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Continuous dielectrophoretic sorting of liquid beads

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ABSTRACT ARTICLE INFO Keywords: We report the concept of continuous sorting of liquid beads using dielectrophoresis (DEP) in a microfluidic Liquid beads device. Liquid beads are liquid droplets encapsulated in a hard polymer shell, which provides a unique core-shell DEP structure crucial for applications such as digital polymerase chain reaction (dPCR), digital loop-mediated Drug delivery isothermal amplification (LAMP), drug delivery, cell cultures and microreactors. A population of consistent Microfluidics microscale liquid beads are required for these applications. We utilised microfluidic methods to continuously Micro elastofluidics produce beads with a narrow size distribution. However, this technique requires a precise control of flow rates to prevent the formation of core-less beads. We generated monodispersed liquid beads with trimethylolpropane trimethacrylate (TMPTM) shell and hydrofluoroether (HEF) core. The device produced about 20-30 % core-less solid beads, which needs to be removed from the population of liquid beads. We first examined the dielectrophoretic (DEP) responses for liquid beads and solid beads. Using a relatively simple setup with a steel needle and indium tin oxide (ITO)-coated glass electrodes, we demonstrated that beads exhibit both positive and negative DEP under alternate-current (AC) and direct-current (DC) electric fields. Subsequently, we designed and tested a microfluidic sorting device to leverage the differential DEP responses for continuous sorting of liquid beads and solid beads. In a DC field, solid beads experience positive DEP, whereas liquid beads show negative DEP, enabling their separation with an efficiency of approximately 80 %. Our sorting method provides a precise, controllable, label-free, and scalable solution for obtaining consistent liquid beads for further applications.

1. Introduction

Micro elastofluidics is an emerging field that focuses on the manipulation and control of fluid in interaction with flexible, elastic materials in the microscale [1]. Utilising the principles of fluid dynamics and flexible materials, microelastofluidics enables the development of a variety of fluid handling systems. Accordingly, the field is divided into continuous-flow micro elastofluidics and digital micro elastofluidics. In continuous-flow micro elastofluidics, fluids are transported and manipulated in a continuous manner. In contrast, digital micro elastofluidics focuses on manipulating small, discrete encapsulated liquid volumes, including droplets [2], liquid marbles [3], hydrogel beads [4], and liquid beads [5]. Among the digital platforms, liquid beads promise a range of potential applications due to their robust structural integrity and ease of manipulation.

Liquid beads are core-shell particles with a solid shell and a liquid core. In contrast to liquid beads, liquid marbles have a soft powdered coating as shells [6]. The shell material of liquid beads can be organic

polymers or organic-inorganic composites [7]. Liquid beads, with their unique core-shell structure, have a range of applications that leverage their combined properties across various applications in industry and research, including drug delivery [8,9], cell cultures [10,11], microreactors [12,13], cosmetics [14,15], and food additives [16,17]. These applications require a large collection of uniformly sized beads with consistent properties. In a previous work, our group demonstrated manual techniques for fabricating millimetre-sized liquid beads. Sreejith et al. introduced a method where core material was injected into a liquid marble, resulting in the formation of the core-shell structure [18]. Aditya et al. created liquid beads by injecting liquid core into a curable, levitated polymer droplet [19]. These methods provided a straightforward approach, featuring a simple setup and fabrication process. However, they were limited to producing large millimetre-scale beads with significant size variation. Additionally, the manual nature of these methods limited its scalability due to the lack of options for automation.

Microfluidic techniques have emerged as a versatile and modern approach for generating uniformly sized double emulsion droplets [20,

