FLUIGENT

MODULAR INSTRUMENTATION FOR CAPILLARY ELECTROPHORESIS USING PLUG-AND-PLAY MICROFLUIDIC, ELECTROPHORETIC, AND OPTIC MODULES

INTRODUCTION

After almost 40 years of development an instrument commercialization, capillary electrophoresis (CE) is now among established analytical techniques and becomes a method of choice for several classes of analytes, notably DNA, glycans, therapeutic proteins, chiral molecules, and inorganic ions. Today, several bench-top commercial instruments exist to perform CE. Although well standardized, robust, and offering great automation, these systems are highly expensive and cumbersome. They are thus often not accessible to academic research and laboratories with a limited budget and modest infrastructure. As a consequence, in-house built CE have been implemented to fulfill the urgent demand for affordable and simple analytical devices. Simple CE systems with syphoning injection have been implemented, only requiring a high voltage (HV) module, one capillary, and vials. However, the injection mode is not reproducible, and the manual flushing using plastic syringes causes high risks of sample contamination. More advanced CE systems exist, but they often require electronic and mechanical skills, that are not always accessible in analytical laboratories with routine operations. With the goal to improve CE popularity, the Institut Galien Paris Saclay (University Paris Saclay) has put effort on the development of an easy to build and high-performance CE system. They developed a “Lego CE”, inspired by the Lego toy concept, in which the user can easily assemble a CE system from different commercially available components[1].

We present here the Lego CE system consisting of available ready-to-use electrophoretic and microfluidic modules, including pressure-based flow controllers. The instrument is coupled with a laser induced fluorescence detector (LIF), and is demonstrated for separations of labelled oligosaccharides.

MATERIALS AND METHODS

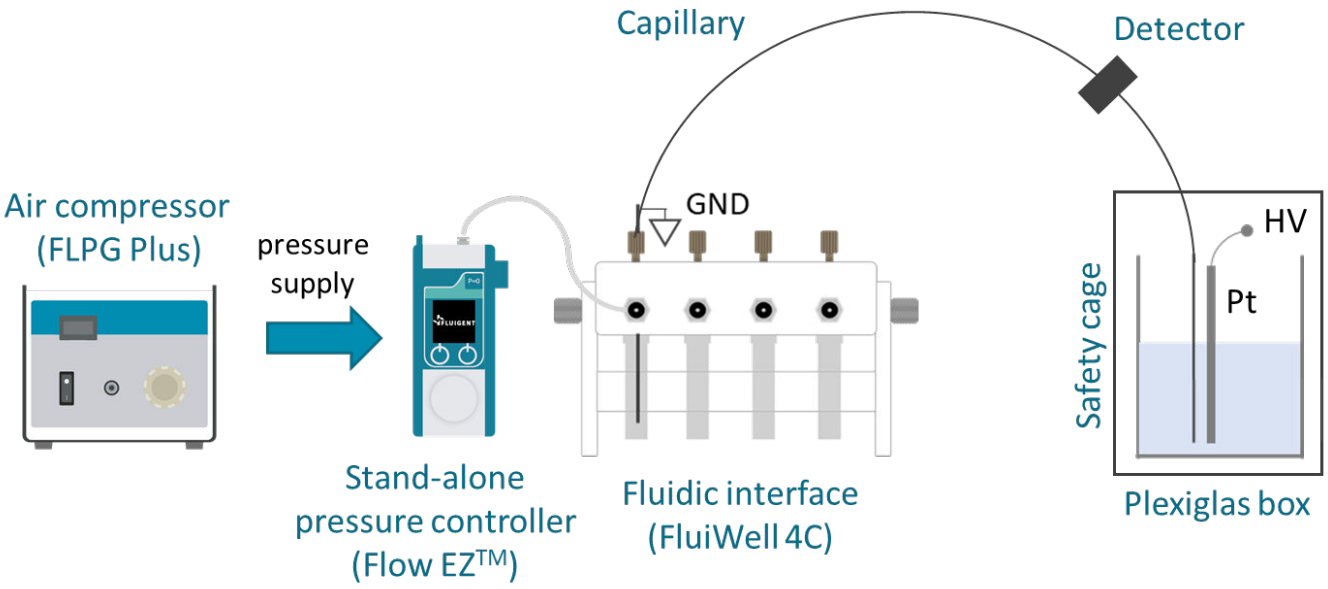

Figure 1: Schematic drawing of Lego CE design. (GND: Ground electrode; HV: high voltage)

I. Materials

i. CE instrumentation

Pressure source

FLPG Plus is a lab-bench-scale compressed air solution, which can produce, without any maintenance, a high quality air for all types of microfluidic experiences. It is a single device including a pressure pump source, a manual regulator, and a pressure sensor and display.

