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PAPER

A 'microfluidic pinball' for on-chip generation of Layer-by-Layer polyelectrolyte microcapsules†

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Inspired by the game of "pinball" where rolling metal balls are guided by obstacles, here we describe a novel microfluidic technique which utilizes micropillars in a flow channel to continuously generate, encapsulate and guide Layer-by-Layer (LbL) polyelectrolyte microcapsules. Droplet-based microfluidic techniques were exploited to generate oil droplets which were smoothly guided along a row of micropillars to repeatedly travel through three parallel laminar streams consisting of two polymers and a washing solution. Devices were prototyped in PDMS and generated highly monodisperse and stable $45 \pm 2 \mu\text{m}$ sized polyelectrolyte microcapsules. A total of six layers of hydrogen bonded polyelectrolytes (3 bi-layers) were adsorbed on each droplet within <3 minutes and a fluorescent intensity measurement confirmed polymer film deposition. AFM analysis revealed the thickness of each polymer layer to be approx. 2.8 nm. Our design approach not only provides a faster and more efficient alternative to conventional LbL deposition techniques, but also achieves the highest number of polyelectrolyte multilayers (PEMs) reported thus far using microfluidics. Additionally, with our design, a larger number of PEMs can be deposited without adding any extra operational or interfacial complexities (e.g. syringe pumps) which are a necessity in most other designs. Based on the aforementioned advantages of our device, it may be developed into a great tool for drug encapsulation, or to create capsules for biosensing where deposition of thin nanofilms with controlled interfacial properties is highly required.

Introduction

In the classical Layer-by-Layer (LbL) technique, oppositely charged polyelectrolytes are alternatively deposited onto a bulk or colloidal template to form polyelectrolyte multilayers (PEMs). Adsorption of these polymer electrolytes (hence the term 'polyelectrolytes') is mainly a result of electrostatic interactions occurring between polycationic and polyanionic electrolytes on a charged colloidal template.¹ Alternatively, LbL

encapsulation can also be achieved by forming interpolymer complexes which rely on donation–acceptance of protons such as hydrogen bonding LbL.² The encapsulation of various templates was reported, such as enzymes or organic crystals, vitamins, cells or hydrogel beads.^{3–7} Nanometre sized films of polyelectrolytes can be obtained after dissolving the colloidal core⁸ or core LbL shell constructs and can be used for various applications in the biomedical industry^{9,10} or the food processing industry.^{11–14}

Current methods for the automation of the LbL encapsulation process utilize conventional macro-scale reactors which are time consuming non-continuous processes requiring bulky and expensive equipment.^{15,16} These reactors not only extend the duration of the LbL process, but also impart problems such as non-uniformity and aggregation of microcapsules requiring further centrifugation, washing and re-suspension steps. Consumption of reagents is also higher in such batch processes which can be an expensive drug in some cases.^{9,10} For such conventional cases, an alternative method of membrane filtration was suggested by Voigt *et al.*¹⁶ to sequentially add polyelectrolytes and a washing solution to the colloidal particles while subjecting the container to continuous mechanical stirring. Although the authors reported a reduced severity of the aggregation problem, the method was still a batch process and

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† Electronic supplementary information (ESI) available: Flow chart for device fabrication (Fig. S1), SEM and optical images of the device (Fig. S2–S3), droplet size distribution (Fig. S4) and movies showing droplet guiding in the device (Video 1 and 2). See DOI: 10.1039/c0lc00381f

required changing the membrane filter depending on the size of the colloidal particles. Attempts to form polyelectrolyte microcapsules with an oil core were made by Sivakumar *et al.*¹⁷ who achieved oil encapsulation and PEM release chemically by forming polyelectrolyte shells on silica particles, infiltrating oil through semipermeable walls of the PEM capsule and eventually disassembling the polyelectrolyte layers with exposure to the solutions with pH 7.5. Grigoriev *et al.*¹⁸ demonstrated the direct encapsulation of dodecane emulsion but the microcapsules formed were poorly monodisperse and showed flocculation. Moreover, both techniques still suffered from tedious and lengthy preparation procedures and depended upon hazardous chemicals in some cases (*e.g.* chloroform¹⁸).

