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Continuous extraction of proteins with a miniaturized electrical split-flow cell equipped with suspended splitters fabricated by dry film lamination

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ABSTRACT

This article describes the development of an electrically driven Split-Flow Thin microsystem suitable for continuous-flow protein purification or concentration. We used a technology based on dry film resist, Ordyl SY 355, to realize 3D suspended structures in a microchannel (150 μm), including patterned Cr/Pt electrodes. The device developed for this research is the first electrical miniaturized dry film SPLITT system including suspended splitters and represents a good miniaturization effort of the ideal SPLITT cell, thus expanding the possibilities of realizing split-flow fractionation microfluidic network based on dry film technology. A suitable design and fabrication process for the realization of such a kind of device is reported. The purification/concentration performances of the system have been tested by infusing a Bovine Serum Albumin solution (0.1 wt percentage) through the inlets and evaluating the protein content at the outlets after the application of a pulsed electric potential. Experimental conditions have been changed during the testing phase, by varying the applied potential and the duty cycle of the square wave and the flow rate to find suitable conditions for protein extraction. The best-achieved purification/concentration rate was around 60%, measured as concentration difference between the two output channels normalized on the input concentration, thus confirming the viability of device for protein enrichment or purification for sample preparation. The device has a potential for integration in micro-TAS, thanks to its reduced dimensions, for applications involving protein analysis or bio-sensing in matrices including proteins interfering with the measurement.

1. Introduction

Protein purification and analysis has been a widely investigated field of studies over the last two decades. The rising interest in proteomics has led to the development of protein separation techniques and equipment over the years, for instance to explore protein's biochemical role or to analyse protein's properties like the tertiary structure e.g. by growing crystals of proteins from pure protein [1,2]. Protein purification is also exploited in protein production applications [3,4], in drug screening and enzyme engineering [5] and for industrial purposes, including the preparation of commercial products such as nutritional proteins (soy protein isolate) [6] or insulin [7].

Over the years, in addition to the development of increasingly performing laboratory equipment, a lot of research has been directed towards the implementation of chromatography, fractionation and electrophoresis at the microscale, through simple microfluidic devices realized with low-costs materials and techniques [8].

In the literature, there is a wide consensus on the need for "point of care" devices for local, rapid and reliable sensing of a wide range of targets in several fields of application. Some examples are the detection of microorganisms whenever it is impossible to carry out laboratory tests (space applications, for example) or to increase the rapidity and reduce the costs of the analysis with respect to the current standard methods (food and blood analysis, tumours markers detection, sensing of toxins and drugs). In micro TAS (micro-total analysis systems), it is usually necessary to couple a sample purification dedicated system to the sensing part to improve robustness and reliability of the analysis.

Matrix interference is a known issue in immunoaffinity methods. For instance, in immunoaffinity purification columns, the presence of proteins usually results in binding site inactivation and reduced capture capability of the functional surface, often requiring at least sample dilution or an extraction step for low concentration analytes [9]. The same issue also affects performances of immunoaffinity biosensors. In the scientific literature, the most used solution is to dilute the sample in an appropriate measurement buffer. For instance, in [10,11] a dilution around 1:10 is used to reduce the protein content in milk below a target threshold, above which proteins overcome the passivation capability of the engineered biofunctional layer. However, the dilution also reduces

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the sensor detection limit; therefore, a quite large background response is often found in measurements. Please see [11] and [12] as examples. In this perspective, the sensor calibration and accuracy become critical, because the substantial background signal requires a very precise management of blank reference measurement or signal subtraction. Any improvement of matrix purity can reflect directly on the interference rejection and ultimately on detection limit and measurement robustness.

As an example of required detection limit in dairy applications, the maximum residual limits (MRL) of antibiotics in milk are in the range of 1–1000 μg/l, while toxins range in the 25–500 ng/l depending on intended use of milk and region. Proteins in milk are typically about 35 g/l depending on feed, stage of lactation and breed [13]. For sample preparation in medical applications, the range of total protein content in biological samples is not very different from milk: in blood it ranges from 45 to 85 g/l [14], while in saliva the protein content is between 0.7 and 12.9 g/l [15]. Therefore, it is possible to evaluate a similar approach also in medical applications.

In sensors with good selectivity, which is usually found with labelled detection or with an amplification scheme like in [11] for instance, analytes are detected even at very low concentration (e.g. toxins in milk) with a "simple" 10x dilution and filtering to reduce interferents like proteins. In perspective, a 10x purification by microfluidic sample processing method not involving dilution can provide similar protein content reduction, without affecting the toxin content.

Label-free sensors typically have a higher detection limit and lower selectivity, e. g. a rejection of interferents in the order of 1:100 like in [16,17]. In this case, proteins need to be lower than 0.1–100 mg/kg to permit MRL detection of antibiotics in milk, thus requiring a purification rate in the order of 1:1000. Chemical extraction of protein by curdling with acids or enzymes like Rennet and filtration allows a 10-fold to 100-fold reduction of proteins (authors' data, unpublished), and an additional microfluidic sample preparation can cover the remaining 10x purification gap.

