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A New Capillary Electrophoresis Device with Deep UV Detector Based on LED Technology

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Abstract: During the last three years, the College of Engineering and Architecture of Fribourg has developed, in collaboration with the School of Pharmaceutical Sciences, University of Geneva - Lausanne, a low-cost analytical capillary electrophoresis (CE) device, equipped with a new deep UV detector based on LED technology. The aim is to use it for educational purposes and/or basic pharmaceutical-analytical services, including identification or quality control assays in developing countries.

Keywords: Capillary electrophoresis · Counterfeit drugs · LED

Fig. 1. Picture of the newly developed low-cost analytical capillary electrophoresis device.

Introduction and Motivation

According to the World Health Organization (WHO), counterfeit medicines are part of the broader phenomenon of substandard pharmaceuticals – medicines manufactured below established standards of quality and therefore dangerous to patients' health and ineffective for the treatment of diseases. For the past three years, the College of Engineering and Architecture of Fribourg has been developing, for this purpose, in intense collaboration with the School of Pharmaceutical Sciences, University of Geneva - Lausanne, a low-cost analytical device, namely capillary electrophoresis (CE) equipped with a new deep UV detector based on LED technology.

Three EC prototypes have been built, the first one, within the period 2006–2007, where the mechanical and electronic issues were addressed, including an original detection device built in Fribourg on the basis of diode technology. The second prototype, within the period 2007–2008, is actually working and the development of methods for drug analysis was the subject of a diploma work that a student of the 'Tecnologico de Monterrey Mexico' carried out in Switzerland from May to October 2009. This work was supervised by Professor Claude Rohrbasser (College of Engineering and Architecture of Fribourg) and Dr Serge Rudaz (UNIGE).[1,2] In this period, particular attention was paid to the robustness of the system in order to anticipate the problems that can be encountered when dealing with analytical methods in developing countries in Africa. The third one has now been sent to Mali for a cooperation project supported by the Swiss Agency for Development and Cooperation (SDC project N°7F-01984.02.66).

This new CE device (Fig. 1) is dedicated to conventional quality control of drug material (active principal ingredient, API) and drugs products (pharmaceutical formulations). CE should be successful implemented in routine analysis due to its short analysis time and simple method development, robust instrumentation, low sample and solvent consumption as well as reduced operating costs.

The most important issue of this project regards counterfeit medicines where CE is perfectly adapted to rapidly evaluate the quality of the drugs and to give evidence for the presence of significant amounts of degradation impurities or absence of the active principle.

The aim of this project is to analyze a range of essential medicines from a list compiled by the WHO (*www.who.int/medicines*). All these substances have a vital importance in their healthcare system. For this purpose, in collaboration with Dr Pascal Bonnabry, chief pharmacist of the University Hospitals of Geneva, a list of 20 frequently analyzed drugs was proposed where the analytical methods need to be optimized. From this list, eight representative active compounds were chosen according to their various physical-chemical properties (acidic, basic or neutral analytes, pKa, logP, *etc.*) as well as their drug formulation to be analyzed by the new CE device. For this purpose, four generic conditions were investigated: Capillary zone electrophoresis (CZE) at acidic pH for quinine and rifampicin, CZE at basic pH for amoxicilline and furosemide and CZE at intermediate pH for the simultaneous analysis of trimethoprim and sulfamethazole namely cotrimazole. The other three compounds, Lamivudine/Zidovudine/Nevirapine, used in HIV-1 infection, were separated thanks to a microemulsion electrokinetic chromatography technic approach (MEEKC).

The Detector

Light-emitting diodes (LED) are semiconductor diodes that emit light when an electric current is applied in the forward direction of the device. LEDs are widely used as indicator lights on electronic devices and increasingly in higher power applications such as flashlights and area lighting. Besides lighting, interesting applications include UV-LEDs (sterilization of water, disinfec-

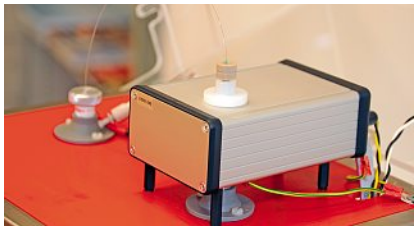

Fig. 2. Detail view of the UV detector based on LED technology.

Fig. 3. Close-up of the UV detector based on LED technology.

tion devices, photosynthesis in plants, phototherapy, UV curing, *etc.*). Recently, the wavelengths attained with LEDs reached 250 nm, and so opens up new possibilities in detection systems for HPLC[3] and capillary electrophoresis.[4–7]

Within the framework of developing a low-cost capillary electrophoresis device, a deep UV detector (@254 nm) based on LED technology was developed. This new type of light source allows a very compact design of the detection system (Fig. 2 and 3). The state-of-the-art electronic control of the instrument including acquisition and data processing were constructed. The stability of the LED and the sensitivity of the detector were found to be remarkable and presented an important potential for further applications such as the analysis of basic compounds at ppm range.