Pressure controller

The Flow EZ is the most advanced flow controller for pressure-based fluid control. It can be combined with a Flow Unit to control flow rate in addition to pressure. It can be used without a PC. One Flow EZ 1000 mbar is used in the setup presented here.

Fluidic interface

The Fluiwell 4-C are specifically designed caps for pressurization in microfluidic experiments. Autoclavable for sterilization, they can be used with different types of tubing and are suitable for long term experiments.

Electrophoresis module

The electrophoresis module was based on a dual polarity high voltage power supply with +/-30 kV maximum outputs.

LIF module

The laser induced fluorescence detector comprises a 488 nm laser module, optical filters and FITC emission filter. The analog signal was recorded with a data acquisition system. More information can be found in the paper written by Liénard-Mayor et. al[1].

ii. Chemicals and reagents

Background electrolyte

The background electrolyte (BGE) is composed of beta-alanine / MES at ionic strength of 50 mM and pH 4.75.

Analyte

Glucose oligosaccharides (dextran ladder) and fluorescent reagents (APTS and fluorescein isothiocyanate FITC).

II. Implementation of the Lego CE and detection of analytes

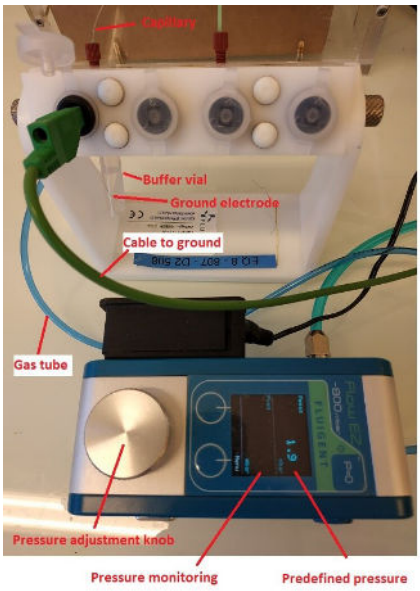

Figure 2: Microfluidic interface. Setup with Electrowell

A schematic of the Lego CE is shown in figure 2. Compressed air generated by the FLPG is driven to the Flow EZ 1 bar to provide highly precise pressure for hydrodynamic injection (usually 30 – 100 mbar) and capillary flushing (1000 mbar). Pressure assistance is also provided to accelerate the analysis and improve the separation resolution. The Fluiwell contains the solutions to be injected in the capillary (i.e. sample, BGE or other generating solutions). In this setup a platinum electrode is already integrated, but with the Fluiwell interface, a steel tubing commonly used for HPLC can be employed for ground connection. All fluid connections were made with 0.02 in. inner diameter (ID) and 1/16 in. outer diameter (OD) Teflon tubing. A fused silica capillary with total length of 45 cm is used. Control and monitoring of the voltage and current during electrophoresis is performed using the power supply unit with -25 kV. The high voltage side was isolated using a Perspex box for cosmetic and arrangement purpose. The custom electrolyte of APTS-labelled oligosaccharides are next injected. The injection is performed using 50 mbar over 10 s. The experiment is repeated with several pressure parameters. The first experiment is performed without pressure assistance. The second experiment is performed with 30 mbar pressure assistance once injection starts. The third experiment is performed using 30 mbar of pressure assistance once injection starts, then 20 mbar after 5 min. Detection is performed using the LIF detector.

