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How to teach students of medical and biological sciences electrophoresis in the spirit of physical chemistry — a laboratory exercise scenario

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Abstract: Gel electrophoresis is one of the most important and most widely used tools in biomedical sciences. However, when students are acquainted with these techniques, information related to practical applications often neglects the physicochemical foundations of the occurring phenomena. The following article proposes a laboratory exercises scenario conducted in the problem-solving and decision-making strategies, which aims to familiarize beginner students with the physicochemical basis of electrophoresis in a simple and accessible way. By analyzing the scheme presented, students will gain knowledge of the basic sciences, as they will learn about the advantages and limitations of the method in addition to its applications. The experiments are designed in a way that allows students to draw conclusions about the parameters affecting the electrophoresis process and the sources of obvious errors. Moreover, the use of simple ionic dyes eliminates the need for complex apparatus and toxic reagents, which may be harmful. The main outcome of the class is to develop students' skill to design their own simple experiments using this commonly used technique.

Keywords: First-year undergraduate/general, problem solving/decision making, electrophoresis, dyes, laboratory scenario, medical education.

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Introduction

All modern medical and biological sciences use tools [1, 2] borrowed directly from the arsenal of physics and physical chemistry. These include techniques based on spectrophotometric or fluorescence measurements (real-time/quantitative fluorescent polymerase chain reaction, qPCR/

QF-PCR; confocal microscopy; enzyme-linked immunosorbent assay, ELISA) or gel electrophoresis (methods for separation and analysis of nucleic acids and proteins) [3, 4], which is the subject of this publication. When teaching students in the biological science, the physicochemical phenomena underlying these methods are often not presented comprehensively and are even neglected. In fact, almost everywhere the teaching of electrophoresis focuses on its applications. This is possible thanks to very well-developed and optimized procedures (e.g., sodium dodecyl sulfate–polyacrylamide gel electrophoresis, SDS-PAGE) [5, 6], which make it easy to perform the experiment without knowing the theoretical foundations of the phenomenon occurring. This approach is justified in part because attachment to procedures ensures reproducibility of results in all laboratories around the world, but on the other hand it prevents self-optimization of the method when the measurement situation becomes unusual.

Another problem in teaching the basics of physical chemistry is the varyig number of hours/classes of chemistry taken by students in chemistry (such as chemistry, medicinal chemistry) and non-chemistry majors (like biology, biotechnology, environmental protection, medicine or medicine and dentistry). On full-time basis, physical chemistry and physics classes are taught only to chemistry majors. Students of biological and medical sciences have proportionally reduced hours in chemistry. Moreover, it can be noted that the number of learning outcomes (and therefore hours) in chemistry is gradually but steadily being reduced in successive regulations of the Polish Minister of Education and Science, which set educational standards for various fields of study, including, among others, the medical and medical-dental faculties [7–9]. In such a case, the question is how to most effectively educate such students in chemistry and how to incorporate as many topics as possible into the curriculum that will be useful to them in their further education. In our opinion, gel electrophoresis is one of the most important topics for these students and should be taught in the future in a way that allows them to become more familiar with its theoretical foundations. Hence, the following article proposes a laboratory exercises scenario conducted in the problem-solving and decision-making strategies, which aims to familiarize beginner students with the physicochemical basis of electrophoresis in a simple and accessible way. By analyzing the outlined scheme, students will gain knowledge of the basic sciences as they will learn about the advantages and limitations of the method in addition to its applications. The experiments are designed in a way that allows students to draw conclusions about the parameters affecting the electrophoresis process and the sources of obvious errors. Moreover, the use of simple ionic dyes eliminates the need for complex apparatus and toxic reagents, which may be harmful.