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21]. The shells of these droplets are cured to form liquid beads. Microfluidic methods produce micrometre-sized droplets with a narrow size distribution at consistent intervals, offering significant advantages over other methods such as bulk emulsification [22] and electro spraying [23]. Microfluidic systems are compact and efficient. However, their fabrication requires specialised equipment and expertise. For the generation of droplets, the channel walls require modification to adjust their wettability. In the case of single emulsion, either hydrophilic or hydrophobic treatment over the entire channel length is sufficient for forming oil droplets in water or water droplets in oil, respectively. However, for water in oil or oil in water double emulsions, dual surface treatment is necessary. One section of the channel surface is made hydrophobic or hydrophilic whereas the remaining section is treated for the opposite wettability. Degradation of such dual coatings poses a major challenge to the long-term consistency of droplet formation process and often requires fine tuning of the flow rates. Despite careful surface treatment and corrective adjustment of flow rates, these devices cannot consistently produce double emulsion and subsequently core-shell liquid beads. Additionally, some double emulsion droplets lose their core during the collection or curing stages, resulting in the formation of core-less solid beads [24]. In a previous work, we reported that up to 50 % of the produced beads could be core-less solid beads [25]. The undesired solid beads pose a major challenge to applications such as drug delivery, which require consistent and identical liquid beads. As a result, sorting core-shell liquid beads from core-less solid ones becomes a necessary step in the work-flow of technologies based on liquid beads.

We found that from the same formation device, liquid beads are 5 % heavier than their solid counterparts [26]. This mass difference could be utilised in flow-based methods to separate liquid beads from solid beads [27]. These methods are classified as either passive or active according to the source of manipulating forces. Passive methods such as pinched flow fractionation [28] and deterministic lateral displacement [29] depend on intrinsic channel geometry or hydrodynamics. Passive sorting methods rely on gravitational or centrifugal forces operate effectively only at low flow rates. In addition, clogging and low sorting efficiency remain a major challenge for passive methods [30]. Inertial microfluidics is an effective passive sorting technique [31]. However, inertial microfluidics requires high flow rates and complex channel geometry for effective sorting. The high flow rate increases the risk of damaging delicate particles. Furthermore, intricate channel designs of inertial microfluidics are challenging to fabricate.

In contrast, active sorting methods allow for a more precise, faster and targeted approach by leveraging a specific property of the beads. Various external fields have been employed for droplet manipulation and sorting, including electric [32,33], acoustic [34,35], pneumatic [36, 37], and magnetic methods [34]. In a previous work, we created magnetic liquid beads by embedding magnetic particles in their shells and demonstrated continuous in-channel separation of magnetic beads from non-magnetic ones [26]. We also observed differences in the magnetic response between magnetic solid and liquid beads. However, we were not able to achieve in-channel sorting due to the relatively low precision of this method. The magnetic response of the beads largely depends on the concentration of magnetic particles, and it was challenging to ensure uniform distribution of the magnetic particles in each bead.

Among the active sorting methods, electric field-based manipulations have attracted a great attention due to their label-free nature, ease of control, quick response, and suitability for microscale integration. Electric methods rely on phenomena called electrophoresis and dielectrophoresis. Electrophoresis refers to the movement of charged molecules or particles through a medium under the influence of an electric field, while dielectrophoresis (DEP) involves the movement of neutral particles in a non-uniform electric field. In a nonuniform electric field, particles experience the dielectrophoretic force due to charge accumulation and polarisation at the interface, resulting from differences in electric permittivity and conductivity on either side of the interface [38]. This phenomenon has been effectively utilised for manipulating both oil-in-water [39] and water-in-oil droplets with high efficiency. However, the manipulation of liquid beads using electric fields has not been explored, likely due to a lack of insights into their behaviour in an electric field. In a nonuniform electric field, beads experience dielectrophoretic forces that can either attract or repel them from the electrodes, depending on core-shell structure and properties of the encapsulated liquid. This makes the non-uniform electric field a promising mean for manipulating liquid beads. Thus, studying the dynamics of core-shell liquid beads and solid beads undergoing DEP is crucial for their continuous sorting or separation in a microfluidic device.

This paper investigates the dielectrophoretic responses of core-shell liquid beads and solid beads. The beads are formed by curing the outer layer of double emulsion droplets produced in a microfluidic device. We then utilised a relatively simple setup with a steel needle electrode and an indium tin oxide (ITO) coated glass as counter electrode to form a non-unform electric field. Our experiments demonstrated that the beads exhibit both positive and negative DEP in a non-uniform electric field under both AC and DC conditions. Finally, we demonstrated the separation of core-shell and solid beads using dielectrophoresis in a microfluidic device.

