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Parallelizable Microfluidic Resistive On-Line Detector of Micrometric Aggregates of Biopharmaceutical Antibodies

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Abstract

A microfluidic device based on the differential measurement of the ionic resistance of a micropore for detection of aggregates of antibodies in biopharmaceutical downstream process is presented. The main novelty of this contribution regards the experimental demonstration that, despite the poor solidness of proteins, their aggregates, in their standard production buffer, can be electrically detected down to 2.4 µm diameter with sub-ms transit time (flow rate of 5 µl/min). Thanks to the simple PDMS fluidic fabrication, compact DC readout circuit and convenient use of the same metallic silver tubing for both electrical and fluidic interconnection, the device can be straightforwardly parallelized in tens of units, thus combining single micrometric sensitivity with larger flow rates (>100 µl/min), suitable for in-line installation in pharmaceutical plants.

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Keywords: pharmaceutical downstream processing, micrometric aggregate detection, resistive pulse sensing, differential microfluidics.

1. Introduction

The efforts oriented to bringing microfluidic technology from the laboratory to the industry can be classified into material development lines, including for instance new patternable materials more suitable for large-scale manufacturing [1], as well as actions aiming at the simplification and standardization of the interfaces between the chip and the rest of the world and of the instrumentation required for the lab-on-chip autonomous operation. Within the last category, we present here the design and experimental validation of a novel microfluidic device for label-free

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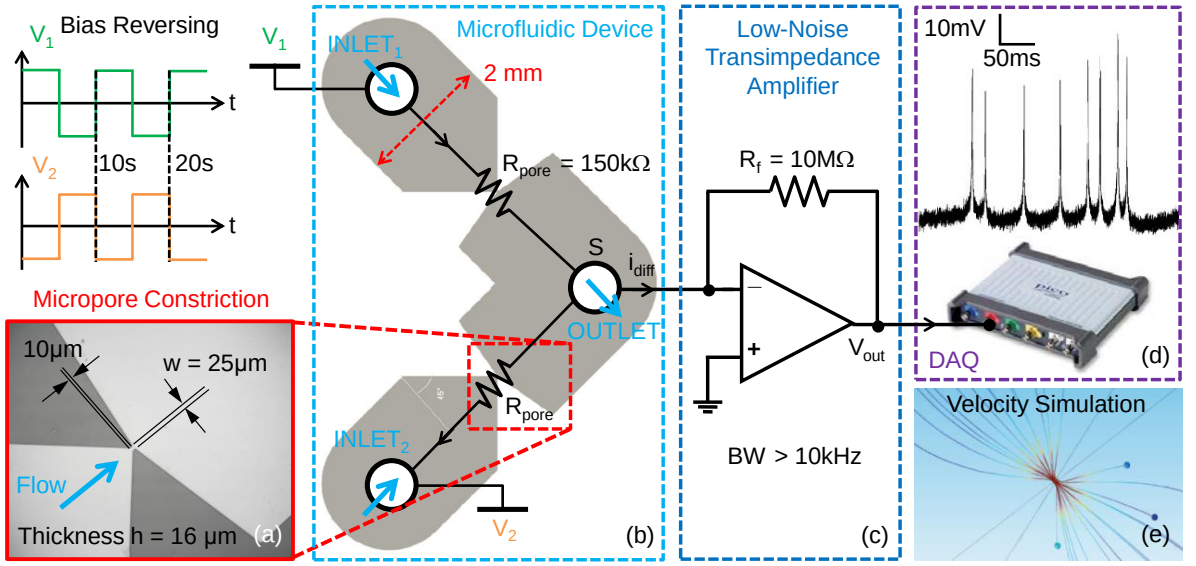

Fig. 1. Aggregate detector based on a micrometric constriction (a), arranged in differential configuration (b) where the differential current i_{diff} is amplified by a TIA (c) and acquired (d). Each particle produces a resistive pulse (ø = 5 µm, Q = 1 µl/min, V_1 = 0.3 V). For flow rates >1 µl/min, the transit time in the channel is sub-ms (e).

and real-time detection of quality of the production of biopharmaceutical products during the purification phase, the bottleneck [2] in alternative to standard chromatographic techniques [3]. In particular, the formation of aggregates of antibodies (with cluster diameter in the 1-10 µm range) during the fabrication of biopharmaceuticals represents a critical issue in terms of yield and must be carefully monitored [4]. The peculiarities of this system are: (i) direct installation in the pharmaceutical pipeline, (ii) unsupervised continuous operation [5], (iii) processing of large flow rates (100 µl/min), in contrast with the typical volumes handled in microfluidic systems. In order to address the last point, i.e. to combine the sensitivity to single micrometric clusters, granted by a micrometric cross section, with large total flow rate, several microfluidic devices are operated in parallel. Thus, the complexity in terms of readout electronics and interconnections should be minimized.