Theoretical background in a nutshell

For a chemist or physicochemist, electrophoresis is a process well described by simple mathematical relationships [10]. It is known that charged particles migrate in an electric field, and this movement is described by the equation (1).

$$v = u \frac{\Delta P}{l} \tag{1}$$

where: v — velocity
ΔP — electric potential difference
l — distance between electrodes
u — ions mobility

In addition, there are friction force and electrophoretic retardation force under non-vacuum condition. The retardation forces (affecting the value of u in equation 1) determine the actual speed for a given type of particle and are governed by the Stokes equation, which describes the force acting on all bodies moving in a viscous medium. The details of these forces, however, are more complicated and, from a non-chemist's point of view, boil down to the instinctive conclusion that smaller particles (lower molar mass, fewer base pairs) will move faster.

Combining these information with Newton's laws of dynamics, we have two balancing forces (one related to inhibition and the other to motion) and one simple equation (1) that allows us to describe the phenomenon with an accuracy acceptable to students of molecular biology. We can affect the movement of a given type of molecule according to the equation (1) by manipulating the potential difference or the distance between electrodes (when ionic strength and pH are constant). The first parameter depends on the power settings, which can be changed at will, and the only limitations should be the device's capabilities. The second variable is not so obvious and can be controlled at the gel formation stage before the actual measurement.

In gel electrophoresis, the gel (almost always a polymer hydrogel) is the non-mobile phase and its properties and structure ensure the selectivity of the method. Polymer hydrogels comprise a macromolecular network that occupies only a few per cent of the volume of the structure and is suspended in water, which fills the rest. From the point of view of the microscale structure, it is a cluster of chaotically connected channels/capillaries in which charged particles move thanks to a potential difference. In such a polymeric hydrogel architecture, smaller particles will not encounter obstacles, while larger ones will have limited freedom of movement (only a few sufficiently wide passages are available to them), ensuring different speeds for different particles.

The last phenomenon important for gel electrophoresis is diffusion [11]. Diffusion is the movement of particles forced by a concentration gradient (particles move from higher to lower concentration). In the case of electrophoresis, this is a very undesirable effect because it takes place in all directions, as opposed to the electrophoretic movement directed toward the appropriate electrode. As a result, the particles of interest occupy a larger and larger area (become diluted) over time, making detection and observation more difficult. Over time, a sample represented as a narrow, well-drawn colored rectangle transforms into an increasingly larger object with blurred borders, which turns into a shapeless smudge, that is often difficult to see. This is why diffusion is the most significant source of errors in electrophoresis-based techniques.

In our opinion, the issues described here represent the minimum knowledge necessary for students of medical and biological sciences to optimize electrophoretic measurements and apply these methods to unusual problems. Here we would like to propose an exercise that will allow students to become familiar with these issues in detail. This is a different teaching strategy from that usually used in medical universities, where the most important thing is the practical application of techniques based on gel electrophoresis. Moreover, at the vast majority of universities, electrophoresis exercises are performed on samples of nucleic acids or proteins. This is understandable, since most often these substances are subjected to electrophoretic analysis during scientific research; however, aforementioned substances are also unstable and difficult to work with. The student should work with them quickly and at the same time precisely to avoid contamination of the sample. This may be too much of a challenge for beginning students, and the high cost of uncontaminated biological reagents (DNA/RNA, proteins) makes it impossible to afford many repetitions. For nucleic acids, there is an additional problem with visualization methods. If we work, as we suggest in this article, with charged dyes instead of nucleic acids,

the movement of objects in gel electrophoresis can be seen with the naked eye. We do not need ultraviolet light sources, which are used in conjunction with mutagenic dyes (such as ethidium bromide [12]) to visualize nucleic acids. This solution eliminates not only the use of harmful reagents, but also radiation, which is very harmful to the skin and eyes. All these facts mean that teaching of electrophoresis is often reduced to demonstration classes, while here we propose a simple lesson scenario to present this method and its theoretical foundations in a simple and convenient way.