2. Materials and methods

2.1. Materials

Silicon wafers were obtained from IBD Technologies Ltd. (Wiltshire, UK). The photoresist SU-8 3050 was sourced from MicroChem Corp (Westborough, USA). PDMS prepolymer and curing agent (Sylgard 184) were from Dow Corning (Midland, MI, USA). Poly(vinyl alcohol) (PVA) with an 87–90 % hydrolysis rate and an average molecular weight of 30,000–70,000 was acquired from Sigma-Aldrich.

The shell liquid or the polymeric outer phase of the double emulsion was prepared by mixing 0.06 g ethyl-4(dimethylamino)benzoate (Merck), 0.05 g camphorquinone (Merck), and 10 g trimethylolpropane trimethacrylate (TMPTMA, Merck. The surface tension, viscosity, and density of this polymer solution were $\sigma_{polymer}=0.032$ N/m, $\mu_{polymer}=0.042$ Pa.s, and $\rho_{polymer}=1.07\times10$ 3 kg/m³, respectively [40]. Hydrofluoroether oil (HFE Novec 7500 from 3 M) was chosen as the core liquid. It has a surface tension of $\sigma_{HFE}=0.015$ N/m, a viscosity of $\mu_{HFE}=1.31\times10^{-3}$ Pa.s, a density of $\rho_{HFE}=1.63\times10$ 3 kg/m³, and a boiling point of 128°C [41]. The outer continuous phase was an aqueous solution with 50 % glycerol (Chemsupply) in water (Milli-Q), with a surface tension of $\sigma_{sol}=0.067$ N/m, viscosity of $\mu_{sol}=6\times10^{-3}$ Pa.s, and density of $\rho_{sol}=1.12\times10$ 3 kg/m³ [42].

2.2. Microfluidic synthesis of liquid beads

We fabricated a PDMS-based microfluidic device for the formation of liquid beads using the method reported by Galogahi et al. [24]. A mask was created with CleWin software and printed on a transparent film. SU-8 3050 was spin-coated on a 4-inch silicon wafer, patterned with photolithography, and annealed to create the mould for microchannels. The channels were 100 µm wide and 120 µm deep, with a constriction width of 30 µm, Fig. 1. The microfluidic device was fabricated using soft lithography. A degassed mixture of PDMS base and curing agent (10:1 ratio) was poured onto the SU-8 mould and cured at 75 °C for 1 hour. The PDMS replica was then peeled off. Inlets and outlets were punched. The PDMS device was bonded to a glass slide after treating both in an oxygen plasma cleaner (PDC-32G-2, Harrick Plasma). Finally, a 1 % polyvinyl acetate (PVA)-deionised (DI) water solution was manually injected into the device and heated at 100° C for 10 minutes. This surface treatment was repeated three times to impart durable hydrophilicity to the channel wall.

The core phase was HFE 7500 oil, while the shell phase consisted of TMPTMA mixed with ethyl 4-(dimethylamino)benzoate, and



Fig. 1. Continuous production of liquid beads. (a) The microfluidic device generates core-shell droplets with an oil core and TMPTMA shell. Inlet 1 introduces HFE 7500 oil, Inlet 2 introduces TMPTMA, and Inlets 3, 4, and 5 introduce an aqueous solution containing 50 % v/v glycerol and 0.06 mM Tween 20. Oil core droplets form at the first junction and move to the second junction, where the shell forms around the core to from double emulsion droplets. In some instances, the TMPTMA droplet fails to encapsulate the oil droplet, resulting in the formation of a single emulsion. A spacer fluid at the third junction prevents interactions among the produced droplets. Images were captured using an optical microscope. The scale bar represents 100 μ m; (b) Liquid bead size distribution.