2. Device design

Starting from the assumption (eventually verified by means of the fabricated device) that the aggregates of antibodies behave like insulating bodies with respect to the surrounding ionic medium, we chose a Coulter-counter approach implemented in current mode. A voltage V_1 is applied across a micrometric constriction and the translocation of the particle occluding the pore produces a current drop proportional to the particle volume. We have optimized the size of a micropore (Fig. 1a, $10 \times 25 \times 16$ µm^3), with the support of FEM simulations, maximizing the SNR and balancing the speed/resolution trade-off in order to maximize also the flow rate Q. We opted for a differential configuration consisting of two micropores fed in parallel and biased by opposite potential $V_1 = -V_2$ (Fig. 1b), allowing: (i) double total flow rate, (ii) immunity the common mode drifts (for instance due to temperature or salinity gradients) and disturbance, (iii) lower dynamic range required by the transimpedance amplifier (TIA) and (iv) reduction of the impact of the voltage generator noise [6].

Another novelty of this work is the use of the same metallic pins (silver tubes) for both fluidic interconnection and measuring electrodes (Fig. 2). The differential bias is periodically reversed (every 5 seconds) in order to grant long-term operation of the chlorinated Ag electrodes, while the voltage bias values V_1 and V_2 can be finely adjusted to compensate the mismatch between the two device branches (R_{pore} ~150 kΩ ± 10%).

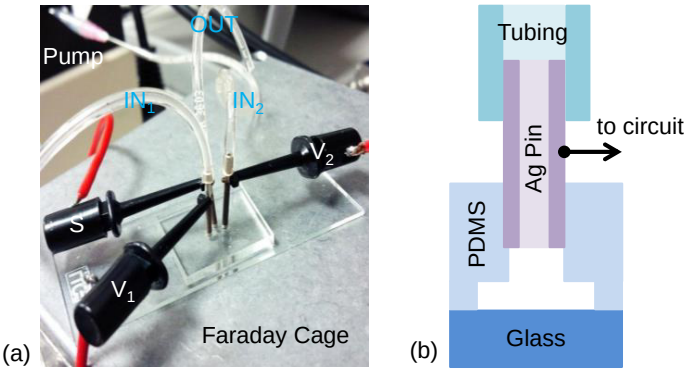

Fig. 2. (a) Photograph of the assembled device in the test setup (including a syringe pump and a Faraday cage), showing the use of Ag/AgCl pins serving (b) as both electrodes and fluidic interconnections between the PDMS device and rubber Tygoon tubings. The Ag pins are chlorinated in HCl with a current of 5 mA/cm^2 for 5 mins.

3. Experimental results

Prior to the use of antibodies cluster of unknown electrical properties, the device has been validated in PBS solution (σ = 1.5 S/m) with polystyrene beads of different calibrated diameters (8, 5 and 2 µm). As reported in Fig. 3, a good size discrimination capability down to a minimum detectable diameter of ~2 µm (at Q = 10 µl/min, with a SNR = 3 and counting rate up to 1 kbeads/s) is demonstrated. It is comparable with what achievable with sophisticated state-of-the-art resonant detectors [7].

Then, the pharmaceutical buffer alone has been characterized, showing a conductivity of 0.35 S/m (i.e. ~PBS/4) and no peaks (Fig. 4a), thus showing the absence of impurities. The observed periodical bipolar spikes correspond to the bias inversion transients and are not sampled by the acquisition system. Instead, when the buffer containing partially purified antibodies (provided by Lek Pharmaceuticals) is injected in the device, resistive pulses are clearly detected (Fig. 4b) down to a minimum estimated diameter of 2.4 µm at Q= 5 µl/min. The measured shape of a single peak (Fig. 4c) is asymmetric due to the asymmetry of the pore tapered access sides (Fig. 1a) and in good agreement with numerical simulations (Fig. 4d). The statistical analysis of ~1500 events shows a distribution of peak amplitudes (Fig. 4e) consistent with a typical of natural aggregation processes: there is a higher concentration of smaller aggregates. The largest detected diameter is ~7 µm, demonstrating the potential to cover the 2-10 µm range.