Laboratory exercise scenario

The most important information for students, when familiarizing themselves with a new method, is (i) the main variables that can be manipulated to optimize the measurement and (ii) the main sources of error in the data obtained. It is not reasonable to point out to the student all possible parameters that can affect the course of electrophoresis, but only those in which the minimal change made is sufficient to affect the observed rate. In addition, these manipulations must be possible from the point of view of practical implementation (e.g., we could lower the temperature to stop unwanted diffusion, but this would impede any form of movement, so it is impractical). For the exercises presented here, we will use the parameters derived from equation (1). In addition, we will show the undesirable effects associated with diffusion. These are the simplest but most effective parameters to change, and the effect related to diffusion is the most spectacular interference effect, which, if ignored, can make measurement impossible.

Necessary reagents

Ready to use Tris Acetate-EDTA 10x electrophoresis buffer (Sigma Aldrich, St. Louis, MO, USA), 0.02% aqueous solutions (water with 10% glycerin) of safranin, eosin (intentionally two forms Y and b mixed together), crystal violet, and bromophenol blue (all Sigma Aldrich, St. Louis, MO, USA). Agarose general purpose (Sigma Aldrich, St. Louis, MO, USA), salmon sperm DNA (Sigma Aldrich, St. Louis, MO, USA), and distilled water.

Apparatus

Horizontal electrophoresis apparatus and power supply.

Procedure of the experiment

1. Dilute the electrophoresis buffer with distilled water according to the instruction on the bottle. Weigh out the appropriate amount of agarose to prepare 40 mL of 1% (w/v) agarose solution in the diluted buffer. Heat the solution until the agarose crystals are completely dissolved. Cool the solution to about 40°C.
2. Pour the solution into a mold (a transparent U-shaped vessel) placed in a leveled gel caster and immerse the comb in the middle of the mold. After the gel has solidified and the comb has been removed, we get evenly spaced holes in the teeth of the comb.
3. After the gel has completely solidified, place it and the mold in the electrophoresis chamber and fill the chamber with buffer.

4. Remove the comb from the gel and then add 10 μL of each dye solution to the appropriate slot/hole using an automatic pipette — mix the crystal violet solution with 10 mg of DNA and load into one of the slots.
5. Connect the power supply to the electrophoresis chamber, apply 100 V, and start the electrophoresis.
6. After 20 min, measure the distance covered by the dyes.
7. Repeat steps 1–6 increasing the weight of the agarose until a 4% (w/v) solution of it in buffer is obtained.
8. Repeat steps 1–6, applying a constant voltage of 150 V.
9. Place the gel obtained according to steps 1–2 onto a small Paterson developing tray, fill the tray with buffer and remove the comb from the gel. Add three selected dyes into each slot. Leave every second slot unfilled.
10. Leave such prepared gel for 3 h (do not apply voltage). After this time, take a photo of the gel.

Data processing and a laboratory report preparation

1. Prepare photographic documentation of all gels.
2. Calculate the electrophoretic velocity of all dyes for both concentration of agarose (in the case of eosin, two bands should be obtained as this solution is a mixture of two dyes). Measure the distance every 10 minutes — evaluate whether the dyes have moved in a uniform motion based on two measurement points.
3. Calculate how many times the practical distance between the electrodes is increased for the gels used (1 and 4% agarose).
4. Calculate the electrophoretic mobility of safranin, crystal violet, and bromophenol blue (use electrophoretic rates for different voltages).
5. Explain why the dye in a mixture of crystal violet and DNA moved in the opposite direction to the plain dye. Which ion is the colloidal particle and which ion is adsorbed on its surface? Prepare an explanatory figure.

Examples of experimental results

Photographic documentation of the selected gels is shown in Figures 1 and 2. The figures show how the position of the dyes in the gel changes under the influence of the potential difference.

The exact values of the paths travelled by the dye and the direction (relative to the electrode polarity) are given in Table 1.