Microfluidics has witnessed a tremendous growth over the past decade due to its several advantages.^{19–26} Droplet-based microfluidics (a comparatively recent branch of microfluidics) involves the generation and/or manipulation of discrete liquid droplets inside microchannels.²⁷ Droplet microfluidics can play an important role in miniaturizing LbL technique by imparting benefits of time and reagent reduction,²⁸ high monodispersity of capsules²⁹ and automating the entire LbL encapsulation procedure into one continuous process.³⁰

Previously, our group utilized microfluidics to report the deposition of four alternative layers of polyelectrolytes (PSS/PAH–FITC) on mineral oil droplets,³¹ where the droplets were generated by a flow focusing geometry and travelled through various bifurcation regions for actual deposition. Non-adsorbed polyelectrolytes were removed by exploiting the Zweifach–Fung effect³² while the colloidal droplets remained in the main channel. More recently, this technique was refined by guiding the generated droplets through different streams of polyelectrolytes and bumping them on obstacle pillars.³³ Priest *et al.*³⁴ reported the deposition of three layers of polymers (PMA/PVPON) on liquid crystal droplets using similar microfluidic techniques.^{31,33} However, for all the above-mentioned literature, the system size and interface complexities are proportional to the number of polyelectrolytes being deposited, as each layer of polyelectrolyte requires its own microfluidic circuitry components (*e.g.* pumps and channels), thereby enlarging the overall system.

In this work, we present a novel LbL deposition method termed as “microfluidic pinball” which utilizes micropillars to guide discrete droplets. Similar to the game of pinball in which a metal ball rolling down a slope lands on ramps, rollovers and other guiding structures, the droplets generated in our microdevice are also guided and diverted to the downstream direction smoothly by repeated unit rows of fabricated micropillars. By miniaturizing this “pinball” concept, we achieved six layers of polyelectrolyte deposition on oil droplets in less than 3 minutes by guiding discrete droplets through parallel laminar streams of two polyelectrolytes/polymers and a washing solution. The polyelectrolyte coated oil microcapsules were collected outside the microdevice and analyzed by fluorescence and atomic force microscopy. A linear increase in fluorescent intensity with every layer of deposited fluorescent labelled polyelectrolyte indicated the successful deposition of PEMs and AFM images confirmed a thickness of ~ 2.8 nm deposited per polyelectrolyte.

Experimental

Mould fabrication

The mould pattern was drawn using Cadence® Virtuoso® software and printed on a plastic photomask for photolithography. Standard one step photolithography was used to fabricate the mould for PDMS soft lithography by using SU-8 2100/2035 (negative photoresist). SU-8 was spin-coated on top of a silicon wafer, baked and patterned using UV photolithography.³⁵ The final mould was hard baked for 15 minutes at 200 °C to impart strength and was subjected to chemical vapour deposition (CVD) of fluoroctyltriethoxysilane (FOTES), a fluorosilane to form a self-assembled monolayer. This coating reduces the surface energy of the SU-8 mould, making peeling of PDMS easier during the soft lithography process.³⁶ The entire process is summarized in ESI, Fig. S1†.

Soft lithography

Polydimethylsiloxane (PDMS) was prepared by mixing the base and curing agent in a 10 : 1 ratio. The mixture was mixed thoroughly and degassed for 30 min to remove any remaining air bubbles. The final mixture was poured on the SU-8 mould and cured inside an oven at 80 °C for 2 hours. The mould was peeled off carefully to avoid damaging the micropillars. The micropillars in PDMS were observed with Scanning Electron Microscopy (SEM) (ESI, Fig. S2†). Through holes having 1 mm diameter were punched to serve as inlet and outlet ports. This patterned piece of PDMS was bonded to a flat piece of PDMS by treating them in oxygen plasma for 60 seconds at 70 W.

Preparation of reagents

Colour dye solutions were prepared in distilled water by mixing them in 1% Tween 20 (v/v) surfactant. Polyacrylic acid (PAA) ((C₃H₄O₂)_{*n*}, 250 kDa) was used as a negative polyelectrolyte and polyvinylpyrrolidone (PVPON) ((C₆H₉NO)_{*n*}, 40 kDa) was used as a neutral polymer. PAA was labelled with Rhodamine 123 by a conjugation protocol as described.³⁷ Mineral oil was chosen as the colloidal phase to form the template drops. All polymer solutions were prepared in a sodium acetate buffer (20 mM, pH 4) and 0.05% Tween 20. An actual picture of the device can be seen in ESI, Fig. S3†.

