ELECTROWETTING-ON-DIELECTRIC (EWOD) DIGITAL MICROFLUIDIC DEVICE FOR SYNTHETIC ORGANIC CHEMISTRY

by

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Presented to the Faculty of the Graduate School of
The University of Texas at Arlington in Partial Fulfillment
of the Requirements
for the Degree of

DOCTOR OF PHILOSOPHY

THE UNIVERSITY OF TEXAS AT ARLINGTON

December 2019
To my parents, Hossein and Sorayya, my sister, Aitin

for their endless love & support
Acknowledgments

Undertaking this PhD has been a truly life-changing experience for me, and it would not have been possible to do without the support and guidance that I received from many people, and I am forever indebted to them.

Foremost, I would like to express my sincere gratitude to my PhD supervisor Dr. Hyejin Moon. I have been lucky enough to have a chance to spend a few years of my life working under her supervision and holding this research. I specifically appreciated her extensive personal and professional guidance and provision of precious scientific knowledge. I am uniquely indebted to her invaluable support and advice when I faced various difficulties. Without her tireless leadership and continuous feedback, this PhD would not have been achievable. I learned from her “SUCCESS IS THE ABILITY TO GO FROM FAILURE TO FAILURE WITHOUT LOSING YOUR ENTHUSIASM.”

My thanks also go out to the support I received from the collaborative work I undertook with the Jeon Laboratory. I am especially grateful to Dr. Junha Jeon, for giving me the opportunity to complete my research in his lab and learn from his immense knowledge.

I am also appreciative of all the advice and support I received from my supervising committee members Dr. Purnendu (Sandy) Dasgupta, Dr. Miguel Amaya, and Dr. Wen Shen. I am deeply thankful to all other faculty members at the University of Texas at Arlington Department of Mechanical and Aerospace Engineering, especially my valuable professor and the department chair, Dr. Erian Armanios, who has always been genuinely supportive.

I am also grateful to the facilities provided by Shimadzu Institute Nano Technology Research Center at UTA, where I fabricated devices for my experiments. Likewise, I am thankful for all the research funding sources at UT Arlington, and the National Science Foundation (NSF) that supported my research.

I want to express my gratitude to my lab-mates and fellow graduate students. Mun Mun Nahar, Shubhodeep Paul, Ali Frazbod and all other fellow doctoral students for their feedback, cooperation, and, of course, friendship, with a special mention to all my research collaborators Parham Asgari and Udaya Dakarapu.

I would like to thank my father and mother, Hossein and Sorayya, who supported me emotionally and financially throughout these challenging years. It was never possible to earn this
PhD without the endless love, inspirations, and sacrifices that my parents made for me from my first day on earth until now. They advocated me through thick and thin during all the hardships of immigration and enabled me to pursue my dream. I learned from them, "YOUR HARD WORK WILL PAY OFF. THE RIGHT PEOPLE WILL SHOW UP. YOUR DREAMS WILL COME TRUE."

I am forever thankful to Aitin, my beloved sister, whose love and care have made me not to be complacent with life; the impossible becomes possible. I am truly obliged to her for all the love and encouragement she gave me during the tough times. Without her endurance and unconditional love, I would not have had the courage to embark on this journey in the first place. From her, I learned, "FAMILY IS THE BEST TEAM YOU COULD EVER HAVE."

And last but by no means least, I would like to thank my auntie, Soheila, who encouraged and helped me at every stage of my personal and academic life, and longed to see this achievement come true. She has made countless sacrifices to help me get to this point.
Abstract

ELECTROWETTING-ON-DIELECTRIC (EWOD) DIGITAL MICROFLUIDIC DEVICE FOR SYNTHETIC ORGANIC CHEMISTRY

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Over the past decades, microscale chemical reaction technology has been attractive in diverse areas of chemistry. It allows the precise control of quantified reagents and highly efficient heat and mass transfer, because of a large interface-to-volume ratio—particularly in case of the exothermic reaction and mixing—, reduced consumption of toxic or expensive agents, improved reaction profiles, and enhanced selectivity compared to macro-scale reactions.