camphorquinone. The continuous phase and spacer fluid consisted of 50 % v/v glycerol and 0.06 mM Tween 20. The flow rates of all liquids were based on our previously reported work on core-shell liquid bead production [24]. The shell and core phases were introduced into the microfluidic device through inlets 1 and 2 at 30 µl/hr and 100 µl/hr respectively, Fig. 1. Flow rates were controlled by a syringe pump (NEM-B101-03 A, CETONI GmbH, Germany). At the first junction, oil droplets were dispersed into the shell phase due to its shearing action. At the second junction, the continuous phase flowing at 400 µl/hr through inlets 3 and 4 broke up the shell phase, forming channel-sized double emulsion droplets. These droplets were produced in a core-shell configuration, with the TMPTMA shell encapsulating the HFE oil core. To prevent droplet coalescence, a spacer phase is introduced at the third junction through inlet 5 at 500 µl/hr. Finally, the formed droplets exited the device at a rate of 150-200 per minutes and were collected in a Petri dish on a gently oscillating plate to further limit coalescence. The collected core-shell droplets were exposed to a 24-W blue light source (450-490 nm) for 20 minutes to harden and form the liquid beads with oil core. The formed beads were tested for their high mechanical and thermal stability in our previously reported work [21].

Droplet formation in a flow-focusing geometry is governed by three main forces: interfacial tension resisting droplet break-up, shear force exerted by the continuous phase, and hydrostatic pressure. Initially, interfacial tension dominates, causing the interface to expand radially and axially toward the channel. As the interface starts blocking the continuous phase against the channel wall, the pressure builds up, enabling shear force to overcome the interfacial tension. This process allows for the droplet breakup. For double emulsions, the droplet formation rates at junctions 1 and 2 must be precisely synchronised. A faster core flow rate leads to incomplete encapsulation by the shell phase, while a slower core flow rate produces too few cores for the double emulsion, resulting in single-emulsion solid beads.

2.3. Microfluidic device for in-channel sorting of liquid beads

After microfluidic generation, the liquid beads were collected with a syringe and reinjected into a PDMS sorting device. The sorting device was fabricated using a 1-mm thick polymethyl methacrylate (PMMA) sheet as a mould, designed with CoralDRAW. Channels were cut into the sheet using a laser cutter (Rayjet 300, Trotec), glued to a glass slide, and placed in a Petri dish. A mixture of PDMS and curing agent was poured onto the PMMA mould and cured at 75 °C for 1 hour. The PDMS replica was peeled off, and inlets and outlets were punched. The PDMS part was then bonded to a (50 mm imes 50 mm) ITO coated glass slide (Adafruit industries, USA), completing the fabrication process. The microfluidic device had two straight channel sections (35 mm imes 1 mm and 15 mm imes0.5 mm). A flow-focusing configuration at the inlet focused the beads into a single line for sorting. The channel height was determined by the 1-mm thick PMMA sheet. A needle-shaped steel electrode (1.5 mm diameter) and an ITO-coated glass electrode (50 mm \times 50 mm) formed the electrode pair. The sharp needle tip was inserted into the PDMS device and positioned 1 mm above the ITO glass. Both AC and DC voltages were applied to the electrode pair to generate an electric field in the channel. The beads were then flowed into the channels near the electrodes for sorting. Images were captured with a digital camera (Nikon D7500, Japan).

2.4. Analytical model

A polarisable particle in a medium experience the DEP force of a nonuniform electric field if its electric properties differ from those of the surrounding medium. The two response types of the particle are positive and negative DEP. In positive DEP, the particle, being more polarisable than the medium, moves toward regions with a higher electric field intensity. Conversely, in negative DEP, the particle is less polarisable than the medium and moves toward regions with a lower field intensity. Fig. 2(a) illustrates the positive and negative DEP experienced by a liquid bead and a solid bead. We used a single shell particle model to analyse the dielectrophoretic response of core-shell beads and a homogeneous particle model for solid beads [43].

In a nonuniform electric field, the particles undergo non-uniform polarisation, equivalent to a dipole. The interaction of the AC electric field with the equivalent dipole moment results in a DEP force [44], expressed as:

$$\langle F_{\text{DEP}} \rangle = 2\pi \varepsilon_0 \varepsilon_1 R_1^3 \text{Re} [f_{\text{CM}}] \nabla |E_{\text{rms}}|^2$$
(1)

$$f_{CM} = \frac{\widetilde{\epsilon}_{23} - \widetilde{\epsilon}_1}{\widetilde{\epsilon}_{23} + 2\widetilde{\epsilon}_1}$$
(2)