Experimental setup

The microfluidic device was mounted under an inverted microscope (Olympus IX71). Liquid reagents were loaded into plastic syringes of various sizes (3, 5, 10 ml) from Becton Dickinson and delivered through syringe pumps from KD Scientific, Inc. The syringes were connected to the microdevice with suitable plastic tubings. A total of five syringes were used. Additional polyelectrolyte layers can be added in our design without increasing the syringe count. Images were acquired using a Rolera-XR camera from QImaging Corp. and saved using Image Pro Express software. Fluorescence images were captured with an Olympus BX61 microscope.

Sample preparation for AFM measurements

PEM microcapsules obtained from the device were pipetted onto a flat mica sheet (10 mm diameter) and dried at room temperature for 30 min. Once dried, the microcapsules were immersed in *tert*-butyl methyl ether for 24 hours to dissolve the oil core. Afterwards, the samples were subjected to AFM measurements in the tapping mode to determine the surface topology and thickness of PEM films.

Results and discussion

Fig. 1a represents a schematic overview of the device. It consists of two main parts: (i) T-junction for the generation of oil droplets and (ii) main channel consisting of a single row of micropillars arranged in a zigzag configuration to guide the movement of oil droplets as shown in Fig. 1b and c. The oil droplets were generated by shearing a mineral oil stream ($9 \mu\text{l h}^{-1}$) with a continuous aqueous stream ($150 \mu\text{l h}^{-1}$). The droplets travelled through a small side channel to enter the main channel. The main channel consisted of 3 inlet and 3 outlet ports for injecting three different solutions (PVPON polymer solutions, washing solution and PAA polymer solution). Micropillars ($150 \mu\text{m}$ height by $40 \mu\text{m}$ diameter) in the main channel were fabricated with soft lithography techniques and guided the droplets into the different streams. The micropillar row angle was empirically determined to be 30° with the channel wall for achieving smooth droplet guiding.

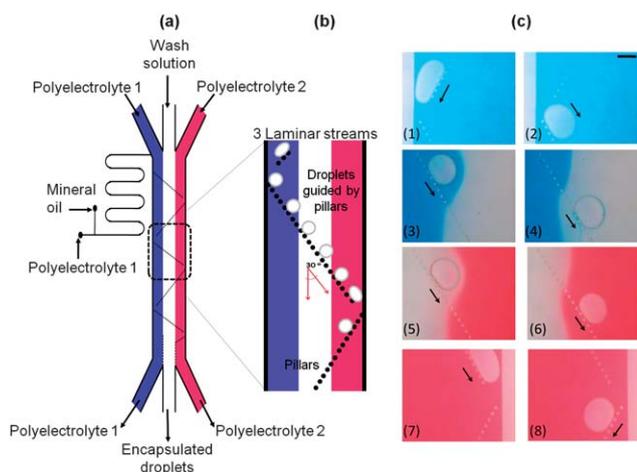


Fig. 1 Overview of the device for continuous generation of polyelectrolyte microcapsules by Layer-by-Layer (LbL) deposition of polyelectrolytes: (a) schematic view (not to scale) shown with the required inputs and outputs for the deposition of six layers of polyelectrolytes and (b) expanded view of a single unit of pillars in zigzag arrangement. The pillars have a diameter of $40 \mu\text{m}$ separated by a distance of $40 \mu\text{m}$ and oriented at an angle of 30° with the channel wall. Circles mark the droplet position whereby the subsequent time-series images were taken. (c) Colour dye system to visualise the 3 liquid streams—micrographs of droplet getting incubated in the first polyelectrolyte (PE) and changing the PDMS ladder within the same PE [(1) and (2)]; droplet entering the washing solution which removes the non-adsorbed polyelectrolyte [(3) and (4)]; droplet entering into the second PE for deposition after the wash solution [(5) and (6)]; deposition of second PE on droplet surface and droplet changing the PDMS ladder within the second PE [(7) and (8)]. Scale bar = $200 \mu\text{m}$.

