

Full Length Research Paper

Improvement of electrophoresis performance by spectral analysis

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This paper describes a new design of standard agarose gel electrophoresis procedure for nucleic acids analysis. The electrophoresis was improved by using the real-time spectral analysis of the samples to increase its performance. A laser beam illuminated the analysed sample at wavelength with the highest absorption of gel components. Thus, DNA band is detected well before it may exits a gel plate. A modified horizontal electrophoresis device was designed, developed and tested.

Key words: Horizontal electrophoresis, laser, spectral analysis, agarose gel.

INTRODUCTION

Gel electrophoresis is a standard technique used in biological laboratories that results in the separation of charged molecules, for example negatively charged DNA (Wang et al., 2009). The molecules are moved in agarose gel by electric current. Small molecular size nucleic acid fragments may move faster than expected and they may exit a gel plate before the end of separation. This often causes errors in the experiment and may negatively influence its results.

In our laboratory, electrophoresis method was re-designed with the use of photoelectric effect. Concentrated monochrome spatial coherence beam (laser) was applied on the opposite end of the gel plate which the samples were immigrating toward. We positioned a photo-detector in the obverse side of the plate. When the loading dye which precedes slightly the first DNA band passes in front of laser, the absorption increases by at least 50%. A user-programmed microcontroller was made to stop the electrophoresis by switching off high-voltage source from the electrodes and giving sound and light alert. The percentage absorption varied according to

gel concentration and color of the used dye. For example, the often used bromophenol blue and xylene cyanol dyes migrate through agarose gels at roughly the same rate as double-stranded DNA fragments of 300 bp and 4000 bp, respectively (Voytas, 2001).

MATERIALS AND METHODS

Spectral analysis

To achieve our aim to improve electrophoresis procedure, we had to search for suitable wavelength(s) for optimal photodetection. We prepared four types of samples for two concentrations of the gel to find their maximum absorption. The first sample contained agarose gel only without any loading dye and it was transparent. The second sample contained agarose gel and ethidium bromide without loading dye and it was transparent as well. The third sample contained agarose gel and ethidium bromide with bromophenol blue dye and it had blue color while the fourth sample contained agarose gel and ethidium bromide with xylene cyanol dye and it had green color.

We examined the samples in Varian 2200 gas chromatograph - mass spectrometer (Agilent Technologies, USA) in visible and ultraviolet spectral ranges. Varian 2200 chromatograph/spectrometer ran on auto gain mode during spectral scanning. The transparent agarose sample without ethidium bromide was considered as reference to assay the spectrum of agarose sample with ethidium bromide, then the preceding was considered as

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reference to obtain the absorption spectrum of dyed samples (bromophenol blue + agarose gel + ethidium bromide) and (xylene cyanol + agarose gel + ethidium bromide). The absorption was studied in wavelength range from 450 to 700 nm.

Factors affecting molecules' migration

Mobility of molecules and its migration in electrophoretic electrical field depend on the following factors, according to Deyl (1979-1983): 1) concentrations of gel sample (T , C) where T is concentration of individual agarose molecules and C is concentration of agarose solvent; 2) sample size and its shape; 3) concentrations of buffer solution; 4) applied electric field strength; 5) proportion of hydrophobic molecules; 6) ionization intensity; 7) buffer temperature; 8) gel pore size which is resultant from variation of gel concentration.

These factors affect molecules mobility in gel and their diffusion coefficient. The diffusion coefficient of sample molecules in free solution D_0 and electrophoresis mobility in buffer solution U_0 are proportional by constant rate a , Andrews (1993):

$$D/D_0 = U/U_0 = a$$

Where, U is electrophoresis mobility in gel, D is diffusion coefficient of sample molecules in gel and a is called impedance coefficient. The distance d that molecules pass during electrophoresis is proportionate with time t [sec] and potential deviation g [$V \cdot cm^{-1}$]. The distance is defined by:

$$d = U \cdot g \cdot t = U \cdot i \cdot t / k$$

Where, i is the current density measured in $A \cdot cm^{-2}$ and k is the qualitative conductivity measured in $\Omega^{-1} \cdot cm^{-1}$.