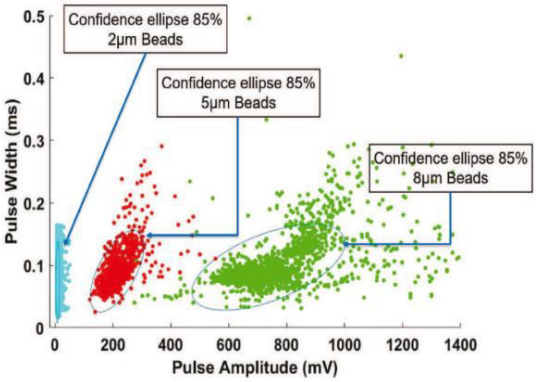

Fig. 3. Scatter plot of the validation tests with calibrated polystyrene beads of 8, 5 and 2 µm diameter (Q=10 µl/min, V_1=1 V) in PBS solution (σ_{PB} =1.5 S/m) showing a linear dependence of the pulse amplitude with the bead volume and straightforward sorting capability down to 2 µm (SNR=3).

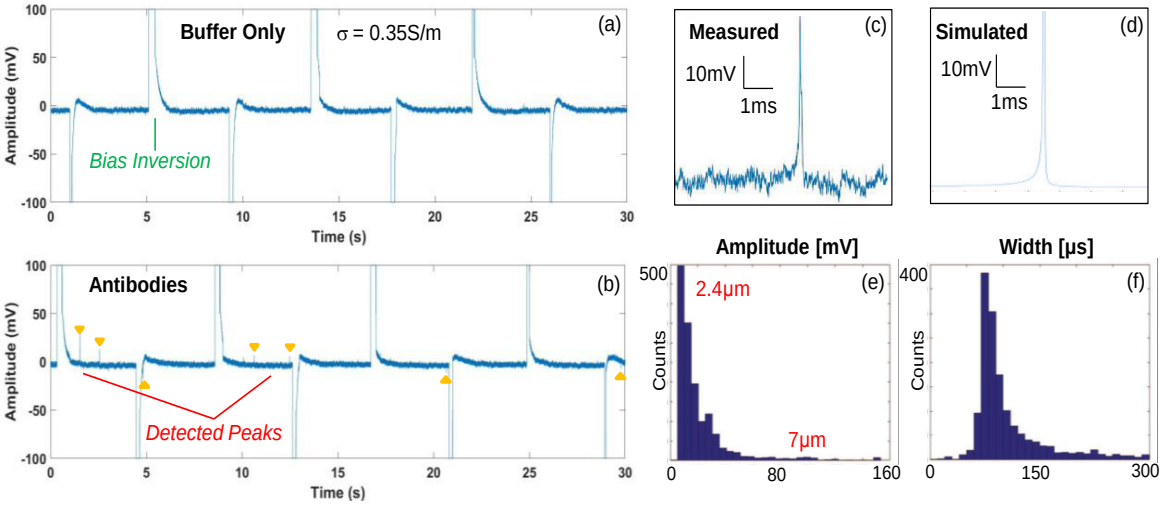

Fig. 4. Comparison between the absence (a) and the presence of aggregates of antibodies in their buffer (b), demonstrating single particles sensitivity (c), matching numerical simulations (d), down to a minimum detectable diameter of 2.4 µm in a statistics of 1500 peaks with consistent distributions of features (e-f) (Q=5 µl/min, V_1=1 V).

The distribution of pulse durations (Fig. 4f) is consistent with a parabolic velocity profile typical of the laminar flow inside the channel: the maximum velocity doubles the average (average pulse width is 100 µs), with the aggregates travelling close to walls experiencing a less probable and longer transit time. As expected, it was also verified that the amount of observed peaks was related to the temperature and to the thawing speed. At a temperature of 23°C, an average of 0.05 peaks/s are counted, while at 37°C and 60°C, the counts increase to 1.8 peaks/s and 16 peaks/s respectively.

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