For proof-of-concept demonstration, colour dye experiments were initially performed to visualise the liquid streams. Blue and red dyes were used to represent two different polymer solutions, whereas plain water was used to represent the washing solution. As captured by a series of frames in time (Fig. 1c), an oil droplet flowed through the first stream of blue solution, guided with the help of micropillars and received its first coating (Frame 1). As the droplet reached the end of the row, it changed direction by changing onto the new downstream ladder smoothly (Frame 2) and stayed in the same solution stream until it finished the remaining half of its travel. To make the next layer deposition possible, it is important to remove the excess non-adsorbed solution which also helps in reducing coagulation of droplets. In this case, as the droplet crossed the boundary of the first solution (Frame 3), it was made to pass through a washing solution (Frame 4). The droplet entered the second red dye laminar stream with very little perturbation which is essential to minimize mixing (Frame 5). Upon entering the second solution, the droplet repeated its initial movements and received a second coating (Frames 6–8).

Afterwards, the droplet was once again guided into the washing solution to remove the excess non-absorbed second layer coating. This entire cycle (unit cycle) only allows the deposition of two layers (single bi-layer) on the droplet surface and makes for one subunit of the whole device. The smooth gliding movement of the droplet along the micropillars through the coloured dye laminar streams can be observed in the ESI (Video S1†) provided with this paper. Multiple bi-layers can be deposited in a similar manner by extending the length of the channel without introducing any extra interfacial complexities.

The design approach using micropillars has the following advantages: (a) at the T-junction, if any small satellite droplets are generated, they pass through the gaps between the pillars ($40 \mu\text{m}$) and get screened out and are collected through the leftmost outlet port of the first polyelectrolyte. Thus, our design acts as an automatic filter to sort out satellite droplets from the targeted size droplets which improves monodispersity. (b) The zigzag arrangement of the micropillars allows twice the incubation time for polyelectrolyte deposition as that of the washing step, which provides sufficient residence time for polyelectrolyte deposition and (c) since, the number of zigzag turns of the arrangement of micropillars decide the number of layers that get deposited on a droplet, input interface components remain constant irrespective of the number of polyelectrolyte layers to be encapsulated.

It is worth mentioning the importance of the washing step here, as it is an important technique in conventional LbL processing and consists of numerous sub-steps such as centrifugation, removal of unadsorbed polyelectrolyte supernatant and resuspension of particles. Droplet microfluidics offers unique advantages in manipulating individual droplets and constricting all sub-steps into a single step. The sheering washing buffer removes the non-adsorbed excessive polyelectrolytes from the surface of the droplet as explained earlier. It also keeps the polyelectrolytes separated.

For the microdevice, it was crucial to achieve suitable control on the number of droplets being generated and released into the main channel as uncontrollable generation of droplets led to droplet overcrowding, laminar stream blocking and change of

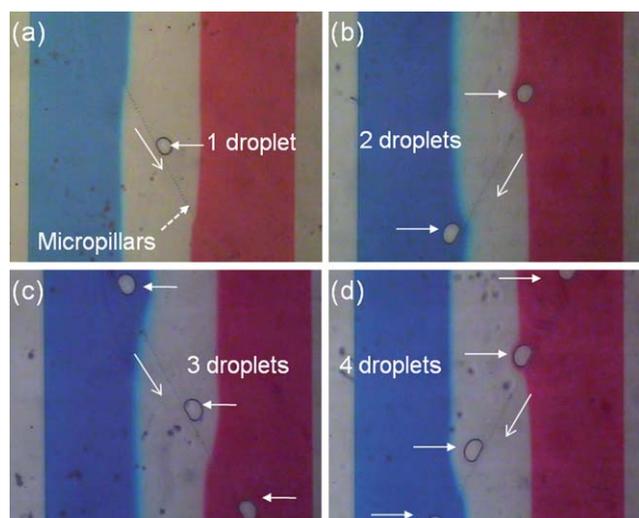


Fig. 2 Four frames during colour dye experiments representing solutions of PAA (blue stream), washing solution (colorless) and PVPON (red stream). Droplet size and frequency were controlled by varying flow rates. Control on the number of droplets is crucial to maintain stability of the laminar streams. Droplets travelled smoothly through stable parallel laminar streams to represent coating of PAA and PVPON with an intermediate washing step.

flow profiles within the channel. In some case, the latter even resulted in convective mixing of the streams, adversely affecting the deposition process (data not shown). To achieve suitable control, a second set of experiments were performed in the colour dye system where flow rates for droplet generation were optimized. As can be seen in Fig. 2, we achieved a high degree of controllability on the number of droplets per row of pillars. More information can be found in the ESI (Video S2†).