Microfluidic devices allow the extraction of proteins in a continuous-flow mode. A continuous flow process is especially needed to monitor parameters in real time in a stream or to provide flexible adaptation to the needs of the measurement process, i.e. tailoring extraction parameters and volume to the specific sample under process. There are several approaches to the separation of chemical species in a continuous-flow device [18]. In the specific case of proteins, it is well known that molecules carry a net charge depending on the pH of the electrolyte and this property is widely used in electrophoresis for analytical purposes.

One of the purification techniques that can exploit applied electric potentials is the Split Flow Thin Fractionation (SPLITT). The SPLITT theory was explained for the first time by J. C. Giddings in 1985 [19] and studied at the microscale from early 2000's. According to this concept, two different fluids are injected into a separation channel by means of two inlets: a sample to be purified and a carrier solution. Under the action of a driving force, including gravitational [20,21], centrifugal [22], electrical [23] and magnetic [24,25] forces, the particles that we want to separate from the sample migrate towards the carrier stream and are flushed out from a dedicated outlet (Fig. 1).

The advantage of lateral transport or the SPLITT technique is the fast extraction in continuous flow, through a transverse application of the fields (electric, magnetic etc.). The aim of this technique is to reach a high field in order to have higher migration speed of particles. Hence, the advantage is to have the electric separation along the shortest distance (higher electric fields with a few volts). Moreover, the splitters enhance the laminar flow inside the channel and the separation of streams at outlets. Therefore, it is possible to achieve protein removal or enrichment at a flow rate in the ml/min range, which is much faster than capability of traditional electrophoresis, where longitudinal transport is provided by electrophoresis coupled with electro-osmosis. Thus, the SPLITT technique can process a larger volume of sample in a shorter time. The approach is particularly suitable for analytical methods needing purification and quick analysis not compatible with traditional preparation, which typically requires several minutes. Consequently, the primary aim of this miniaturised system is fast protein extraction, enrichment or removal rather than to separate different proteins in a mix, because although the latter is possible [26], the resolution is lower than with traditional electrophoresis.

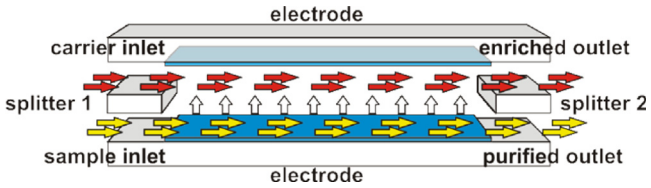

Fig. 1. SPLITT system scheme. The splitters play a key role to maintain the laminar flow. The stream flowing from the extracted particles outlet (red arrows) is enriched in protein, while the other (yellow arrows) is purified. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

In our study, we combine a microfluidic splitter structure with a pulsed vertical electric field imposed between two electrodes. The use of pulsed field allows a faster electrophoretic drag by increasing the potential beyond hydrolysis potential, without the formation of gas bubbles and the need for complex electrode degassing structures as shown for instance in [26]. Since the applied pulsed field is asymmetrical, proteins are attracted preferentially by one of the electrodes and they move from the sample to the carrier stream. Therefore, the carrier solution (higher concentration) and the purified sample (lower concentration) are collected at the two different outlets. In laminar-flow conditions, the sample and the carrier travel simultaneously through the separation chamber one above the other, without mixing. Indeed, there are no turbulences in the microstructure, because of the very small channel size and consequently the very low Reynolds number (< 10). As shown by Giddings [19], the presence of two structures at both ends of the separation channel, called splitters, is fundamental to prevent mixing and preserve the laminar flow.

Narayanan et al. presented examples of microfabricated SU-8 based electrical SPLITT systems tested with polystyrene nanoparticles [23] using a simplified "offset" SPLITT structure, which is a trade-off of performances and fabrication complexity. With our work, we demonstrate the fabrication of an electrically-driven multi-layered SPLITT device with multiple layers of laminated resist (Ordyl SY 355), including suspended microstructures (splitters) within the separation microchannel; in addition we tested the extraction performances with real biomolecules (Bovine Serum Albumin, BSA).

As already mentioned, the splitters play a key role in preventing the mixing of the streams coming from the two inlets. The function of these suspended structures is widely discussed in the pioneering works about SPLITT fractionation from the already cited research group of Giddings [19,26] and has been confirmed by Narayanan et al. In particular in Narayanan's work, some simulations concerning splitters structures are reported. Comparing the different computational results, the SPLITT cell equipped with splitters is considered as the "ideal design" for the split-flow thin fractionation. Narayanan states that the simplified "offset splitter" design is just an approximation of what ideally a SPLITT cell should be to prevent mixing phenomena, and he adopted the "approximated" design because of the difficulty of realizing the cell with the splitters with an SU8-PDMS based technology.

The use of the dry film technology to fabricate microfluidic devices with included electrodes has been discussed in the literature, showing very encouraging results [27–32]. However, to our knowledge, our work is the first implementation of real suspended splitter structure at the microscale including electrodes, which was achieved by a fine tuning of the lamination process of the different layers and appropriate design of the device geometry.

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