$$\widetilde{\varepsilon}_{23} = \widetilde{\varepsilon}_{2} \left[\left(\frac{R_{1}}{R_{2}} \right)^{3} + 2 \left(\frac{\widetilde{\varepsilon}_{3} - \widetilde{\varepsilon}_{2}}{\widetilde{\varepsilon}_{3} + 2\widetilde{\varepsilon}_{2}} \right) \right] \Big/ \left[\left(\frac{R_{1}}{R_{2}} \right)^{3} - \left(\frac{\widetilde{\varepsilon}_{3} - \widetilde{\varepsilon}_{2}}{\widetilde{\varepsilon}_{3} + 2\widetilde{\varepsilon}_{2}} \right) \right]$$
(3)

where $\langle F_{DEP} \rangle$ represents the time-averaged DEP force, f_{CM} is the Clausius–Mossotti (CM) factor, E_{rms} is the root-mean-square value of the

electric field, and $\tilde{\epsilon}_{23} = \epsilon_i + \sigma_i/j\omega, i = 1, 2, 3$ is the complex permittivity of the materials. ϵ_1 , ϵ_2 and ϵ_3 are the relative permittivities of the suspending medium (DI water), TMPTMA shell, and HFE oil core, respectively; σ_1 , σ_2 , and σ_3 are the conductivities of the suspending medium, shell, and core, respectively; and $\tilde{\epsilon}_{23}$ is the equivalent complex permittivity of the bead. The sign of the DEP force is determined by the factor f_{CM} , with $f_{CM} > 0$ resulting in a positive DEP force and $f_{CM} < 0$ leading to a negative DEP force. The sign of f_{CM} depends on the radii R_1 , R_2 and the permittivity and conductivity (ϵ_i , σ_i) of the bead-suspending medium system.

We used MyDEP software to calculate f_{CM} values for both solid and core-shell beads [43]. The software includes pre-defined particle models: a homogeneous model for solid beads and a single-shell model for core-shell liquid beads. Users can input values for size and electric properties, Table 1. The calculated f_{CM} values were plotted against the input frequency to guide the sorting strategy, Fig. 2(b). We noted that the solid beads exhibit positive f_{CM} values at lower frequencies, while core-shell beads exhibit negative f_{CM} values across all frequencies. Consequently, we selected a DC electric field for in-channel sorting of core-shell liquid beads from solid ones.

3. Results and discussion

3.1. Manipulation of floating liquid beads

Initial experiments were carried out to demonstrate the DEP responses of floating core-shell and solid beads in a non-uniform electric field. Fig. 3(a) illustrates the experimental setup that comprises of a 1-mm thick DI water layer confined into $\sim 20 \times 20$ mm area on the top

Table 1

Parameters and dimensions for CM factor calculation and electric field simulation.

Symbol	Implication	Value
ε ₀	Permittivity of free space	$8.854\times 10^{-12}~\text{F/m}$
ϵ_1	Relative permittivity of DI water medium	80
ε2	Relative permittivity of TMPTMA	2.5[45]
ε3	Relative permittivity of HFE oil	5.8[46]
σ_1	Conductivity of DI water medium	$5.5 imes10^{-10}~\text{S/m}$
σ_2	Conductivity of TMPTMA	10^{-8} S/m[45]
σ_3	Conductivity of HFE oil	$10^{-12} \text{ S/m}[47]$
R_1	Shell inner radius	25 µm
R_2	Shell outer radius	50 µm
δ	Shell thickness	25 µm
L	Steel electrode tip diameter	0.1 mm
L_{s}	Electrode distance	1 mm
w	Channel width	1 mm



Fig. 2. DEP responses of particles in a non-uniform electric field. (a) Homogeneous and single shell particle models experiencing positive and negative DEP forces; (b) Clausius–Mossotti (CM) factor for core-shell and solid beads, overlaid with geometric and electrical properties of the beads.