RESULTS

Separation and detection of APTS labelled oligosaccharides

Glucose-oligosaccharides are often used as the ladder reference for analyzing N-glycans released from glycoproteins, serving for quality control of therapeutic glycoproteins and diagnostic purposes[2,3]. The Lego CE-LIF was used for separations of APTS-labelled oligosaccharides. Figure 3 shows the electropherograms obtained without pressure assistance (figure 3A), using pressure assistance of 30 mbar t = 0 s (figure 3B), and using pressure assistance of 30 mbar at t=0 s and 20 mbar at t = 5 min (figure 3C). We can observe that excellent peak shapes and separation resolutions are achieved for glucose units GU1 till GU6. To compensate for the peak retardation when using beta-alanine/MES BGE, pressure assistance can be applied during electrophoresis, which is not difficult when using the Flow EZ pressure controller. As we can see in figure 3B, the peaks arrived faster to the detector and more glucose units could be visualized under the pressure assistance at 30 mbar. To finely tune the electrophoresis, it is also possible to use a pressure gradient. By applying a pressure of 30 mbar at 0s and then 20 mbar at 5 min, the fast arrival of the first four peaks could be maintained, whereas separation resolution for the slower ones, which could correspond to the sizes of large N-glycans of glycoproteins, was improved (see figure 3C).

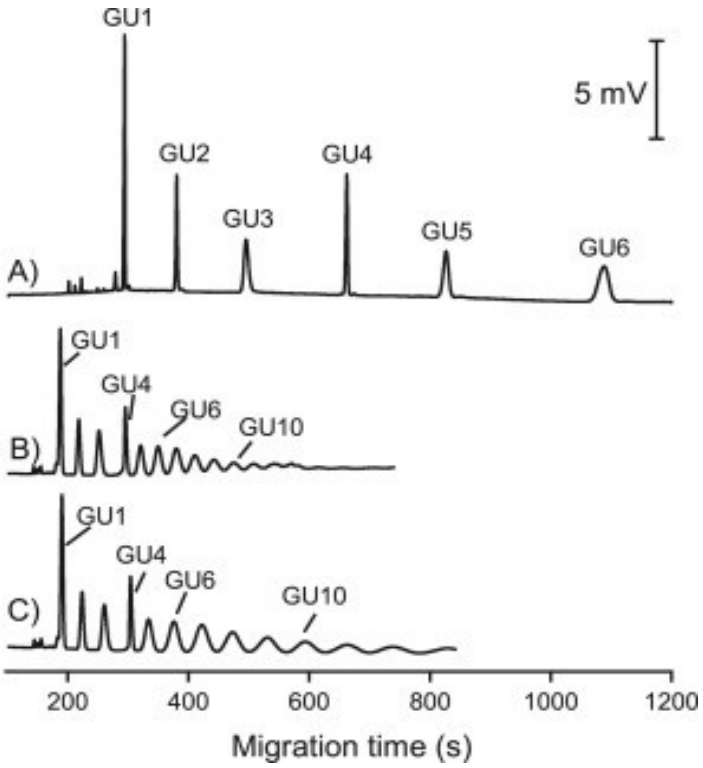

Figure 3: Electropherograms for CE-LIF separations of oligosaccharide ladders using the Lego CE-LIF instrument; hydrodynamic injection at 50 mbar over 10s. A) Without pressure assistance; B) With pressure assistance at 30 mbar from t = 0s; C) With pressure gradient: 30 mbar at t = 0s, then 20 mbar at t = 5 min[1]

With this demonstration, the authors expect to open a door for various applications exploiting both hydrodynamic and electrokinetic principles with Lego CE-LIF. This tool that can be tuned to get it adapted for any kind of prospective glycan analysis. Indeed by playing on voltages and pressures one could achieve the best separation performances whatever the type of glycans to be analyzed (i.e. N- or O-glycans, small or longer ones or even a mixture of these types).

CONCLUSION

A new Lego CE, that can be fully constructed using commercially-available products, was successfully developed. The need for electronic and mechanical skills, which is often a barrier for constructing in-house CE, is remarkably reduced. The great functioning of this system was demonstrated by separating and detecting fluorescent oligosaccharides. By using pressure assistance provided by the Fluigent instruments, it is possible to accelerate the detection, while keeping a good separation between each peak. Additional experiments have been performed using this system, including testing of different electrolyte, comparing the performance with a commercial CE system. All these results and more information concerning this Lego CE system can be found in the great paper written by Liénard-Mayor et. al1. The Lego design would allow the users to setup their own analytical devices at a cost at least 70 % cheaper than the purchase price of a commercial system while keeping a high degree of standardization (i.e. a 'standard' setup) and facilitation of technology transfer that are not offered by in-house-made versions.

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