Analytica Chimica Acta 988 (2017) 50–57

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Controlled, synchronized actuation of microdroplets by gravity in a superhydrophobic, 3D-printed device

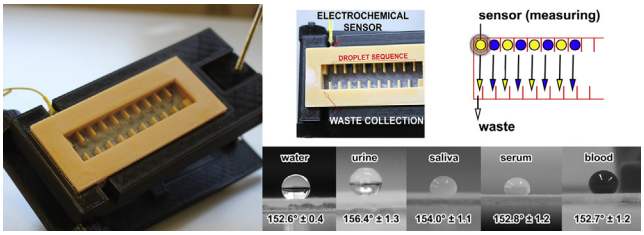
P.E. Oomen [a], J.P.S.H. Mulder [a], E. Verpoorte [a, *], R.D. Oleschuk [b, **]

[a] Pharmaceutical Analysis, Groningen Research Institute of Pharmacy, University of Groningen, Antonius Deusinglaan 1-XB20, 9713 AV Groningen, The Netherlands
[b] Department of Chemistry, Queen's University, 90 Bader Lane, Kingston, Ontario K7L 3N6, Canada

HIGHLIGHTS

- A 3D-printed device was coated with a superhydrophobic spray and placed in a holder.
- Controlled actuation of 10–50 μL droplets was possible by gravity alone.
- The superhydrophobic coating allowed actuation of various biological fluids.
- Using an integrated microelectrode, glucose in droplets of rat serum was measured.
- The device is readily customizable for other applications.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 24 April 2017
Received in revised form
21 July 2017
Accepted 4 August 2017
Available online 14 August 2017

Keywords:
Superhydrophobicity
Droplets
3D printing
Gravity actuation

ABSTRACT

Droplet manipulation over open surfaces allows one to perform assays with a large degree of control and high throughput, making them appealing for applications in drug screening or (bio)analysis. However, the design, manufacturing and operation of these systems comes with high technical requirements. In this study we employ a commercial, low-friction, superhydrophobic coating, Ultra-Ever Dry®, on a 3D-printed microfluidic device. The device features individual droplet compartments, which allow the manipulation of discrete droplets (10–50 μL) actuated by gravity alone. Simply by angling the device to normal in a 3D-printed holder and rocking in a "to and fro"-fashion, a sequence of droplets can be individually transferred to an electrochemical microelectrode detector and then to waste, while preserving the (chronological) order of samples. Multiple biological fluids (*i.e.* human saliva, urine and rat blood and serum) were successfully tested for compatibility with the device and actuation mechanism, demonstrating low slip angles and high contact angles. Biological matrix (protein) carryover was probed and effectively mitigated by incorporating aqueous rinse droplets as part of the analysis sequence. As a proof-of-concept, the enzyme-coupled, amperometric detection of glucose was carried out on individual rat serum droplets, enabling total analysis in ≈30 min, including calibration. The device is readily customizable, and the integration of droplet generation techniques and other sensor systems for different analytes of interest or applications can be realized in a plug and play fashion.

* Corresponding author.
** Corresponding author.
E-mail addresses: p.e.oomen@rug.nl (P.E. Oomen), j.p.s.h.mulder@rug.nl (J.P.S.H. Mulder), e.m.j.verpoorte@rug.nl (E. Verpoorte), richard.oleschuk@chem.queensu.ca (R.D. Oleschuk).

http://dx.doi.org/10.1016/j.aca.2017.08.010
0003-2670/

1. Introduction

Precise control over (sub-)microliter-size volumes of fluids is one of the principal benefits of microfluidics. The predictability of fluid movement in complex geometries, created through micro-engineering, allows for a large degree of on-chip experimental control and versatility. Device functionality can be further increased by incorporating (microfabricated) sensors. Droplet-based platforms differ from continuous flow-based microfluidics in that the small volumes manipulated are in fact discrete droplets [1–3]. This presents a number of advantages. For example, generating higher throughput in a system is relatively facile through parallelization. Without the need to significantly increase size or complexity, multiple analyses can be run on a single device [1,2]. Droplets containing analytes or reagents can be actuated and mixed with each other with remarkable precision, either over surfaces [2,4] or through channels in multiphase systems [1,3] (based on the immiscibility of liquids or gases). Furthermore, the high surface-area-to-volume ratios of the droplets enable fast reaction times, *e.g.* for mass transfer or diffusion processes [4,5]. As a result, droplet microfluidics has been utilized for high-throughput applications in biochemical assays [6–8], drug development [9,10] and in point-of-care diagnostic tools [11]. The simplicity and robustness of droplet microfluidic devices combined with biosensors [12] has also been exploited to develop a droplet-based diagnostic tool for malaria [13,14]. We focus on systems where droplets are actuated over hydrophobic surfaces. In these systems parallelization for high-throughput, reproducible analysis and manipulation of samples and reagents can be performed with the greatest flexibility, since the droplets are separate, discrete containers [2,4]. This is especially interesting for the manipulation, maintenance and sequential actuation of droplet series (*e.g.* samples from continuous-flow based external devices).