Fig. 3. DEP response of a floating liquid bead. (a) Experimental setup for measuring the velocity of a floating bead in AC and DC fields; (b) Trajectories of core-shell and solid beads demonstrating negative and positive DEP respectively. Scale bar is 200 μm; (c) Simulated 2D electric field distribution near the steel electrode at 35 V DC potential difference, showing field lines as curves and DEP forces as arrows. Includes a free body diagram of the floating bead under positive DEP; (d) Average velocity of core-shell beads under negative DEP; (e) Average velocity of solid bead undergoes negative DEP for input voltages and frequencies, except at 1 Hz and 200 V; (f) Average velocity versus applied AC voltage at 100 Hz; (g) Average velocity versus applied DC voltage. Solid beads exhibit positive DEP, while core-shell liquid beads exhibit negative DEP.

of 50×50 mm ITO coated glass. A steel needle was placed at the centre of the layer and 1 mm above the ITO glass. This setup formed a needle-to-plate electrode configuration with 1-mm gap, where the needle is positively biased and the ITO glass in connected to ground [48]. Beads were floated gently on the water surface at 10 mm away from the needle tip. Subsequently the electric field was turned on and bead motion is

recorded from the top using a DSLR camera (Nikon D7500). The images were extracted from the recorded videos, and further analysed using the TrackMate plugin in ImageJ software [49].

When a liquid bead was gently placed on the surface of a water-filled container, it floats at the air-water interface due to the equilibrium between the downward gravitational force and the upward forces of buoyancy and surface tension. Jin at el. reported the DEP trapping of a floating liquid marble as a digital microfluidics platform utilising DEP forces for controlled movement [50]. Here, we focused on floating core-shell and solid liquid beads, examining factors influencing their motion such as applied voltage and frequency of the electric field. We aim to exploit the gained knowledge for in-channel sorting of the beads. While we understand that the interaction of an electric field with a bead suspended in a medium differs from that with a floating bead, we selected the floating bead setup due to its ease of input voltage and frequency optimisation and the savings in resources. Since the DEP force required to move a floating bead is greater than that for a suspended bead [51], the optimised voltage and frequency parameters for the floating bead will reliably ensure bead motion within a microfluidic channel.

At a distance of 1 mm from the electrode in a nonuniform electric field, the liquid beads experience either a positive or negative DEP force due to the electric field gradient, Fig. 3(a). The horizontal component of the force $F_{\text{DEP}-r}$ serves as a driver that pulls or pushes the floating bead toward or away from the electrode. Frictional force opposes the movement of the bead. Fig. 3(b) illustrates the trajectories of a core-shell and solid bead, demonstrating their positive and negative DEP responses in a 35 V DC field. The floating bead also experiences upward forces, including the vertical component of the DEP force $F_{\text{DEP}-z}$, buoyancy, and the surface tension of the carrier liquid, while its weight acts as a downward force. The force balances in the *r* and *z* directions (with upward and toward the electrode considered positive) are described as:

$$F_{\rm DEP-r} - F_{\rm f} - m \frac{\mathrm{d}\nu}{\mathrm{d}t} = 0 \tag{42}$$

$$F_{\text{DEP}-z} + F_{\text{b}} + F_{\text{s}} - G = 0$$
 (5)

Here, *m* is the mass, and *v* is the velocity of the floating liquid bead. $F_{\text{DEP}-r}$ and $F_{\text{DEP}-z}$ represent the horizontal and vertical components of the DEP force, respectively. $F_{\rm f}$ denotes the frictional force, $F_{\rm b}$ the buoyancy force, F_s the surface tension force, and *G* the weight of the bead. Fig. 3 (c) shows the free body diagram of the forces acting on the bead. Fig. 3 (c) also demonstrates the simulated electric field and DEP force vector between the electrodes and around the steel electrode (inset). The simulation was carried out in COMSOL Multiphysics 5.6.

We plotted the average velocities of the core-shell beads (Fig. 3(d)) and solid beads (Fig. 3(e)) against input frequencies in an AC electric field. Positive velocities indicate positive DEP, whereas negative velocities indicate negative DEP. The input frequencies ranged from 1 Hz to 20 kHz. We set the voltage range between 200 V and 800 V; below 200 V, the DEP force was too weak to move the beads. Above 800 V, the potential difference caused instant hydrolysis of the surrounding DI water. Hydrolysis also occurred at low frequencies for 300 V, 500 V, and 800 V, so we could not obtain the bead velocities under these conditions. The velocity of both bead types generally decreased as the frequency increased. The average velocity was calculated by taking the difference between the mean velocity with the field on and the mean velocity with the field off. The positive velocity at 200 V and 1 Hz input corroborated $f_{\text{CM}}>0$ condition. Fig. 3(f) showed that the velocity of both solid and core-shell beads increases as the input AC voltage rises at 100 Hz. However, only the solid beads displayed a linear relationship with the applied voltage.