A characteristic feature of eosin is that it is usually available in two forms (dominant Y and B), which differ in structure and consequently in color. The use of such a dye in this exercise allows to illustrate the fact that electrophoresis can document contamination of the sample with other substances.

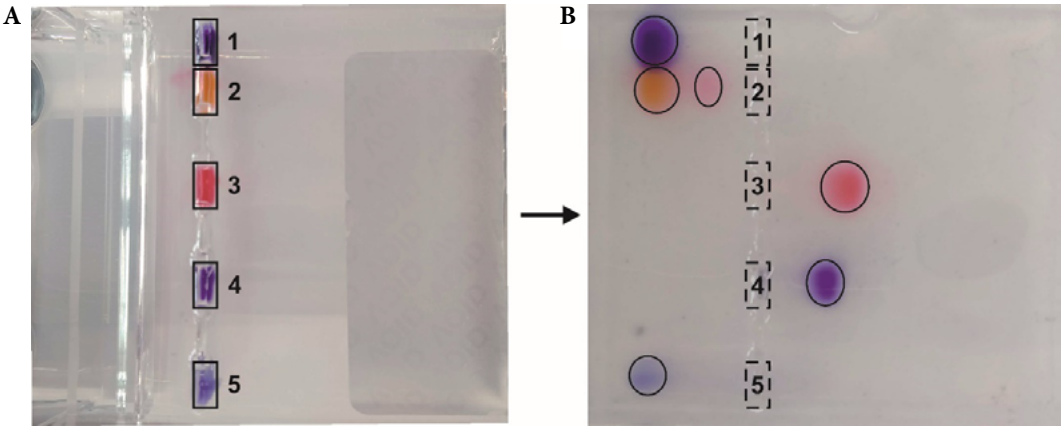


Fig. 1. Photographs of a 1% agarose gel with loaded dye samples: 1 — bromophenol blue, 2 — eosin, 3 — safranin, 4 — crystal violet, 5 — mixture of crystal violet and DNA before (A) and after (B) electrophoresis conducted at 100 V for 10 minutes.

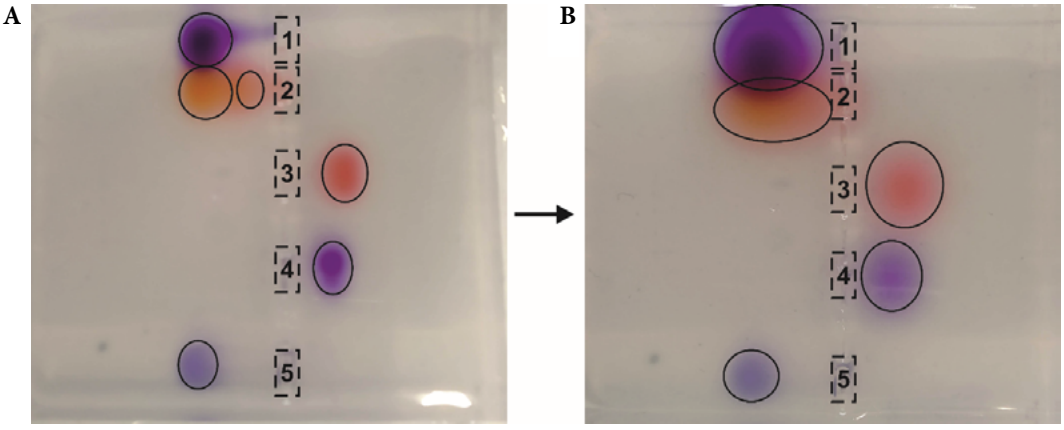
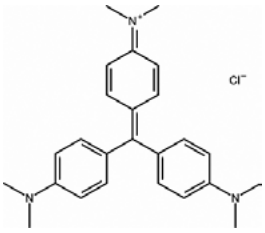
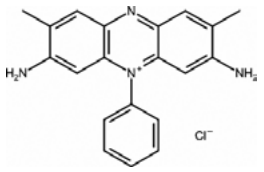
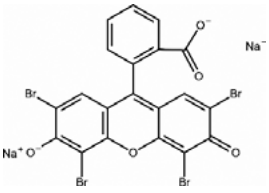
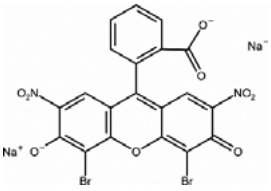
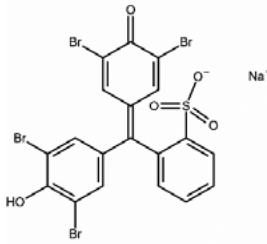