The mainstream microscale reaction processes were established using continuous microchannel flow systems, which has emerged as a powerful complement to batch chemistry on the laboratory scale due to relative advantages, some of which include: increased safety, more accurate temperature control, ease of reaction scale-up, and amenability to automation.

Despite of successful demonstration from prior studies, microchannel-based approaches suffer from several limitations. For example, clogging of the channels by products or byproducts may cause the difficulty of maintaining a constant hydrodynamic pressure, thus stable flow. Requirement of complex flow network and cross-contamination due to unwanted diffusion through channels are also concerned. Moreover, solvent-swapping processes pose very challenging problems in microchannel reactors. Another drawback of microchannel reactors presents in combinatorial chemistry—a powerful tool for lead compound discovery and optimization of new drugs and materials. Since a combinatorial synthesis through either batch or flow reactors requires
as many reactors as the number of all possible combinations of reactants, the reactor system tends to be excessively complex.

A digital microfluidic platform using electrowetting-on-dielectric (EWOD) principle can be an alternative and/or complement a microchannel reactor. An EWOD digital microfluidic platform eliminates the necessity of predetermined channel network and mechanical pumps and valves. Since it is a droplet-based flow, it can prevent cross-mixing and cross-contamination. Each droplet plays as a batch reactor, which brings the feasibility of performing multi-step reactions that may involve with solvents swapping and combinatorial synthesis. Researchers have taken advantages of these unique features of EWOD microfluidic devices to conduct on-chip chemical reactions, e.g. reactions in ionic liquid droplets, synthesis of radiotracers, and synchronized synthesis of peptide-based macrocycles. Of note, that all these reactions on EWOD chip mentioned above utilized solvent fluids that are movable by EWOD actuation. However, contemporary organic synthesis generally requires non-polar or polar aprotic solvents, and their poor movability in an EWOD chip has been a long-standing problem.

First, this study focuses on movability of different types of fluids other than aqueous solutions in the EWOD microfluidics to be a versatile platform for various applications. An electromechanical model using a simple RC circuit has been used to predict the mechanical force exerted on a liquid droplet upon voltage application. In this study, two important features missed in previous works are addressed. Energy dissipation by contact line friction is considered in the new model as the form of resistor. The phase angle is taken into account in the analysis of the AC circuit. The new electromechanical model and computation results are validated with experimental measurements of forces on two different liquids. The model is then used to explain influences of contact angle hysteresis, surface tension, conductivity, and dielectric constant of fluids to the mechanical force on a liquid droplet.

In the second part of the study, we introduce a novel technique of an “engine-and-cargo” system that enables use of non-movable fluids (e.g., organic solvents) on an EWOD device. With esterification as the model reaction, on-chip chemical reactions were successfully demonstrated. Conversion data obtained from on-chip reactions were used in the demonstration of reaction characterization and optimization such as reaction kinetics, solvent screening, and catalyst loading. As the first step toward on-chip combinatorial synthesis, parallel esterification of three different
alcohols were demonstrated. Results from this study clearly show that EWOD digital microfluidic platform is a promising candidate for a microscale chemical reaction.

Thirdly, we demonstrate in-line, and on-chip workup as a step towards promoting multi-step synthesis on an EWOD device. In this study, we selected an acid-base workup as a model system and successfully shown the consecutive steps, including neutralization reaction, acid-regeneration reaction, evaporation, and recrystallization. Moreover, on-chip recrystallization of benzoic acid and benzophenone manifested the capacity of an EWOD device to handle the solid particles, whether added or formed during the reaction, with neither change in device architecture nor disassembly.

