

Regular Article

Cost Effective Submarine Electrophoresis Apparatus

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ABSTRACT: Electrophoresis is the process of differential movement or migration of ions by attraction or repulsion in an electric field, which has a wide range of application in the field of biological science. In this paper a cost effective electrophoretic apparatus was designed and described, which replaces the conventional platinum electrode with copper and silver electrode. A greater change in PH was observed in cathode side which helps the biological molecule to migrate much faster than in a conventional apparatus. This apparatus was also tested for their effect on plant and animal DNA electrophoresis and compared with the conventional electrophoretic apparatus. Thus the results from our study reveal a cost effective electrophoretic apparatus which do not disturb the migration of biological molecule.

Introduction

The process of electrophoresis is defined as 'the differential movement or migration of ions by attraction or repulsion in an electric field'. In practical terms, a positive (anode) and negative (cathode) electrode are placed in a solution containing ions. Then, when a voltage is applied across the electrodes, solute ions of different charge, i.e., anions (negative) and cations (positive), will move through the solution towards the electrode of opposite charge. The history of Capillary Electrophoresis can be traced back over a century, as noted by Li (Li, 1993). In 1800, Nicholson and Carlisle discovered electrolysis which is the decomposition of a compound into its ions by the passage of an electrical current through a solution of the compound (Abramson, 1934). In 1791 Luigi Galvani discovered electrical activity in the nerves of the frogs that he was dissecting. He thought that electricity was of animal origin and could be found only in living tissues. A few years later, in 1800 Alessandro Volta discovered that electricity could be produced through inorganic means. Soon thereafter, in 1808, Reuss (Abramson, 1934) reported an interesting phenomenon involving electricity and its mobilizing effect on liquid matter. In fact, by using small sheets of copper and zinc and cloth spacers soaked in an acid solution, he built a battery - the first apparatus capable of producing electricity. Electricity has a central role in our lives and to this day Electrochemistry is a standard course of study. The same nature of electrical conductance and transmittance of the biological molecules can be used in their separation process by the technique named electrophoresis. For many years the phenomenon of electrophoresis has been studied, and much work has been done in designing an apparatus for better measurements of mobility, zeta potential and zero charge of minerals. There are a number of complications in the theory of electrophoretic motion. These complications are mainly due to electrophoretic retardation, surface conductance and relaxation (Kruyt, 1952; Overbeek, 1959 and Frumkin, 1946). The process of DNA electrophoresis has a wide range of application such as restriction pattern analysis, DNA quality and quantity analysis, polymorphism study and has a considerable demand for large-scale purification of gene vectors will arise if the relevant authorities approve on-going therapy trials (Tiainen et al., 2007). The usage of platinum electrode is costlier and has higher resistivity than the copper and silver electrode. So in this paper the author has described a cost effective electrophoresis apparatus which employs copper and stainless steel as electrodes.

Materials and Method

Apparatus design

This apparatus consists of three main parts: a power supply unit, a conducting unit and two electrodes. The power supply used has a range of 0 to 300 v DC and 0 to 50 milliamps DC. This unit was designed with two sets of electrodes: cathode made of copper and the anode made of stainless steel for electrophoretic mobility when high accuracy. The conducting unit is a glass tank of dimension filled with the buffer system. The experiment was performed at room temperature and sufficient care was taken to reduce the effect of joule heating

Agarose gel Electrophoresis

Agarose gel of 1% was casted with agarose with low electro endo osmosis (EEO) in the 1X TAE buffer used for electrophoresis. DNA extracted from the plant source was separated in this gel with TAE buffer with a constant voltage of 100Volts for 1 hour and the same protocol was evaluated with a commercial electrophoresis unit which exists in the lab. After electrophoresis the gel was stained with a solution of EtBr (0.5ug/ml) for 30 minutes and visualized under UV transilluminator.

Measurement of PH and temperature

During the process of electrophoresis the change in PH was measured on the both anode and cathode side with Elico PH meter with glass and calomel electrode. Change in temperature in both the electrodes was measured at different time intervals 10th, 20th, 40th and 60th minutes of the electrophoresis with a standard lab thermometer. The same experiment was compared with a commercial electrophoresis unit which exists in the lab.

Measurement of time for electrophoresis

During the process of electrophoresis the distance traveled by the tracking dye was measured at 10th, 20th, 40th and 60th minutes of the electrophoresis and expressed in centimeters. The set of experiment was continued with a commercial electrophoresis unit which exists in the lab.

Statistical Analysis

All the experiments were conducted as three different individual experiments and expressed as the mean of the experiments. The mean value was expressed with \pm SD, which was calculated with the Microsoft Excel programme.

Result and Discussion

The apparatus designed and described in the experiment was very simple to construct with the materials which exist in our day today life. This apparatus makes use of two commonly used metal wires (copper and stainless steel) as electrode for electrical conductance and electrophoresis. When comparing with the usual platinum electrodes, the metal used as electrode in this experiment has less resistance towards the electrical conductance. The flow of electrons is easier through metals with higher conductivity or lower resistivity. Generally metals with chemically inert nature which can donate or accept electrons are preferred as the electrode material. Platinum satisfies these conditions but it is costlier, hence copper electrode in purest form has been used in this project due to its lower cost, higher conductivity and easy availability. In our experiment the author measured the time required for the electrophoresis process.