Fig. 2. Photographs of a 1% agarose gel with loaded dye samples: 1 — bromophenol blue, 2 — eosin, 3 — safranin, 4 — crystal violet, 5 — mixture of crystal violet and DNA immediately after electrophoresis (A) conducted at 100 V for 10 minutes and 3 hour after the process (B).

Table 1. Migration direction and travelled distance of the dyes tested after 10 minutes of electrophoresis conducted in a gel with different agarose concentrations (1% or 4%) and potentials (100 V or 150 V).

Dye name	Chemical structure	Electric charge / migration direction	Distance travelled [cm] agarose conc. / potential difference		
			1% / 100 V	4% / 100 V	4% / 150 V
crystal violet		+ / –	0.640	0.430	0.648
safranin		+ / –	0.310	0.290	0.830
eosin Y contaminated by eosin B	 	– / +	0.750	0.610	1.238 (0.510)[a]
bromophenol blue		– / +	0.760	0.650	1.310
crystal violet and DNA		– / +	0.540	0.430	1.438

[a] — value for eosin B

Obligatory learning objectives

The most important knowledge that the student will acquire after performing the proposed laboratory exercise boils down to the variables affecting the rate of movement of particles in gel electrophoresis. These can be described as four issues, namely: ion charge/Zeta potential, external potential difference, gel concentration and diffusion. These factors are presented and discussed in the following subsections of this publication.

Diffusion and electrophoresis

Thanks to diffusion, we can smell blooming flowers or freshly baked bread, but at the same time it is a major obstacle to electrophoresis [11]. Diffusion occurs independently of electrophoresis, including when there is no potential difference and no ordered movement forced by it. Diffusion causes movement of particles in the gel, but unlike electrophoresis, it takes place in all possible directions. It can be said that diffusion results in dilution of the molecules applied to the gel (the driving force behind diffusion is the concentration difference, while in electrophoresis it is the potential difference). Therefore, it is difficult to detect them over time (Fig. 2), and ultimately detection is impossible. These arguments make diffusion the most significant obstacle of proper gel electrophoresis, and only efficient and quick measurements give us any chance of success.

Ion charge/zeta potential

The application of electrophoresis is limited to the separation of charged objects. The sign of the charge tells us in the direction of which electrode the particle will move (Fig. 1 and Table 1). This part is simple and instinctive, since opposite charges should attract each other. Therefore, cations migrate to the negative electrode and anions to the positive electrode. Unfortunately, when it comes to electrophoresis, it is not always that simple. In common theoretical models, we treat a single inorganic ion as an object with an infinitesimally small volume, so its charge practically does not depend on its surroundings. In electrophoresis, we almost always work with larger particles, where this approximation is not correct, and the concept of charge should be replaced by the more general zeta potential. It takes into account not only the particle itself, but also its surroundings, in particular other ions in its environment. It is necessary to explain why, in the presence of some ions, we observe a change in the direction of movement of the object during electrophoresis (instead of migration to the negative electrode, we observe migration to the positive electrode or vice versa), as in the case of a mixture of crystal violet dye and DNA (Fig. 1). Here we observe zeta potential overload, a phenomenon caused by the fact that the grouping of two types of apparently independent particles should be treated as a single object [13]. Most importantly, here we cannot consider their combined charge as a simple sum, but must use the geometry-dependent zeta potential, considering the motion caused by the electric field generated during electrophoresis. On the application side, it is enough to know that the direction of motion can be changed, and we do not need to present the student with the entire theoretical basis of the phenomenon, which is far beyond his knowledge of physics. This effect is used in one of the most widely employed electrophoretic techniques, SDS PEGE, where it allows positively charged proteins and those that originally have no charge to be given a negative charge so that they can move in an electric field all toward the same electrode. This situation facilitates the identification of proteins on the basis of parameters other than electrical charge.