The EWOD DMF device, by having robustness, chemical compatibility, and ease of use, is an alternative or a complementary tool to microreactors based on continuous channel flow for organic synthesis, and it can provide a broader range of chemistries and operating conditions.
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Chapter 1

Background

1.1 Emergence of Microreaction Technology

The rise of microreaction technology began with the development of flow chemistry. In the past decades, flow chemistry has emerged as a powerful complement to batch chemistry on the laboratory scale due to relative advantages, some of which include: increased safety, more accurate temperature control, ease of reaction scale-up, and amenability to automation. Flow chemistry has offered the capacity to optimize processes and remote monitoring reaction reiteration 24/7. Such capability provided new potentials for laboratories that may use these new machine-assisted approaches to create a more sustainable working environment [1].

Furthermore, there has been a significant reduction in the waste generated in the flow chemistry laboratory, which can be attributed to the least demand for silica gel chromatography to effect product clean-up, which is often mandatory in conventional synthesis applications.

Last but not least, the capability of producing unstable intermediates in-line and directly consume them in a downstream transformation is also a reward of the flow chemistry principles when compared with batch mode performance. A significant example of such capability is for the synthesis of Curtius, where acylazides are generated as intermediates, which then undergo a thermally controlled post-process and consequently quenched to yield the final products [2].

A general continuous flow chemistry set-up is shown in Figure 1-1, where the reagents are separately pumped through a micromixer into the reactor. The reactor is equipped with a temperature-controlled unit, and it can be customized for different reaction conditions. Based on the demands for a reaction, the reactor may own different types, including coils (polytetrafluoroethylene (PTFE), perfluoroalkoxy (PFA), and stainless steel to microchips, fixed-bed reactors, agitating microsphere reactors and tube-in-tube reactors.

Rapid growth in the employment of flow chemistry induced a revolution in laboratory instruments; however, restrictions have continued in adapting with many organic and inorganic
transformations, particularly for highly oxidizing, corrosive, and particle-laden flows. Low-temperature reactions in batch mode usually require large quantities of cryogenic materials, which are expensive both in terms of price and energy consumption. Moreover, solvent intrusion and accurate temperature control for lengthy operation are issues that necessitate further actions. These difficulties do not go away by moving to flow chemistry.

Figure 1-1 General scheme for a continuous flow system [3].

Microscale chemistry allows reactions to be chosen that require less extreme reaction conditions—shorter reaction times, ambient pressure instead of high pressure, and reduced temperature requirements. The laboratory method involving microscale chemistry allows the use of altered reagents, new reaction pathways, and alternative solvents, yielding less hazardous products and by-products.

Microscale chemistry is a laboratory-based green chemistry approach accomplished by using microstructured devices and significantly reduced amounts of chemicals. Microscale chemistry can be implemented without jeopardizing educational standards or analytical rigor, and its techniques are amenable to industrial R&D applications. Since its modest beginning at three institutions in the early 1980s (Bowdoin College, Merrimack College, and Brown University), microscale chemistry has experienced a rapid growth in the U.S. and around the world.
Originally, microscale chemistry was introduced in the organic chemistry laboratory at Bowdoin College, Maine. It was later expanded to cover general, inorganic, analytical, and environmental chemistry. The National Microscale Chemistry Center was established at Merrimack College in 1992–1993 as the first center to offer formal microscale chemistry training to teachers and chemists at all levels from elementary school to university. The introduction of microscale organic chemistry by Mayo et al. [4] has created a revolution in a manner in which organic laboratory instruction is carried out. In the traditional inorganic laboratory, the high costs associated with macroscale preparations have limited experiments to the use of only the least expensive metals (chromium and cobalt, for example). Also, many of the materials used in the traditional inorganic chemistry laboratory pose significant health and ecological risks [5].

Figure 1-2 shows a comparison of the growth of the number of patent publications in different technological areas [4]. Nanotechnology and Lab-on-a-chip are significant innovation drivers who have been utilized in various fields of chemical and biological applications.

