

# Dosing and separation of tracking dyes in glass chip for capillary gel electrophoresis

**Streszczenie.** W artykule przedstawiono wyniki dozowania i separacji barwników śledzących w szklanym chipie do kapilarnej elektroforezy żelowej. Barwniki o różnych kolorach oraz masach cząsteczkowych i objętości rzędu pojedynczych nanolitrow zostały rozdzielone w ciągu kilku minut z wykorzystaniem elektrohydrodynamicznego mechanizmu zarządzania przepływem. Łatwość obserwacji tak małych objętości za pomocą prostego systemu monitoringu pozwala wykorzystać barwniki śledzące do optymalizacji mikroprzepływów oraz konstrukcji chipów mikrofluidycznych. (Dozowanie i separacja barwników śledzących w szklanym chipie do kapilarnej elektroforezy żelowej).

**Abstract.** In this paper, results of dosing and separation of tracking dyes in a glass chip for capillary gel electrophoresis are presented. Dyes of various colors, molecular mass and volume range of single nanolitres were separated in a few minutes, utilizing electrohydrodynamic mechanism of flow management. Easy observation of such small volumes using simple monitoring system allows for tracking dyes application in optimization of microfluidic chips construction and management of microfluidic flow.

**Słowa kluczowe:** elektroforeza, lab-on-a-chip, CGE, separacja, barwniki śledzące.

**Keywords:** electrophoresis, lab-on-a-chip, CGE, separation, tracking dyes.

## Introduction

Capillary gel electrophoresis (CGE) is a high performance separation method, especially useful for genetic and proteomic analysis. In this method, electric field is used to force migration of particles in a gel-filled capillary. Due to sieving mechanism of the gel, particles are size-separated to fractions (bands) and identified.

As standard CGE apparatus are bulky and maintenance-expensive, a new generation of miniature CGE devices utilizing lab-on-a-chip (LOC) technique are recently investigated. LOC technique provides integration of electric-controlled microfluidic injectors and separators in a single CGE chip, resulting in high separation efficiency, low sample/reagents consumption and automation of analysis.

Efficient and repeatable on-chip management of nanolitre-range sample volumes requires optimization of correlative parameters, e.g. electric field strength, flow time. This task is challenging, as systems for on-chip sample monitoring are mostly complex and microfluidic flow is observed locally.

We propose a new approach to monitor and design microfluidic flow in CGE LOCs, utilizing tracking dyes. These color markers are commonly used in standard slab-gel electrophoresis for DNA run monitoring, as nucleic acids are not visible during separation. Dyes of various colours, molecular mass and electric charge are generally available, low-cost and provide easy visual observation in LOC utilizing simple monitoring system.

In this work, dosing and separation of selected tracking dyes in glass LOC for CGE has been presented and discussed.

## Experimental

**Method principles.** CGE separation mechanism provides very effective sorting of electrically-charged particles in a gel medium. At the beginning, sample plug is injected to the inlet of a gel-filled capillary (Fig. 1a). Afterwards, electric voltage is applied to capillary terminals, enforcing electrohydrodynamic flow of particles towards the outlet and their size- and charge-dependent separation in a gel sieving matrix (Fig. 1b). Identification of distinct bands is provided by relevant detection techniques, e.g. absorbance, fluorescence, electrochemical.

The separation efficiency rises as the injection volume decreases and therefore dosing of small sample plug is required. In standard CGE instruments the sample is

sucked into the capillary inlet from an external reservoir and therefore a minimum injection volume is relatively high.

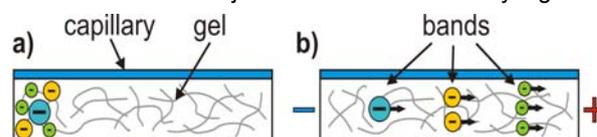


Fig. 1. Scheme of CGE separation of anodic particles: a) injected sample plug, b) separated bands

LOC technique provides integration of capillary-like injectors and separators in a network of microfluidic channels. This solution enables significant reduction of the sample plug and provides very high dosing repeatability.