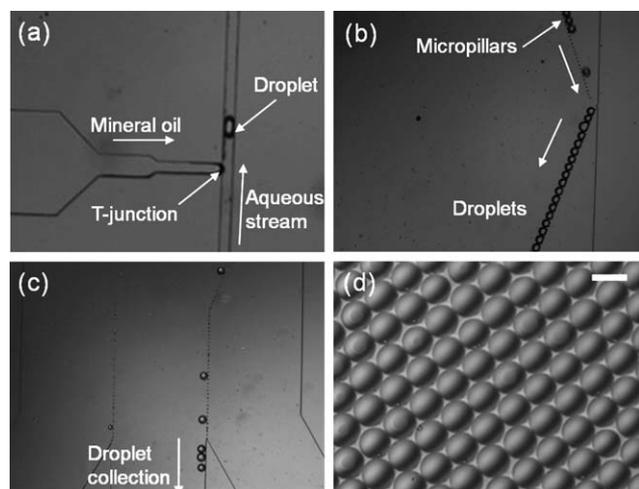


Fig. 3 a) Generation of an oil droplet in PAA stream. The droplet generation frequency and size can be controlled by changing flow parameters. (b) Movement of droplets guided with micropillars (80 μm height, 20 μm diameter). (c) Droplets undergo a final rinse solution and are collected through the middle stream. (d) The generated droplets ($45 \pm 2 \mu\text{m}$) have a high rate of monodispersity which is important to obtain stable droplets. Scale bar = 50 μm .

Fig. 3a presents the actual generation of an oil droplet at the T-junction for polyelectrolyte deposition. Once generated, the droplets were introduced in the PAA stream of the main channel where they continuously adsorbed the negatively charged PAA until they were guided into the sodium acetate washing buffer. The polyelectrolyte PAA was conjugated with Rhodamine (fluorescent dye) to optically confirm polyelectrolyte deposition on the droplet surface. At the end of the washing step, droplets entered the adjacent laminar stream of PVPON which got deposited on the PAA coated oil droplets because of a hydrogen bond formation between carboxyl group of PAA and the lactam group of PVPON³⁸ (Fig. 3b). The entire process was repeated three times to get six layers (3 bi-layers) of LbL coating. The coated droplets were collected through the middle stream and subsequently analyzed (Fig. 3c). Highly monodispersed droplets of size $45 \pm 2 \mu\text{m}$ were achieved as indicated by the tightly packed “array” in Fig. 3d (ESI, Fig. S4†).

The collected droplets exhibited uniform fluorescence indicating successful PAA deposition (Fig. 4b). To confirm subsequent PEM adsorption, separate devices were fabricated to generate microcapsules having 2, 4 or 6 PEMs. Experimental conditions were kept consistent in all the devices. A linear increase in fluorescence intensity with increasing layer number confirmed the successful adsorption of PEMs (Fig. 4c).

To determine PEM thickness, collected microcapsules were analysed by atomic force microscopy (AFM) as shown in Fig. 5. Oil capsules were placed on mica sheets and the mineral oil core was dissolved in *tert*-butyl methyl ether solvent. The microcapsules fell flat on the mica surface as the oil core dissolved in the solvent and was removed, predominantly due to diffusion.³⁹ The microcapsule thicknesses were measured using AFM in tapping mode and were found to be approximately $11.2 \pm 2.1 \text{ nm}$, $20.8 \pm 3.9 \text{ nm}$ and $31.4 \pm 4.5 \text{ nm}$ for 2, 4 and 6 layers respectively. For

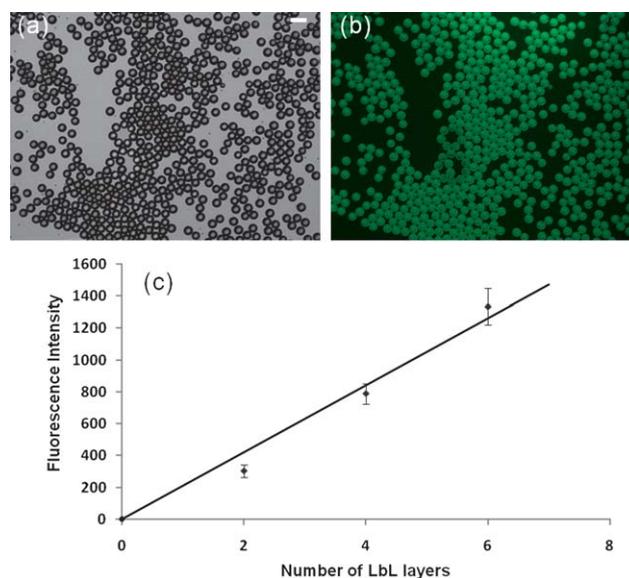


Fig. 4 PEM-coated oil droplets: (a) bright field image and (b) fluorescence image. PAA was conjugated with Rhodamine and the fluorescence serves as an indicator of polyelectrolyte adsorption. The droplets were collected through the middle stream and analyzed under a microscope. (c) Fluorescent intensity increased linearly as the number of PEMs increased. Scale bar = 100 μm .

