INTEGRATION OF FLUORESCENT PH SENSORS IN MICROFLUIDIC FREE-FLOW ISOELECTRIC FOCUSING PLATFORMS USING AUTOMATED INKJET PRINTING

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ABSTRACT

In this work, we present the integration of fluorescent pH sensor arrays, into microfluidic free-flow electrophoresis (µFFE) chips. The sensor cocktail consisted a fluorescein derivate, which was linked to a hydrophilic acrylate based polymer. Optical pH sensor arrays were generated by automated inkjet printing and integrated into µFFE platforms using a fabrication cycle based on multiple photopolymerisation. The functionality of microfluidic free-flow isoelectric focusing (µFFIEF) platforms was demonstrated in separations of three P503 labeled proteins via monitoring of sample and sensor fluorescence in different spectral channels. These multifunctional microanalytical devices allow non-destructive online monitoring of isoelectric points (pI).

KEYWORDS: fluorescent chemical microsensors, on-chip pH monitoring, microfluidic free-flow isoelectric focusing, optical detection

INTRODUCTION

µFFIEF enables the continuous separation and preparation of minute amounts of biomolecules under mild conditions. However, monitoring of the pH gradient can only be performed indirectly or by subsequent offline analytical methods. Recently, we demonstrated that these constraints can be overcome by the integration of a fluorescent pH sensor layer into an µFFE microchip [1]. Therein, isoelectric points could be monitored directly via a photopolymerised pH sensor layer, but the approach was limited to photopolymer-based sensor matrices and suffered from only moderate response times. Herein, we present a novel straightforward approach applying automated piezoelectric inkjet printing for fast generation of microscale pH sensor structures without photopolymers and demonstrate their integration, characterisation and application in a µFFE chip platform.

THEORY

In free-flow electrophoresis an electric field is applied perpendicular to hydrodynamic buffer and analyte flows. For separations based on isoelectric focusing a pH gradient is established in the separation bed by an ampholyte mixture and analytes are located at the point of zero net charge (isoelectric point, pI). Fluorescent pH sensors work via the reversible protonation and deprotonation of probe compounds embedded into hydrophilic polymeric matrices that change their emissive properties. Inkjet printing uses piezoelectric actuators that deform upon application of an electric charge and is therefore able to deliver very small droplet volumes that are expelled from the printer in this process..

EXPERIMENTAL

µFFE structures were generated using photopolymerisation of oligoethyleneglycol diacrylates (OEG-DA) between two glass slides applying a light-impermeable mask. One glass slide was acrylate-modified and bonded covalently to the acrylic photopolymer. The other one was unmodified and could be removed and replaced by an acrylated glass slide with printed pH sensors after generation of the microfluidic structure. For bonding, photolithography with the acrylated slide containing a pH sensor array was applied in a subsequent step (Fig. 1a). In a typical procedure, a sensor matrix in solution is printed in spherical spots onto the glass using a piezoelectric microdosing system (desktop robot & PICO Valve MV-100, Nordson) (Fig. 1b). Fluoresceinisothiocyanate (FITC) was covalently linked to polyhydroxy-ethylmethacrylate (pHEMA) by stirring a solution of pHEMA and FITC in N,N-dimethylacetamide at 75 °C for 3 hours, precipitated as well as washed three times with 50 mL BRP pH 6 and applied as an 5% solution in ethanol and water (9:1, v/v).

The pH sensitivity of the sensor structure was calibrated with Britton Robinson buffers (BRB) in the pH range from 3 to 10 that were of equal conductivity to the ampholyte mixture (Fig. 3a). Furthermore, response time and leaching of the sensor array were characterised measure fluorescence intensity by changing the pH between 3 and 10 as well as at a constant flow of 50 µL/min using a photomultiplier tube (PMT).