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It was observed that the system described in this paper requires less time than the convention one (Fig-2). DNA of larger size were analyzed in 15 agarose gel with field strength of 200 to 400 V/cm with a novel method for stretching a long DNA molecule in agarose gel with alternating current (AC) electric fields (Kaji et al., 2002).

Thus the electrophoresis unit equipped with electrode which enables high conductivity and low resistance can help design the electrophoresis units for separation on long stretch of DNA with high electric-field.

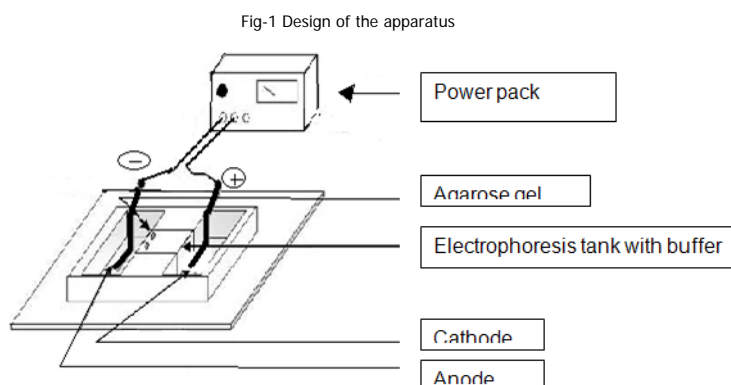
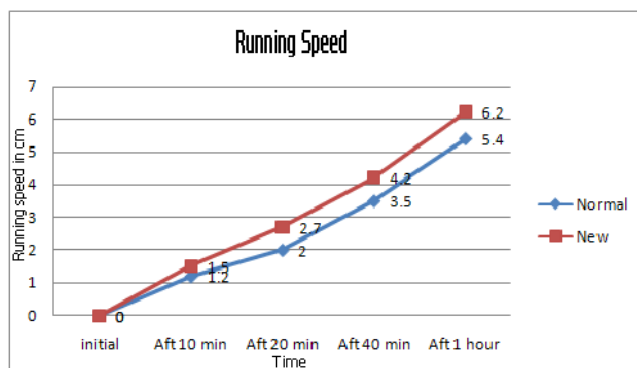


Fig-2 Measurement of time required for electrophoresis



Change in pH of the buffer during the process of electrophoresis was measured near both the electrodes and it was compared with the conventional electrode with platinum electrode. In cathode side of both the conventional and the electrophoresis unit described in this paper it was observed that the PH increases with time. Till 20 minutes after initiation of electrophoresis process the change in pH was equal in both the units, but from 40th minute slight increase in PH was observed in the electrophoresis unit described in this paper, which was greater than the conventional one (Fig-3). In case of cathode side the result is vice-versa and a marginal difference in PH was observed between the both electrophoresis units (fig-4). This may be due to the movement on H⁺ ions which travels from anode and reaches cathode and their accumulation increases proportionally with time. This indicates the movement of ions will be much faster in the electrophoresis unit described in this paper when compare to the conventional one.

Fig-3 pH variation in cathode side

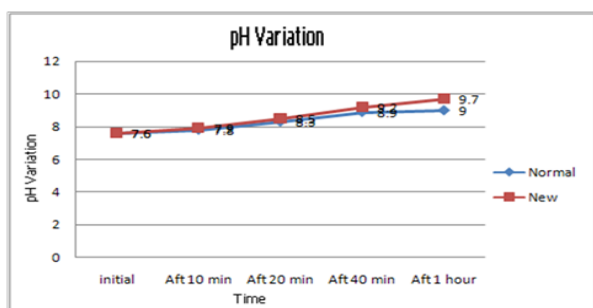
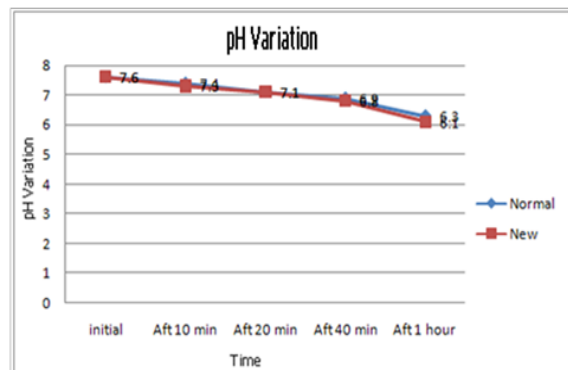
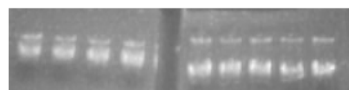


Fig-4 pH variation in anode side



The apparatus described in this paper was used to test their ability to electrophoresis of DNA from both plant and animal source. It was observed that no DNA degradation was observed with this system and the movement was faster than that of the conventional one. (Fig-5)

Fig-5 Comparison of electrophoresis of Plant and animal DNA



In conclusion a simple electrophoresis system was designed and developed to teaching and demonstrate the process of submarine gel electrophoresis in Molecular biology. The expensive platinum electrode was replaced with readily available copper and stainless steel electrode and compared with the conventional electrode it terms of change in PH in buffer system at both the electrode, time required for electrophoresis and effect of DNA damage. The system described in this paper will help the educators to use in hands-on classroom teaching with principle and components on a basic electrophoresis system, which does not require platinum electrodes. The use of common copper wire and stainless steel in place of platinum electrodes provides a simple, inexpensive, and highly reproducible system that is adaptable to instructional needs.

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