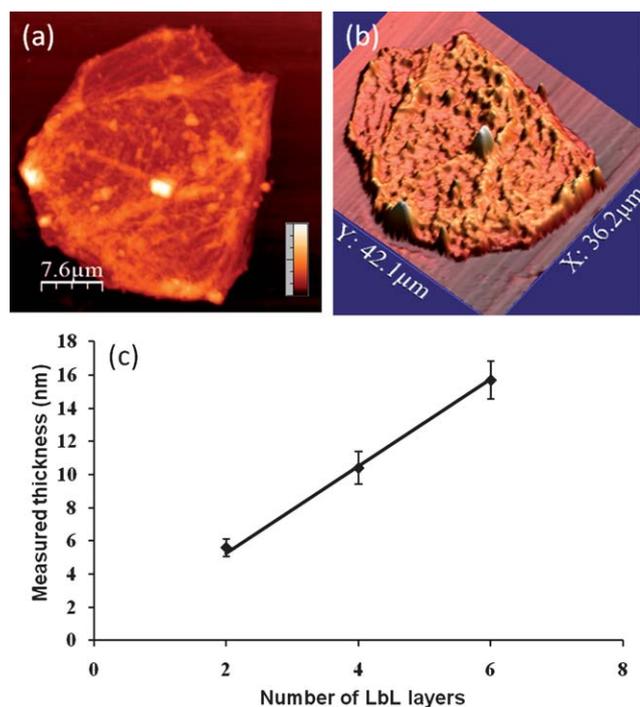


Fig. 5 a) AFM image of a collapsed microcapsule after oil core removal. Three bi-layers of PAA/PVPON (Z-scale bar = 1000 nm). (b) 3D view of the same collapsed microcapsule. (c) Measured thickness of PEMs after the removal of the oil core for 2, 4 and 6 layer capsules.

the collapsed microcapsule, these results represent double the true bi-layer thickness. The individual bi-layer thickness can be calculated as 5.6 ± 1.1 nm, 10.4 ± 2.0 nm, 15.7 ± 2.3 nm and the thickness of a single polymer layer can be calculated to be ~ 2.8 nm. This thickness is consistent with the average thickness obtained for single layer of conventional Layer-by-Layer polyelectrolyte capsule, which is reported to be 2 to 3 nm.¹⁷ The linear increase of the microcapsule thickness shows the buildup of polymers on the droplet surface with each subsequent coating. The AFM 3D view of the flat capsule (Fig. 5b) shows the membrane of the capsule after treatment with organic solvent. The rough domains and folds on the membrane surface have been previously recognised in conventional LbL approaches^{40–42} and are attributed to the reorganization of polymer complexes within Layer-by-Layer assembly due to the organic solvent treatment.

In this work we demonstrate LbL deposition based on hydrogen bonding between the polymer pair PAA and PVPON. However, we see no limitation in extending our approach towards other LbL methods including those based on electrostatic interaction, *e.g.* the widely studied PSS/PAH system. The separation of two oppositely charged polyelectrolyte streams with a central washing buffer steam, in our approach, avoids mixing. Therefore eliminating the formation of an interfacial complex as reported previously.⁴³

Conclusion

We are reporting a novel microfluidic approach termed ‘microfluidic pinball’ to generate LbL polyelectrolyte microcapsules.

Our approach utilizes rows of micropillars arranged in a zigzag fashion to guide oil droplets through laminar streams of polymer and washing solutions. We successfully demonstrated the deposition of 3 bi-layers of polyelectrolytes which was achieved in less than 3 minutes with a thickness of ~ 2.8 nm per layer. Higher number of PEMs can be achieved with our approach without adding any extra interfacial complexities which is a limitation in other devices. We believe that our technique paves the way for a fast, continuous and automated microcapsule production process which is highly required in drug encapsulation and drug delivery fields.

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