Electrowetting on dielectric (EWOD) is a common technique for droplet actuation over open surfaces. Here, the macroscopic contact angle (CA) of a droplet on a hydrophobic surface is changed when a potential difference is applied between the droplet and a dielectric-coated electrode. This facilitates precise droplet manipulation when an electrode array is utilized [4,15,16]. However, the use of EWOD devices in diagnostic tools is limited, as contamination of the hydrophobic surface due to protein adsorption is regularly reported, necessitating employment and optimization of surfactants [17]. Another disadvantage is the high level of technical requirements for laboratories that aim to produce and operate EWOD-based devices. A different approach to droplet actuation in microfluidic devices employs magnetic fields. In this case, droplets consisting of a liquid with high magnetic susceptibility are actuated by applying a magnetic force in a desired direction. Alternatively, paramagnetic microparticles added to the droplets can be used to move these droplets with applied magnetic force. In this approach, known as magnetofluidics, external, permanent magnets [18,19] or built-in electromagnets are used [20,21]. Magnetofluidics, too, is most often performed on hydrophobic surfaces (CA $\geq$ 120°).

Superhydrophobic (SH) surfaces, inspired by dust- and water-repellant wax coatings seen in nature (*e.g. Lotus* leaves [22]), give rise to CAs greater than 150° for water droplets. As a consequence, an oil phase layer, which is often used to surround droplets on hydrophobic surfaces to further decrease friction and prevent evaporation of the water phase, is no longer necessary. Like their naturally occurring counterparts, synthetic SH surfaces exhibit high CAs due to micro- and nanometre scale features. This phenomenon has been thoroughly described in the Cassie-Baxter model [19,23]. The advantages of SH surfaces have also been recognized in the context of analytical devices. One example is the enhanced analysis of biological fluids using paper spray-based mass spectrometry, facilitated by the use of SH paper substrates [24]. Patterned SH surfaces have also been applied for sample-confinement on MALDI-TOF mass spectrometry target plates [25] and as substrate for protein micro-arrays [26]. Interestingly, magnetofluidics on a commercially available SH coating has also been demonstrated [19,27,28]. In the work of Mats *et al.* [19], amorphous polytetrafluoroethylene (PTFE, or Teflon® AF, a hydrophobic coating commonly used in DMF), *Colocasia* leaves (a natural SH surface) and Ultra-Ever Dry® (UED, a SH formulation of fluorinated silica nanoparticles) were compared with respect to their suitability for actuation of droplets using superparamagnetic particles. Both the natural and commercially available SH surface enabled magnetic droplet actuation at relatively low magnetic particle concentrations, and forces as low as 900 nN were required to actuate a 10-μL droplet. The PTFE surface exhibited higher friction, however, which prevented easy droplet actuation at the particle concentrations studied.

In fact, the dramatic decrease in surface-droplet adhesion when using SH substrates makes actuation of droplets by mere gravity possible. Gravity actuation has the potential to significantly decrease the complexity, improve the robustness and thus enhance the potential of (disposable) point-of-care diagnostic devices, especially in low-resource settings. This study presents a simple, yet elegant, compartmentalized, 3D-printed device to sequentially deliver droplets by gravity-actuation to an electrochemical detector. The device is aerosol-coated with UED to minimize droplet adhesion. It is placed in a holder that positions it at an angle relative to normal, to establish a potential gravitational energy. The device is then rocked in a "to-and-fro" fashion, to transfer droplets between offset compartments along opposite sides of the device. With each rocking motion, each droplet is brought one compartment closer to the detector compartment at the end of the device. Discrete transport of a sequence of droplets towards a detector housed within one of the compartments of the device can now be accomplished. Moreover, during the actuation of the sequence, the (chronological) order of the droplets in which droplets were sampled or added is preserved. As a proof-of-concept, an electrochemical sensor is integrated and used for glucose measurement in droplets of rat serum with free enzyme. The 3D-printed device we present is readily customizable and would be compatible with a variety of detection methodologies.

2. Materials & methods

2.1. Superhydrophobic surface coating

UED was purchased from Jeroen van Beurden Special Products (Vlijmen, The Netherlands) as a two-component coating set, an adhesive base coat and a top coat containing fluorinated silica nanoparticles. Standard glass microscope slides (Thermo Fisher Scientific, Breda, The Netherlands) were aerosol-coated with the base coat and left to cure for approximately 3 h at room temperature. The glass was then coated with the SH top layer, which was left to cure overnight, also at room temperature. Contact angle measurements and assessment of protein sorption on the surface were performed on surfaces treated in this way (see Section 1 of the Supplementary Information (SI) for detailed methods).

2.2. 3D-printed device and holder

To allow droplets to be actuated by gravity, a compartmentalized device was designed to envelop the glass substrate. Design of the device was done in SolidWorks (Waltham, MA, USA) – see Fig. S1 in SI. The device was printed in polylactic acid (PLA) (EasyFil filament, d = 1.75 mm, Formfutura, Amsterdam, The Netherlands)