Fig. 3(g) presents the velocities of core-shell and solid beads in a DC electric field, with input voltage limited to 30 V to 45 V. Below 30 V, the beads did not respond to the electric field. Above 45 V, hydrolysis occurred at the electrode. In a DC field, the obtained velocities vary linearly with the applied voltage. Solid beads exhibited positive DEP, and core-shell beads exhibited negative DEP, consistent with previously calculated f_{CM} values at 1 Hz. At high voltages, the induced dipole moment in the beads reaches saturation due to limited polarizability of the bead material. In presence of a highly distorted electric field, the DEP force acting on the saturated beads deviates from its expected

quadratic dependence on field strength, Eq. 1. This nonlinear effect, characterised by large variations in bead velocity, is more pronounced in core-shell beads than in solid ones, as shown in Fig. 3(f). At frequencies of 1 Hz or lower, core-shell and solid beads exhibit distinct DEP responses. Solid beads transition from negative to positive DEP at 1 Hz, Fig. 3(f) and maintain positive DEP for all DC input voltages, Fig. 3(g). In contrast, core-shell liquid beads consistently exhibit negative DEP across all input voltages and frequencies. This led us to use a DC electric field for in-channel sorting of the beads.

3.2. Sorting of in-channel liquid beads

After curing, the beads were collected, redispersed in water, and transferred to a glass syringe through polytetrafluoroethylene (PTFE) tubing for in-channel sorting. To prevent the beads from adhering to the syringe and tubing walls, a 10 % Poly (vinyl alcohol) (PVA) solution was flushed through the system for 10 minutes, followed by a 15-minute baking at 100° C. The process was repeated three times to achieve the desired hydrophilicity.

Two streams were introduced into a central channel: one containing a mixture of solid and core-shell liquid beads dispersed in water, and the other serving as a buffer with DI water. The flow in the main channel was diverted into outlet 1 and outlet 2 downstream, Fig. 4(a). The optimal flow rates for the buffer and bead-dispersed medium were set at 6 ml/hr and 3 ml/hr, respectively. The optimised flow rates and flowfocused channel design ensured a well-separated single-bead stream, preventing chain formation that could interfere with bead sorting. In this design, outlet 1 (0.5 \times 1 mm) branches off at an angle of 30° from the straight path of outlet 2 (1 \times 1 mm). The steel electrode was introduced at the junction of the two channels. The design ensures that beads experiencing positive DEP are attracted towards the steel electrode and exit through outlet 1, while unaffected or repelled beads exit through the straight channel toward outlet 2. The bead motion in the channels is governed by DEP and hydrodynamic drag force, with DEP directing beads towards or away from the electrode and hydrodynamic drag force moved them along the channel. We neglected electro-osmosis (EO) effects on the beads, as these weak electrokinetic forces act over only a few micrometres, which is negligible compared to the millimetre-range working distance of the DEP [52]. Additionally, the low ionic concentration of DI water used as the buffer further diminishes the EO effect. Fig. 4 [a(i)] show the beads trajectory in a DC field. Solid beads experienced positive DEP, moving towards the electrode and exiting through outlet 1. Core-shell liquid beads experienced negative DEP, moving away from the electrode and exiting through outlet 2. In an AC field, all beads experienced negative DEP and were deflected to outlet 2, Fig. 4[a (ii)]. In an AC field, all beads experienced negative DEP and were deflected to outlet 2, Fig. 4[a(ii)]. As a result, we opted for a 35-40 V DC field for sorting the beads instead of an AC field.