Figure 1-2 Comparison of the growth of the number of patent publications in different technological areas [4].
About the year 2000, fabrication of microreactor began utilizing glass, ceramics, silicon, or steel, shown in Figure 1-3. In spite of that, such microstructured devices were costly to fabricate, and some of them were prone to plugging by small particles or degradation due to reagent incompatibility.

Figure 1-3 Examples of microreactors beginning with primary microreactors from about the year 2000 (left), through current laboratory microreactor systems (middle) to ongoing microreactor scalable development and production equipment. (a) Thin film silicon microreactor for partial oxidation [6]. (b) Miniature glass-silicon gas liquid packed bed reactor [7]. (c) steel evaporator [8]. (d) microfabricated stainless steel bubble column reactor. (e) with glass microreactor chip (f) Vapourtec laboratory tube reactor system (g) ThalesNano hydrogenation system (H-Cube) with self-generated hydrogen and packed bed microreactor (h) Chemtrix development and numbered-up production systems with micro structured glass reactors (i) Corning micro structured glass reactors (G1–G3) illustrating the scaling principle and a numbered-up G1-system [9] (j) Microstructured stainless steel development [10].

Microreactor technology have shown unique potential advantages over conventional reactors in chemical and nanomaterials synthesis due to the scale-dependent processes of heat and mass transfer, the fluidic regimes of low Reynolds number, and large ratios of surface area to volume.
Rapid Reaction. Researchers have shown that most often microfluidic reactors offer "faster reaction" compared with those demonstrated on the lab-scale. This is especially true for very fast reactions where the reaction rate is much faster than the rate of mixing, and for reactions where molecular diffusion determines the rate of reaction. Such micro-chemical synthesis is mainly governed by a mechanism of molecular diffusion, of which its rate correlates with the product of the diffusion coefficient and the local concentration gradient. Notably, microfluidic reactors, in comparison with the bulk reactor, have higher space-time yields (product formed per reactor volume and time).

Small Volume Consumption. Microscale chemistry brings an advantage to the informatics section of pharmaceuticals and chemical process industries; That is so due to a distinguished cost-benefit in handling small volumes of expensive reagents where minimal amounts may be available for research & development. To gather the same (and in most cases, more) chemical information, microfluidic reactors consume far less reagent than bulk systems.

Green Credentials. Microfluidic reactors provide a host of the small instantaneous volumes mean that reactions involving reactive, toxic, or explosive intermediates for the benefit of dangerous chemistry. Furthermore, the high surface-area-to-volume ratios within the channel allow the rapid transport of heat during exothermic reactions and the enhancement of wall quenching in radical-mediated systems. Additionally, the miniaturized chemical reactions have taken the advantages of close monitoring, which brings a possibility to determine the reaction endpoint.

Point-of-use for Microfluidic Synthesis. It should be noted that the true product from microfluidic reaction systems would be information, rather than a more tangible substance or intermediate. Having said that, many industrial laboratories established microfluidic reactor division, where many aspects of a reaction are optimized, including kinetic reaction rate, temperature, and reagents flow. Microfluidic reactors are capable of controlling heat and mass transfer and can thus precisely define the process of particle nucleation and growth. The integrated microfluidic system by offering miniaturization of the library formation revolutionized the execution of screening discipline and led discovery through in situ click chemistry more accessible, well-built, and cost-effective. The manifested record in the last few decades constitutes
a proof of principle for performing sequential chemical processes, multistep reaction, and parallel or combinatorial synthesis.

1.2 Microfluidic-Based Chemical Synthesis

The mainstream microscale reaction processes were established using continuous microchannel flow systems. Continuous flow chemistry offers many advantages ranging from controlled process conditions to high flow rates and mass throughput.