Configuration of microfluidic channels of CGE chip is presented at Figure 2a. Cross-shaped injector enables dosing of required sample volume into the separation channel. The sample is pipetted into inlet of channel #1 and further operations are electrically-controlled: 1) accurate electric voltage is applied, forcing sample transport through crossing area (Fig. 2b); 2) the voltage is switched, causing injection of the sample plug from the crossing to the separation channel (Fig. 2c); 3) as the voltage is maintained, separation of the sample components occurs (Fig. 2d).

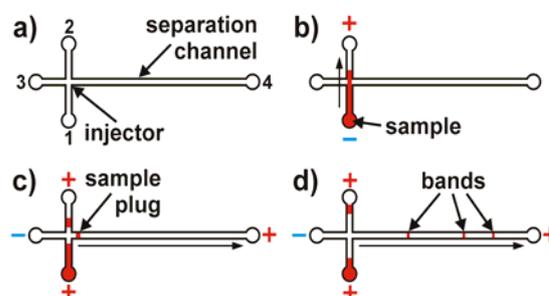


Fig. 2. Layout of CGE LOC: a) functions of microfluidic channels; operation procedure: b) transport, c) injection, d) separation

**Chip design and fabrication.** The chip for capillary gel electrophoresis was fabricated of two layers of borosilicate glass and four glass reservoirs (Fig. 3a). Microfluidic channels were wet etched in the bottom glass layer and inlet holes were drilled in the top layer. Reservoirs and both layers were permanently sealed utilizing fusion bonding

process, at the temperature 650°C. The details of glass chip fabrication procedure were described elsewhere [1,2].

The layout of CGE LOC is presented at Fig 3b. The chip contains microfluidic cross-injector with three dosing channels (5 mm long, 560 µm wide, 30 µm deep each) and a straight separation channel (25 mm long, 560 µm wide, 30 µm deep). The capacity of glass reservoirs placed at the endings of microchannels is 36 µl each.

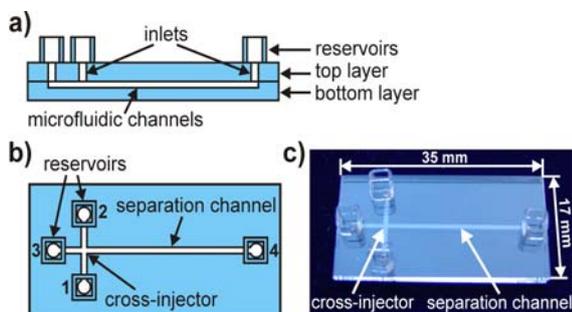


Fig. 3. Glass chip for CGE: a) cross-section scheme, b) layout and functions, c) chip at a glance

Entirely glass construction of the chip provides high transparency and easy visual observation of sample flow, as well as simple cleaning and full reusability (Fig. 3c).

**Experimental set-up.** Electrohydrodynamic sample dosing and separation is provided by a developed electric control system, containing: multichannel high voltage power supply, high voltage multiplexer and PC-based management (Fig. 4a). The high voltage power supply utilizes low-ripple proportional DC to HVDC converters providing voltage up to 1 kV per output channel. The channels are connected to the multiplexer, containing reed relays for voltage switching. The multiplexer is controlled by PC with LabView software, utilizing digital acquisition & control unit. Platinum wire electrodes are inserted into reservoirs of the chip. The system allows for easy, automatic and repeatable high voltage management in LOC according to programmed voltage-time settings.

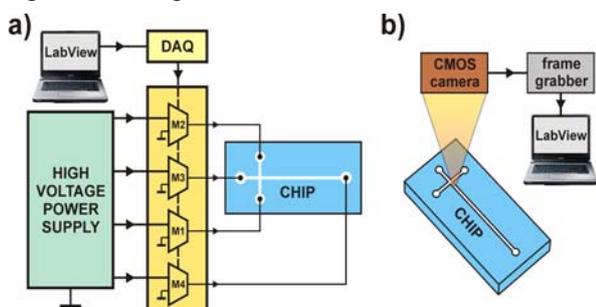


Fig. 4. Scheme of experimental set-up: a) electric control system (DAQ – digital acquisition & control unit, MUX – multiplexer), b) visual monitoring system

The chip was placed under miniature camera with a zoom lens enabling real-time sample flow monitoring (Fig. 4b). Analog data was processed to digital by a frame grabber and transferred to PC. LabView software provided real-time image display and recording.