In nucleic acids applications, electrophoresis mobility in free solution U_0 depends on the weight of the molecules and the viscosity of the medium. D_0 is inversely proportional with the square root of molecular weight of DNA. Richards and Lecanidou (1971) reached the conclusion that the value of U_0 and D_0 is as follows; $U_0 = 31 \cdot 10^{-5} \text{ Cm}^2 \cdot \text{V}^{-1} \cdot \text{S}^{-1}$ in 0.05 mol buffer solution at 25°C. However, D_0 varies from 1.3×10^{-6} to $1.4 \times 10^{-7} \text{ cm}^2/\text{s}$ for nucleic acid molecules which have molecular weight range between 104 to 106 bp. The shape of electrophoretic nucleic acids is considered as fascicular zone during the separation by electrophoresis. This zone could be described by changing the concentration C ($m \cdot l^{-1}$) with the position X along the motion axis. The relation between the highest concentration C_m and the width of the band w_{atc} is given by the following equation:

$$2 w_{atc} = C_m / 2$$

The injected sample volume applied into the unit of gel cross-section area is u . If the potential deviation g in the sample solution is equal to the gel, the primary width of the band at the reference point could be given by the following relation:

$$2 w_0 = a \cdot u$$

The application of the potential deviation g in the sample layer mostly is greater than in the gel, so the width of primary band will be smaller than that suggested in the previous equation. This may be neglected provided that:

$$2w_0 < (2 DT)^{1/2} \text{ or } u < (2 D_0 t / a)^{1/2}$$

The applied sample volume is rarely considered as major factor in design while $2w_0 < (2 DT)^{1/2}$, the relation of band width is given as

the following:

$$2 w = (16 D t \ln 2)^{1/2}$$

Electrophoresis is the movement of an electrically charged substance under the influence of an electric field. This movement is due to the Lorentz force, which may be related to fundamental electrical properties of the body under study and the ambient electrical conditions by the equation given below (Taylor et al., 2004):

$$F_e = q \cdot E$$

Where, F_e is the Lorentz force, q is the charge carried by the body and E is the electric field. The resulting electrophoretic migration is countered by forces of friction such that the rate of migration is constant in a constant and homogeneous electric field:

$$F_t = v \cdot f$$

Where, v is the velocity and f is the frictional coefficient. Migrating ion attains constant velocity at which moving and retarding forces balance:

$$v = E \cdot q / f$$

The electrophoretic mobility μ is defined as follows:

$$\mu = v / E = q / f$$

The expression above applies only to ions at concentration approaching zero and in a non-conductive solvent. Polyionic molecules are surrounded by a cloud of counterions which alter the effective electric field applied on the ions to be separated. This render the previous expression a poor approximation of what really happens in an electrophoretic apparatus. The electrophoresis mobility depends on both the particle properties (surface charge density and size), and solution properties (ionic strength, electric permittivity, and pH). For high ionic strengths, an approximate expression for the electrophoretic mobility is given by the Smoluchowski equation:

$$\mu_e = \epsilon \cdot \epsilon_0 \cdot \xi / \eta$$

Where, ϵ is the dielectric constant of the liquid, ϵ_0 is the permittivity of free space, η is the viscosity of the liquid, ξ is the zeta potential (surface potential) of the particle.

RESULTS AND DISCUSSION

Absorption studies on mass spectrometer in visible and ultraviolet spectral ranges revealed that absorption was 100% in wavelength range from 450 to 500 nm, and the absorption was 80% at 600 nm. As shown in Figure 1 which indicates the visible light spectrum of blue bromophenol dyed sample, there is almost complete absorbance of the light in blue and violet range (below 500 nm). It is self-evident due to the blue color of the sample. On the other hand, there was 80% absorbance at light wavelength 600 nm; which is in the red range.