Potential difference

The difference of applied potentials is the simplest parameter affecting the speed of movement of particles during electrophoresis. According to equation (1), the greater the potential difference, the faster the molecule moves. Based on the previous section, we already know that the longer electrophoresis takes, the more distorted the image is due to diffusion. Based on this, one can assume that the potential difference should be as high as possible, and the only limitation will be the power supply. Unfortunately, the reality is not so simple due to Joule heating, which occurs in all electrical conductors. This phenomenon causes the gel to heat up over time, and the heating rate increases when a higher potential difference is applied. Rising temperature leads to additional large perturbations (temperature increases the diffusion rate and electrical resistance). In extreme cases, this can even lead to gel melting. In addition to the aforementioned thermal effects, high currents also cause other flow disturbances, such as the 'smile effect'. This situation forces us to make a trade-off between the duration of the experiment and the quality of the result obtained.

Gel concentration

When making a gel for electrophoresis, one usually first prepares a solution containing a certain concentration of ingredients, which in the next step gels or polymerizes into a ready-to-use hydrogel. The concentration used directly affects the microstructure of the hydrogel. In the structure of a polymer hydrogel, which is used in gel electrophoresis, we can distinguish two main components: a three-dimensional polymer network and the aqueous solution in which this network is immersed. From the point of view of electrophoresis, the density of the network structure (the amount of polymer per unit volume) is essential, and it depends on the concentration of the starting solution from which the gel was formed. In general, the average density of the network will affect the path the molecules take during electrophoresis; as in a maze, the shortest path to the exit depends on the width of the corridors (Fig. 3). The labyrinth analogy is justified here, since the gel imposes defined micro-pathways on the moving particle within the existing channels in the gel.

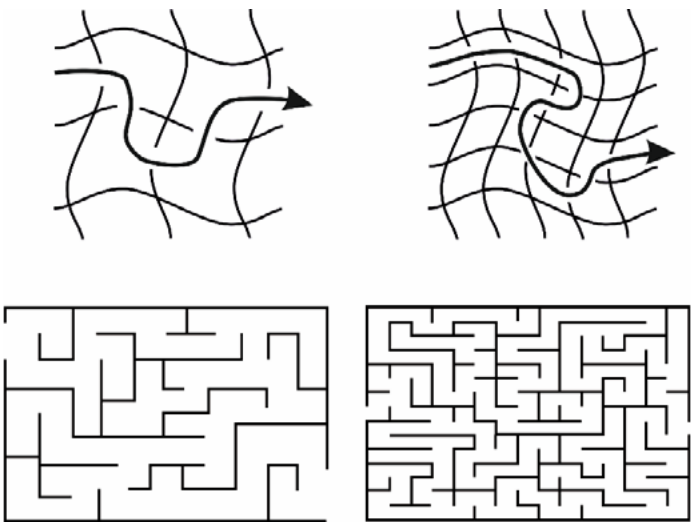


Fig 3. Schematic representation of a polymer hydrogel network as an analogy of a maze — the higher the gel concentration, the longer distance for the dye particles to travel through.

This distance affects the actual distance between the electrodes, so consequently, as shown in equation (1), it affects the speed of particles moving in the electric field. Combining all this information, we can conclude that the speed of particle movement in gel electrophoresis depends on the gel concentration and decreases as the concentration increases. This observation can also be explained by other more complex phenomena concerning the physics of hydrogels (the effect of concentration on viscosity and dielectric constant), but changes in the actual distance between the electrodes can be considered the most significant.