Metric scale with 1 mm resolution was placed under the chip, providing reference for sample flow along the separation channel.

**Samples and reagents.** Colorful tracking dyes, commonly used for optical monitoring of DNA fragments separation in a slab-gel electrophoresis, were utilized (Tab. 1). The colour

of each dye depends on the pH of the solvent. Molecular mass and electric charge are affecting electrophoretic mobility of dyes in a gel sieving matrix, e.g. orange G migrates the fastest. This feature is utilized for DNA monitoring, as characteristic migration speed of dye strictly corresponds to migration speed of DNA fragments of specific length [3].

Table 1. Properties of selected tracking dyes – some information adapted from [4]

Name	orange G	bromophenol blue	cresol red
Colour <sup>1</sup>	orange	blue	red
Molecular mass [u]	452	669	382
Polarity	+	++	+
DNA fragment equivalent [number of base pairs] <sup>2</sup>	50	300	1000

<sup>1</sup>for neutral pH; <sup>2</sup>values given for 1% agarose gel electrophoresis

Selected tracking dyes were dissolved in the deionized water to a required concentration, ranging from 0,1% to 5%. Series of mixtures, containing various concentrations of dyes, were prepared. Loading agent (100% glycerol) was added to every mixture in order to increase sample solution density.

Microfluidic channels of the chip were filled with a separation gel (POP-4, Applied Biosystems) for standard CGE instruments, utilizing pressure of a laboratory pipette. Glass reservoirs were filled with standard electrolytic buffer for CGE analysis of genetic material (1x Tris-Borate-EDTA, A&A Biotechnology).

**Dosing and separation of tracking dyes.** Wire electrodes were inserted in the reservoirs. Voltage-time distribution for every electrode was set in the software. The sample was pipetted in reservoir #1 and afterwards the program was started. The real-time images of sample injection and separation were recorded in the digital database and compared. Series of experiments allowed for estimating accurate voltage ratio and time settings for repeatable sample plug injection and separation (Tab. 2).

Table 2. Optimum voltage and time settings for sample operation

Reservoir	Sample operation	
	transport	injection/separation
1	0 V	500 V
2	500 V	500 V
3	0 V	0 V
4	0 V	1000 V
Time	25-35 s	50-90 s

On the basis of optical density of separated bands, the optimum concentration of tracking dyes in the sample was estimated to: 1% orange G, 1% bromophenol blue and 5% cresol red.

## Results and discussion

Optimized sample concentration and operation settings enabled accurate dosing and separation of tracking dye mixture (Fig. 5). Transport time in a range from 25 to 35 seconds provided repeatable injection of sample plug volume (approx. 9 nanolitres). It has been found, that applied injection technique allows for diminishing the sample plug to 1-2 nanolitres, but for such small sample plug volumes the contrast of separated bands is significantly lower, making visual observation problematic. Increasing concentration of tracking dyes could be the solution, but for presented samples the maximum solubility of dyes in water was nearly achieved and therefore utilizing another solvent is required.

The separation of selected tracking dyes was obtained within approximately 50 seconds after sample plug injection. The bands are distinct and colourful, enabling unambiguous differentiation of dyes. The increment of electric voltage in reservoir #4 allows for shortening of separation time, but the influence of heat generation in the separation channel on gel stability and increased thermal diffusion must be concerned.

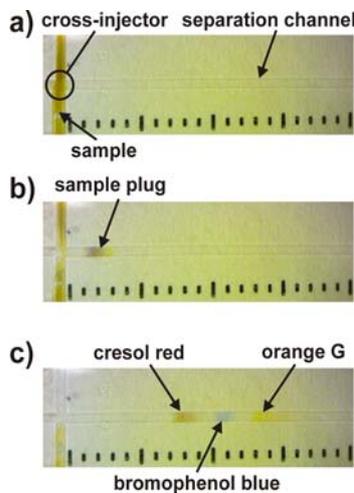


Fig. 5. Real-time view of injection/separation area of glass LOC for CGE during: a) transport, b) injection, c) separation of tracking dyes

For the quantitative determination, the optical density distributions along the separation channel were measured (Fig. 6). Distinct peaks, corresponding to the sample, sample plug and separated dyes, are clearly recognised.