The UV detector shows a high reliability and is ready to be used in professional applications. Currently the limiting factor remains the available wavelength (250–254 nm) of commercial LEDs. The current development will provide UV LEDs having shorter emission wavelengths, in the 200 nm range which will enlarge the number of detectable molecules.

Applications and Results

One of the first practical application was the analysis of the three-ingredient combination Lamivudine/Zidovudine/Nevirapine which is used as a stand-alone anti-retroviral treatment for human immunodeficiency virus (HIV-1) infection. Combination therapy is recommended in HIV infection for all infants, children, and adolescents who are treated with antiretroviral agents. Compared with monotherapy, combination therapy slows disease progression and improves survival, results in a greater and more sustained virologic and immunologic response, and delays development of virus mutations that confer resistance to the drugs being used ('Guidelines for the use of antiretroviral agents in pediatric HIV infection', Oct 2006. National Institute of Health (*http://aidsinfo.nih.gov/*). As shown in Fig. 4, the three compounds can be easily separated with good resolution within a reasonable time of approximately six minutes. The peak shapes for all compounds are of good quality.

A second analytical application is cotrimoxazole, a combination of sulfamethoxazole and trimethoprim supplied in various pharmaceutical forms. Sulfamethoxazole and trimethoprim are both antibiotics that treat different types of infection caused by bacteria, mainly ear infections, urinary tract infections, bronchitis, traveller's diarrhoea, and *Pneumocystis carinii* pneumonia.

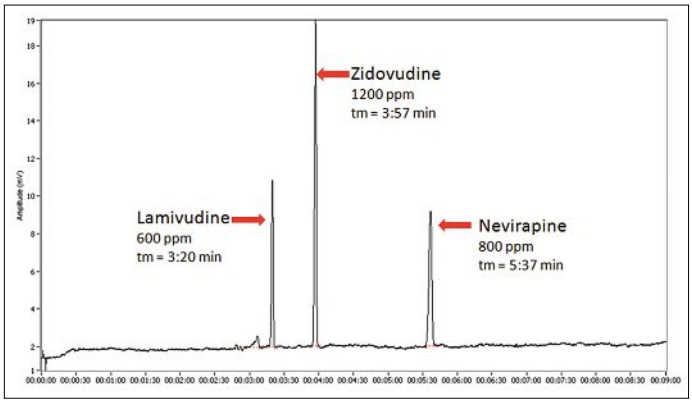

Fig. 4. Analysis of anti HIV drugs (20 kV, 400 v/cm, borate buffer 50 mM at pH 9).

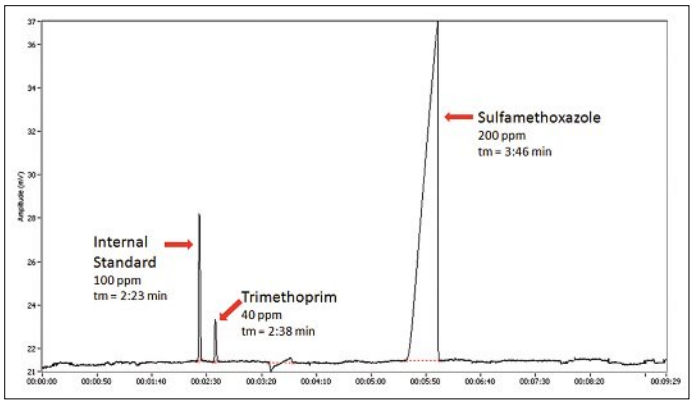

Fig. 5. Analysis of cotrimoxasole of bactrim (20 kV, 400 v/cm, HAC/tris buffer 50 mM at pH 6.1).

Cotrimazol is one of the most commercially successful drugs and is prescribed to millions of people each year. As shown in Fig. 5, with the newly developed low-cost CE device the two ingredients can be well separated within six minutes. More method development work is currently on-going to obtain robust and reproducible methods and to evaluate other drug products.

Conclusion

The device allowed the correct separation and determination of numerous compounds in acidic, basic and neutral media and the micro-emulsion technique has also yielded excellent results. The sensitivity of the detector is perfectly adapted to the concentration range levels required for the analysis of pharmaceutical formulations expected in these type of drugs and the signal-to-noise ration is quite satisfactory. The method of analysis for quinine formulation has been fully validated according to ICH criteria. Complete validation of alternative methods for other drugs is on-going.

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