The relationship described above might suggest that a student could reduce the time required for an electrophoresis experiment by decreasing the gel concentration, but two additional problems limit our freedom of choice. The first is due to the fact that there is a minimum concentration of components in the starting solution necessary to form a stable gel. The rule is that the more polymer, the better the mechanical properties of the gel, so at concentrations that are too low, the gel will not solidify or will be brittle and will disintegrate. The second limitation is due to the '*u*' factor present in equation (1), discussed earlier. As mentioned, it incorporates many of the physical parameters that characterize the system, including the molar mass/size of the particles being separated in the electrophoresis process. This means that the course of electrophoresis will also be affected by the size of the moving particles and will be strongly correlated with the density of the polymer network, which determines the width of the available channels in the gel. For these reasons, the optimal gel concentration should be correlated with the molar mass of the substances, and these relationships are not easy to describe. Therefore, they can be found in the appropriate tables available in the literature or in the instructions provided by manufacturers of ready-to-use kits. It is impossible to calculate these parameters directly, but in can be said with some approximation that we use low gel concentrations for the analysis of large mass particles and *vice versa* for small particles — high concentrations.

Final remarks

This article aims to identify issues that, during a class introducing beginning students to electrophoresis, will give them the tools to optimize simple measurements using these techniques. The lesson scenario is presented in the form of practical laboratory exercises for students to perform on their own and may prove to be a useful teaching tool. The proposed procedure enables students to learn the advantages and limitations of the method, not just its main principle and applications. A student starting to design an electrophoresis experiment or trying to correct an existing one should know that it is possible to directly influence two parameters, namely the gel concentration and the applied potential. This article demonstrates in an accessible way the effects of manipulating these parameters, which will enable students to search thoughtfully for optimal conditions. Another important point that will be communicated to the student in a practical way is the issue of the effect of diffusion on gel electrophoresis. Therefore, after completing the exercise, students should be able to interpret simple measurement results, identify potential problems and propose possible solutions.

In addition, the authors hope that the theoretical foundations of gel electrophoresis presented here will allow students to understand the underlying physics of this method, commonly used in molecular biology, in a way that is accessible to non-physicists. Our approach is to build knowledge in a way that is free from the burden of having a large mathematical and physical knowledge base, using mainly observation and practical experience (the problem-solving and

decision-making strategies). What is more, the scenarios proposed here involve a move away from the toxic chemical (carcinogenic fluorescent dyes) and physical (UV radiation) factors used in advanced biological experiments using gel electrophoresis. We offer the proverbial safe sandbox in which the student can perform many experiments under a variety of conditions, so that they can get a feel for the general properties of the system before proceeding to the real measurements. In this way, students will acquire the resources to use their existing knowledge to conduct/optimize electrophoretic measurements under other experimental conditions without knowledge of the advanced physics and chemistry underlying the method.

There is another, additional advantage of the proposed laboratory exercise — its low cost. Assuming a group of 4 students, the cost of one class can be estimated at 10 euros. 80% of this amount is accounted for by the high price of agarose. This cost can be further reduced if the gel, once used, is melted and reused. With the potential to melt the gel 4 times without over-staining it, the total cost can be reduced to around € 2.5 per class.

Author contributions

Conceptualization, K.K. and M.K.-K.; methodology and investigation, J.G., K.K.; formal analysis, K.K.; data curation, J.G. and K.K., writing — original draft preparation, J.G., K.K. and M.K.-K., writing — review and editing, K.K. and M.K.-K.; visualization, J.G. and K.K.; supervision, K.K. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

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Conflict of interest

None declared.

Informed consent

Informed consent was obtained from all individuals included in this study.

Ethical approval

Not applicable.

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