., 2021; Kumrular et al., 2023); the excellent tensile strength that make it durable during installation (Arnold, Dalstra and Verna, 2016; Engeler et al., 2021). As a result, the life-span is extended. Last but not least, SS orthodontic wire is biocompatible, meaning it doesn't adversely affect biological tissues (Castro et al., 2015; Kokorev et al., 2020). The titanium oxide layer that covers the orthodontic wire makes it suitable for many biomedical devices (Mollabashi et al., 2020). This characteristic is essential since it guarantees that no impurities enter the gel or sample during electrophoresis tests, protecting the accuracy of the findings. However, there are several reasons that caused corrosion of GED electrodes. Galvanic corrosion occurred between the electrode wires due to the direct electrical contact with each other in the presence of an electrolyte (TBE buffer solution). As a result, the anode tends to corrode more rapidly, while the cathode is less affected. Electrolysis and ion migration of the electrode material could be occurred due to the passage of electric current through it. Consequently, this can cause the breakdown of the metal's protective oxide layer, making it more susceptible to corrosion.

Another reason of SS-wire corrosion is the exposure to various chemicals. Buffers, coloring solutions, and other chemicals are frequently used in the gel electrophoresis process. Similarly, orthodontic SS-wire are susceptible to corrosion even in dental applications due to the presence of chemicals of various temperature and acidity (Castro et al., 2015; Nalbantgil et al., 2016; Chepelova et al., 2023). During gel electrophoresis, the electrodes were immersed in a TBE buffer (1X) which is considered a high alkaline environment (8.3 ± 0.15 at 25°C).

From mechanical perspective, even though orthodontic SS-wire have to be made to move teeth with constant force while maintaining their elasticity (Alcaraz et al., 2023), mechanical stress or abrasion during the assembly process may produce micro cracks or imperfections in the metal surface, making it more susceptible to corrosion.

Selection of electrode materials in GED significantly influences both the efficiency and reproducibility of molecular separation techniques. Table 2 presents the results from the present work in comparison with other studies performed for alternative materials.

Table 2. Comparison of materials and electrode poles (+ for anode and − for cathode) for corrosion resistance, longevity, and effectiveness in gel electrophoresis

Material and electrode pole	Corrosion resistance	Longevity	Effectiveness	References
Platinum* (+ , −)	High	High	Excellent	(Sepel and Loreto, 2002)
Stainless Steel (+ , −)	Moderate	Moderate	Good	(Sepel and Loreto, 2002), and (Shirazu, Lee and Abd-Elmissih, 2009)
Nichrome (+ , −)	Moderate	Low (6-8 hr)	Satisfactory	(Aryal *et al.*, 2019)
Aluminium (+) , Nichrome (−)	Moderate	Corrosion was observed in the anode (~6 hr)	Satisfactory	(Aryal *et al.*, 2019)
Copper (+ , −)	low	Influenced by the deposition of $Cu(OH)_2$	Poor, needs regular maintenance	(Sepel and Loreto, 2002),(Britos, Goyenola and Oroño, 2004) (Acut, Magsayo and Curaraton, 2019)
Nickel-Free Stainless Steel (+ , −)	Moderate	Corrosion was observed in the anode (~ 11 hr)	Good	Proposed method

* Platinum is the gold standard material used commercially as electrodes for GED.

SS alloys can be classified according to their crystal structure in ferrite, austenite and martensite. The austenite SS structure is considered superior as it provides higher ductility, higher degree of cold working, and greater corrosion resistance (Ortiz et al., 2011; Brüngger et al., 2019). The presence of Ni in the austenite SS is to provide structure stability and increase corrosion resistance (Nalbantgil et al., 2016; Ahssi et al., 2020). Therefore, for GED application, orthodontic SS- wire with Ni is preferred to increase the life-time of the electrodes.

It is noteworthy that the reuse of the running buffer may facilitate the corrosion process because there may be trace impurities or contaminants that can lead to localized corrosion, even in supposedly pure solutions. Future research should be more systematic by considering the effect of various voltage conditions on electrode life span as it will give valuable information regarding the durability and operating parameters. Finally, it is highly recommended that both

electrode wires should be of the same material (similar electrochemical characteristics) to minimize galvanic corrosion.

4. CONCLUSIONS

In conclusion, nickel-free stainless steel orthodontic wire can be used as alternative for costly and fragile platinum electrodes in gel electrophoresis device. The results showed that an electric field was generated successfully as well as DNA fragments were separated from each other efficiently. This makes it a cost effective solution and also an attractive option for student laboratories or researchers with limited financial support without sacrificing performance. Nevertheless, the anode electrode was oxidized after 11 hours of use. Detailed investigations are necessary to understand the performance of stainless steel electrodes under different voltage settings and gel electrophoresis methods. Having this knowledge would help improve the procedures to extend the lifespan of using this electrode material. Ongoing research to optimize the composition of materials and experimental methods has the potential of this cost-effective solution across various molecular biology uses.

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