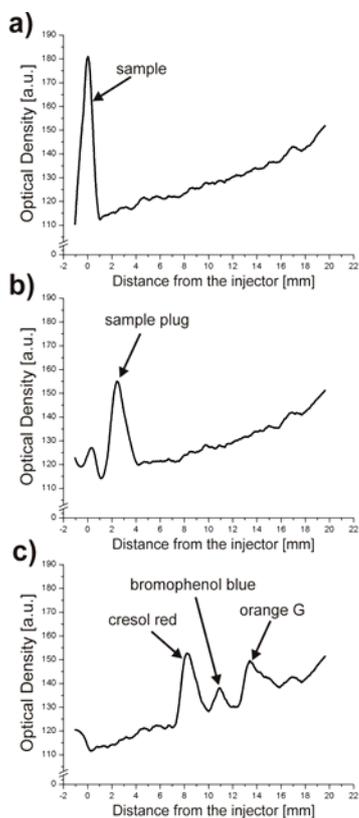


Fig. 6. Optical density distribution along the separation channel during sample: a) transport, b) injection, c) separation

The migration velocity and electrophoretic mobility of the dyes were determined (Tab. 3). The assessment for orange G was not strict, as particles of this dye are smallest, resulting in high diffusion. In a reference to DNA fragment equivalent presented in Table 1, the relation of measured electrophoretic mobilities of the dyes is accurate. However, DNA equivalents are available for slab-gels only and therefore direct comparison of tracking dyes and DNA migration in CGE LOC requires further study.

Table 3. Parameters of tracking dyes electrophoresis

Tracking dye	orange G	bromophenol blue	cresol red
migration velocity [ $\text{mm}\cdot\text{s}^{-1}$ ]	0,28	0,24	0,17
electrophoretic mobility [ $10^{-6}\text{ cm}^2\cdot\text{V}^{-1}\cdot\text{s}^{-1}$ ]	112	96	68

## Conclusions

In this work, application of commonly available tracking dyes for optimization of fluid flow management in microchips for capillary gel electrophoresis has been proposed. Nanolitre-range sample plugs were transported, injected and separated utilizing electrohydrodynamic effect. The mixture of three tracking dyes has been successfully separated within 50 seconds. Distinct bands of colorful dyes are easily differentiated utilizing simple visual monitoring system with camera, as well as with a naked eye.

It must be noted that dosing and separation of selected in this work tracking dyes corresponds to dosing and separation of DNA fragments in a size range of 50-1000 base pairs. Nucleic acids of this size are utilized in genetic mutation detection, noninvasive prenatal diagnosis, pathogen detection and many others. As the multiplicity of tracking dyes is immense, including various molecular mass, polarity, electric charge and colours, they can be applied as dummy samples for simulation of microfluidic flow and separation of other macromolecules, e.g. peptides.

High versatility and accessibility of tracking dyes allows for their wide application in microfluidics, especially for easy optimization of sample flow management, as well as designing of microfluidic LOCs.

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## REFERENCES

- [1] Kubicki W., Walczak R., Dziuban J., Injection, separation and fluorimetric detection of DNA in glass lab-on-a-chip for capillary gel electrophoresis, *Optica Applicata*, 41 (2011), nr.2, 409-416
- [2] Kubicki W., Walczak R., Dziuban J., Miniaturowy system do żelowej kapilarnej elektroforezy DNA z detekcją fluorescencyjną, *Przegląd Elektrotechniczny*, 86 (2010), nr.10, 69-71
- [3] Westermeier R., *Electrophoresis in practice*, Wiley-VCH, Weinheim, 2005
- [4] Sambrook J., Russell D., *Molecular Cloning: A laboratory manual*, Cold Spring Harbor Laboratory Press, New York, 2001

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