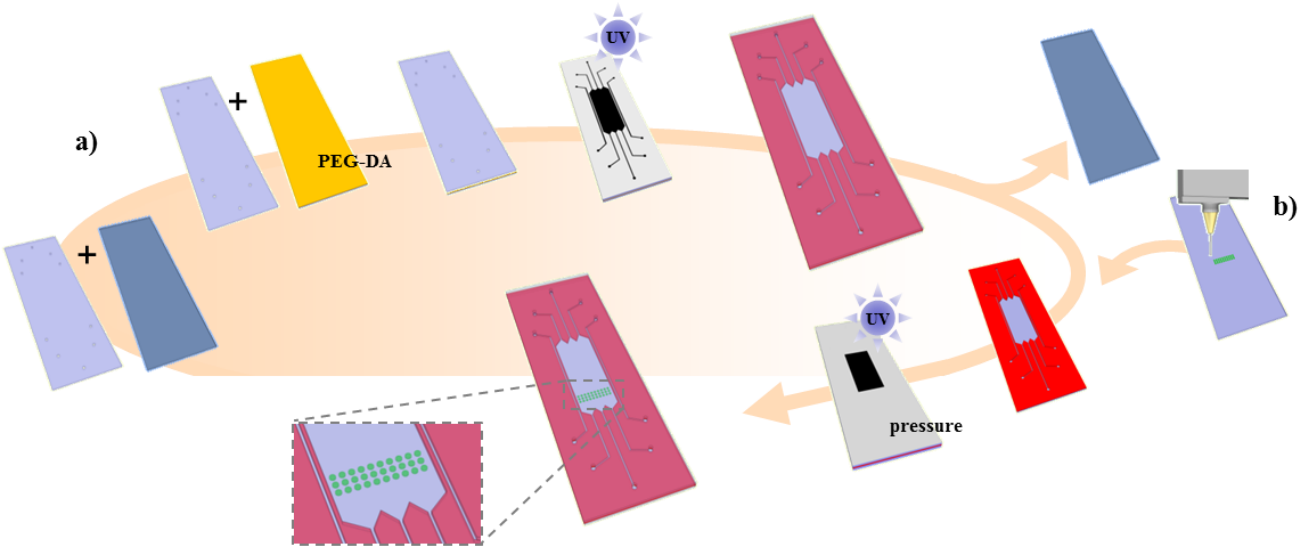

Figure 1: Illustration of the fabrication cycle of microfluidic glass-PEG free-flow electrophoresis chips with an integrated pH sensor array via multiple photopolymerisation and inkjet printing of sensor arrays on glass.

Proteins were labeled with P503, a red fluorescent dye not affecting total analyte charges, by reaction of 1 mL protein solution (3 g/L in 100 mM sodiumcarbonate buffer pH 9) and 5 µL dye solution (25 mM in DMF) for 15 min at RT with an afterwards cleaning using a sephadex G-25 column and dialysis against water. IEF was performed with protein solutions (BSA 87 µg/mL, conalbumin 197 µg/mL, chymotrypsin 367 µg/mL) injected into the separation bed via the central fluidic inlet flanked by channels containing 1% ampholyte (pH 4-7) with 0.1 % Tween20, and acidic as well as alkaline sheath flows (BRP pH 3 and 8) (Fig. 2a). The electric field of 1.4 kV (280µA) was applied via copper electrodes attached to electrolyte channels containing acidic or alkaline buffer (BRB pH 3 and 10). Fluorescence was monitored in two spectral channels for analyte and sensor quantification.

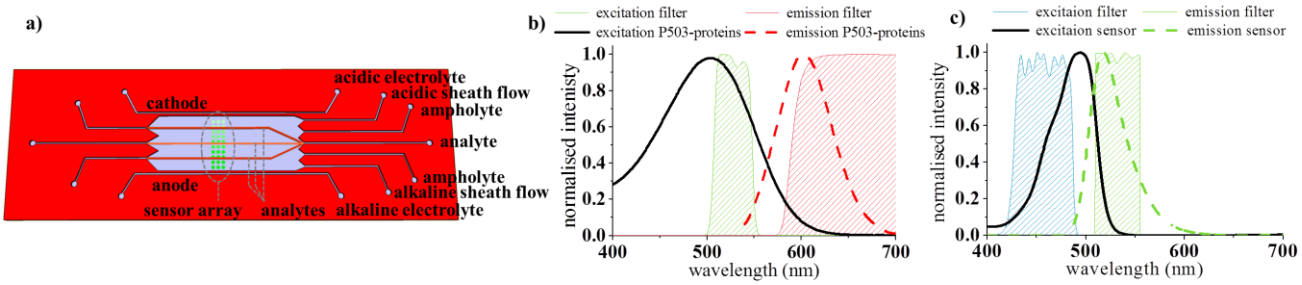

Figure 2: a) Schematic of a µFFE chip with an integrated pH sensor array and fluidic setup. Spectral channels used for protein (b) and pH sensor (c) monitoring, respectively.

RESULTS AND DISCUSSION

The successful fabrication of microfluidic free-flow electrophoresis chips for online pH monitoring capability in the separation bed is shown in this work. The integration of sensor structures in microfluidic platforms could be realised by automated piezoelectric inkjet printing. Best results were obtained by using a dosing needle of 200 µm inner diameter. The polymer is fixed on the glass surface by physical adhesion. With this setup spherical spots with a diameter of 538 ± 83 µm and a height of 2.4 ± 1.0 µm in a sensor array of 11 x 3 functional elements in an area of 18.75 mm² could be produced.