After sorting, the beads collected from outlets 1 and 2 were dispersed in water in separate Petri dishes, and images were captured. We counted the number of beads using Hough circle transform (HCT) in a custommade Python script [53]. We adopted our previously reported algorithm to accurately detect and count both core-shell and solid beads [25]. Fig. 4(b) illustrates the bar graph of the counted beads collected at outlets 1 and 2. We selected three different regions of the collected beads for counting and obtained a sorting efficiency of about 80 %. The mixture of core-shell and solid beads was sorted at the outlets at a rate of about 1000 beads per minute. Approximately 80 % of the solid beads exited through outlet 1, while around 80 % of the core-shell beads exited through outlet 2. Fig. 4(c) illustrates the beads overlaid with white circles. Single circles represent detected solid beads, while concentric circles indicate core-shell beads. Fig. 4[c(i)] shows beads from outlet 1 that are mostly solid. Fig. 4[c(ii)] shows core-shell liquid beads from outlet 2.



Fig. 4. DEP sorting of liquid beads: (a) Schematic of microfluidic device, demonstrating bead sorting with DEP using steel needle and ITO glass electrodes. (i) At 35 V DC, core-shell beads are deflected towards outlet 2, while solid beads are attracted towards outlet 1. (ii) At 500 V Ac and 1 Hz frequency, all beads are deflected to outlet 2. (b) Bar graph showing the sorting efficiency close to 80 %, a total number of 544 solid and 1950 core-shell beads were observed. (c) Images of collected beads at outlet 1 (i) and outlet 2 (ii), with detected beads overlaid by circles, indicating core-shell or solid types (inset). Scale bar is 500 µm.

4. Conclusion

We explored the DEP responses of core-shell liquid beads and solid beads within a microfluidic device, aiming to achieve continuous sorting of the beads. Our investigation was motivated by the need for efficient sorting techniques in applications that require uniform bead populations, such as drug delivery, cell culture, and microreactor. Initially, we fabricated core-shell liquid beads using a microfluidic device by curing the outer layer of double emulsion droplets. We chose the microfluidic method for its ability in producing beads with a narrow size distribution and scalable production. However, the lack of precise fine tuning of flow rates and challenging surface modification led to the formation of up to 30 % core-less solid beads.

Our experiments demonstrated that both core-shell and solid beads exhibit distinct DEP responses in a non-uniform DC electric field, with core-shell liquid beads showing negative DEP and solid beads displaying positive DEP. This differential DEP behaviour is attributed to the difference in dielectric properties of both bead types. A core-shell liquid bead consists of two different materials in contrast to a homogenous solid bead. We fabricated a microfluidic device with a flow-focusing configuration to leverage these DEP responses for bead sorting. The device utilised a steel needle-ITO coated glass electrode system to create a non-uniform electric field within the channel. By applying a DC electric field, we successfully sorted core-shell beads from solid beads with an efficiency of approximately 80 %. The sorted beads were detected and counted using image processing algorithm coded in Python.

Our findings demonstrated the potential of DEP-based sorting for microfluidic applications. The ability to precisely manipulate and sort beads based on their DEP responses offers significant advantages over traditional passive sorting methods, which often suffer from low efficiency and susceptibility to clogging. Additionally, DEP in our sorting device provided label free, quick, precisely controllable and easy to integrate sorting scheme. A cascaded sorting design can potentially enhance sorting efficiency to up to 90 % by incorporating additional stages. Previously, our group demonstrated a 307-fold increase in white blood cell purity by introducing an extra sorting stage [54]. Future work will aim to optimise sorting efficiency and explore the scalability of the DEP-based sorting technique. This will involve studying the effects of bead geometry, such as shape, shell thickness, flow rates, dielectric properties of the buffer, and channel geometries on sorting performance. We also plan to integrate this sorting method with other microfluidic processes to create a comprehensive lab-on-chip system.

CRediT authorship contribution statement

Nguyen Nam-Trung: Writing – review & editing, Supervision, Conceptualization. Galogahi Fariba Malekpour: Writing – review & editing, Investigation. Sreejith Kamalalayam Rajan: Writing – review & editing, Supervision. Tran Du Tuan: Writing – review & editing, Writing – original draft. Yadav Ajeet: Writing – original draft, Validation, Investigation. Roshan Uditha: Writing – review & editing, Writing – original draft. Vashi Aditya: Writing – review & editing, Writing – original draft.

Declaration of Competing Interest

The authors do not have any conflict of interest to declare.

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Data availability

Data will be made available on request.

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