A 620 nm laser was chosen for further testing because of high availability and economical price. We tested wavelength 620 nm in Varian 2200 run on single beam mode to measure transmission. The results are shown in Table 1. The advantage of the described method is the

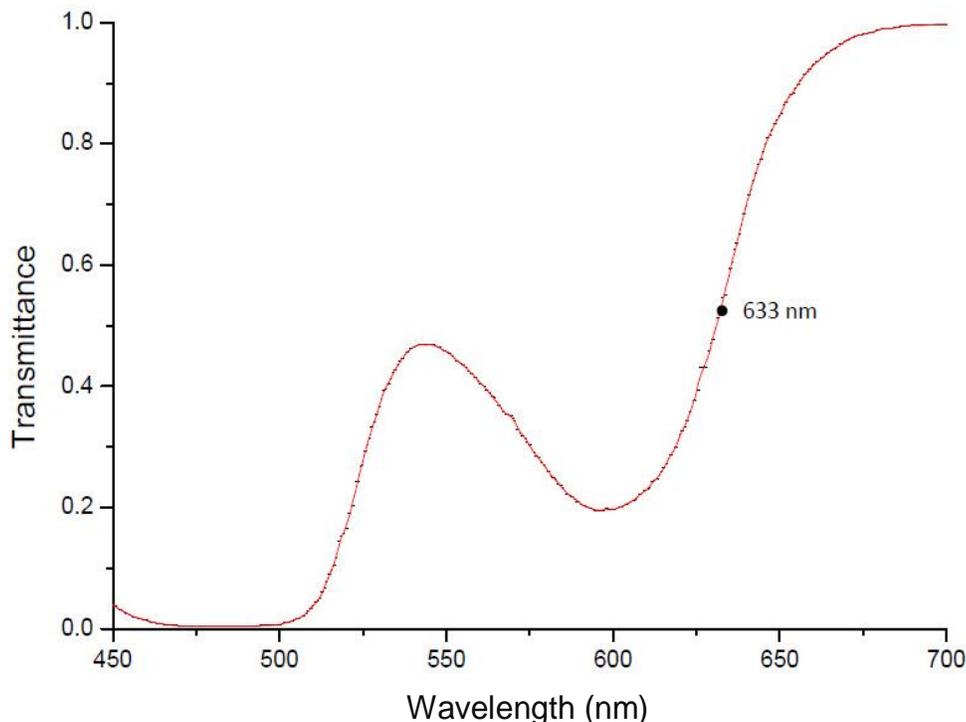


Figure 1. Transmittance of blue bromophenol dyed sample.

Table 1. Transmittance on wavelength 620 nm in Varian 2200 in a single beam mode.

Gel concentration (%)	Color of the sample	Transmission at 620 nm (%)
1	Clear	76
	Blue	31
	Green	37
3	Clear	64
	Blue	29
	Green	34

integrated design of electrophoresis device which includes electrophoresis tank with two electrodes (anode and cathode), rest of gel plate and buffer tank, two dark chambers for laser and photo-detector, power supply and control panel. The device has three control mechanisms: time interrupt, potential selecting, and laser interrupt.

Time interrupt

The time of electrophoresis can be adjusted by the user from 1 min to 99 min using timer countdown. When the time passes, a sound alarm is issued and electrophoresis current is blocked. The time unit consists of display, time up and down buttons and set button. The up and down buttons are used to adjust the timer and the set button is used for setting the time of electrophoresis.

Potential selection

There are four different potential values to be selected (35, 50, 100 and 150 volts) as the most common used potentials in the applications of electrophoresis of nucleic acids.

Laser interrupt

Laser detection is used to detect DNA bands just before they may exit the gel plate. The detection is an optional choice. If the laser interrupt button is switched on and when the loading dye which precedes slightly the first nucleic band is detected by laser, sound alarm is issued and electrophoresis of sample is stopped by turning off the potential applied on electrodes.

Conclusion

Based on the measured absorption spectrum of DNA in ultraviolet range, we can use photoelectric effect in horizontal electrophoresis by application of an array of focused ultraviolet light beams that can determine the exact position of each migrating fragment. Thus, it is possible to develop an integrated and comprehensive device with no need of the use of a UV documentation camera or fluorescence dye such as ethidium bromide, which is a toxic substance causing genetic mutation.

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