The calibration of the sensor array resulted in a strong response to pH with a pKa of 6.99 ± 0.04 (Fig. 3). Response times t_{95} 0.27 ± 0.06 s for the change from alkaline (BRB pH 10) to acidic (BRB pH 3) solution and 1.62 ± 0.19 s for the reverse direction could be achieved which is an improvement of a factor of 9 compared to a photopolymerised pH sensor layer described previously and allowed to monitor pH in microanalytical separations almost in real-time. Leaching experiments showed an intensity decrease of 8% after 22 min when applying a hydrodynamic buffer (BRB pH10) flow.

For on-chip IEF analyses a pH gradient was established in the separation chamber by introducing an ampholyte mixture which can be monitored via the green fluorescence of the pH sensor (Fig. 2c, 3b). Proteins and other biomolecules were separated by IEF and detected in the red channel (Fig. 2b, 3d). An overlay of the two channels allowed the visualisation of both pH gradient and protein bands.

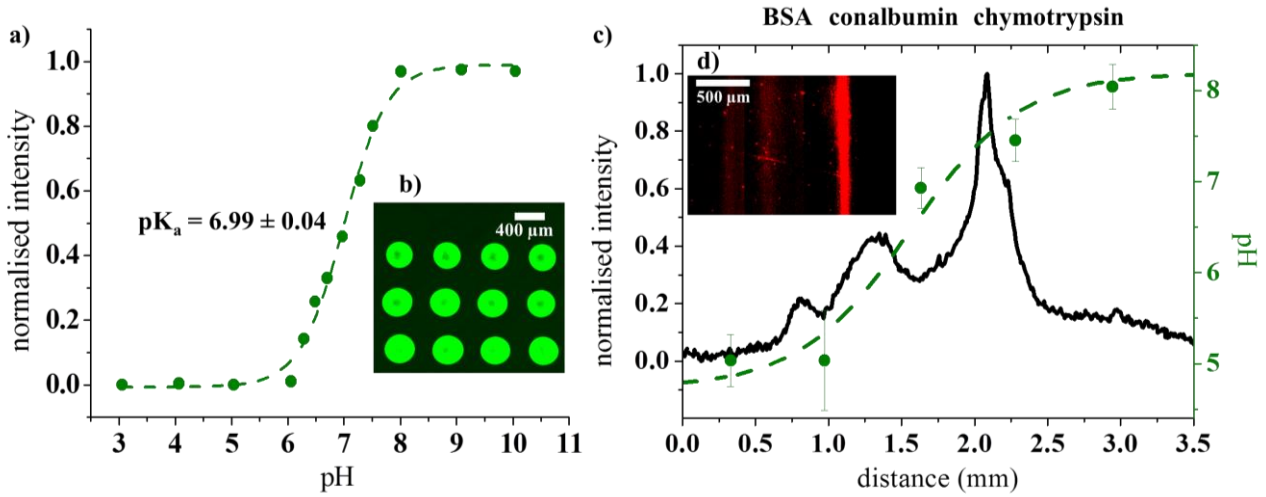

Figure 3: The obtained pH calibration curve (a) of the microchip integrated pH sensor array shown in b. Separation of the proteins BSA, conalbumin and chymotrypsin by µFFIEF (c): baseline corrected protein fluorescence in the separation chamber (black) with online monitored pH by the integrated sensor (green) and d) a false colored fluorescence image of the separation (baseline corrected).

A separation of three labeled proteins of known pI, bovine serum albumin (BSA), conalbumin and α-chymotrypsin using free-flow isoelectric focusing is shown in Fig. 3c and d. The integrated sensor array showed a fluorescence intensity gradient according to the pH gradient in the separation bed (Fig. 3c, green dots). The pI of these proteins could be determined via the integrated sensor array and showed only minor deviations less than 0.6 pH units from known literature values (Table 1).

Table 1. Isoelectric points of proteins and deviations of determination

protein	known pI	calculated pI	deviation
BSA	4.9	5.17	0.27
conalbumin	6.1	5.99	0.11
chymotrypsin	8.3	7.71	0.59

CONCLUSION

In this work the fabrication of µFFE chips with integrated fluorescent pH sensors via inkjet printing and photopolymerization of the microfluidic structure is presented. These chips were applied for µFFIEF of three proteins with online detection of their isoelectric point yielding deviations of less than 0.6 pH values to their literature values.

The demonstrated novel fabrication technique represents a straightforward approach to implement fluorescent sensor structures in microfluidic devices. It is anticipated that these microchips can be further developed e.g. to extend their dynamic pH range and for separation of unlabeled proteins.

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