A continuous-flow microfluidic system based on an internally connected microchannel network is the most straightforward microfluidic configuration that can be constructed by the mechanical assembly of capillary tubes or lithographical fabrication of an on-chip microchannel network [11-13]. Generally, reagents and solvents are introduced into a continuous-flow microfluidic reactor by syringes/syringe pumps or back pressures through tubing connections, and products are collected on the other end of the reactor. The characteristics of a continuous-flow microfluidic system confer excellent efficiency to different chemical reactions. Practically, microfluidic systems have brought tremendous developments to synthetic chemistry, opening up new paths toward the desired product while granting an insight into the mechanism at the molecular level. The existing challenges of batch synthesis and possible solutions from microfluidics are summarized in Table 1-1.
Table 1-1 How could microfluidics help with synthetic chemistry?

<table>
<thead>
<tr>
<th>Operation</th>
<th>Existing challenges</th>
<th>Microfluidic solutions</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operation</td>
<td>The hand-operated operation, lengthy reaction time, high reagents consumption, poor reproducibility</td>
<td>Consecutive operation mode, short residence time, minimal reagent consumption, automation</td>
<td>Ref. [14, 15]</td>
</tr>
<tr>
<td>Regulation</td>
<td>Green credentials, e.g., heat, pressure, and toxic compounds</td>
<td>Close monitoring of reaction parameters and reagent delivery, rapid mixing and heat/mass transfer, immediate use of intermediates, minimum exposure to hazards</td>
<td>Ref. [16, 17]</td>
</tr>
<tr>
<td>Integration</td>
<td>Inadequate monitoring of reaction parameters and conditions</td>
<td>Miniaturized structure, ease of fabrication, accurate and optimized control temperature, pressure, flow path, flow rate, residence time</td>
<td>Ref. [18, 19]</td>
</tr>
<tr>
<td>Integration</td>
<td>The necessity of External apparatus for product detection</td>
<td>inline analytics for real-time monitoring</td>
<td>Ref. [20]</td>
</tr>
<tr>
<td>Integration</td>
<td>Low screening/optimization performance</td>
<td>High-throughput chips, automation, program control, self-optimization</td>
<td>Ref. [21, 22]</td>
</tr>
</tbody>
</table>

Microfluidic systems have been manufactured from a wide range of materials, including polymers, glass, silicon, ceramics, and steel. The choice of materials depends on the chemistry to be performed, operating conditions required for a system, and ease of fabrication. Mechanical micromachining, stamping, and LIGA techniques can be used to create metal microchannels. Metal devices, Figure 1-4(a) has high temperature/pressure resistance, but most metals suffer from incompatibility with strong acids. Ceramic microstructures, Figure 1-4(b), have advantages of being chemically inert and stability at elevated temperatures. Nonetheless, the Microfabrication of ceramics is sophisticated because of the need to balance shrinkage with thermal expansion during
firing. Polymer-based devices, such as those based upon poly(dimethylsiloxane) (PDMS), Figure 1-4(c), and SU-8, take advantage of rapid prototyping and fabrication, but at the cost of dissolving in or swelling by common organic solvents. Recently, fluoropolymer integrated devices have also shown utility due to chemically inert properties. However, the bonding between glass and fluoropolymers or other polymers is weak result in restricted operation at elevated pressures. Alternatively, fluoropolymer capillaries may be utilized but also have temperature constraints. Glass is still a favorite material for the chemist, who is familiar with its chemical robustness and optical transparency, Figure 1-4(d). However, the isotropic etching used to realize most channels in glass could limit the feature density on-chip. Silicon also has much to offer because of its electrical properties and compatibility with a multitude of fabrication processes, including the integration of electrical circuits. Silicon-based microreactors, Figure 1-4(e) and (f), are readily fabricated using photolithography, wet etching, and deep reactive ion etching (DRIE) techniques. The high heat transfer coefficient of silicon also allows precise control over reaction temperature. Cost and device failure with strong bases can, however, be potential limitations.

Figure1-4 Examples of microfluidic systems fabricated from (a) metal [23] (b) ceramic [24] (c) PDMS, (d) glass [25] and (e, f) silicon.
Microscale synthesis via continuous-flow microreactors has been reported in both configurations of single-phase continuous-flow and emulsion (two-phase) micro-droplet/segmented-flow microreactors, shown in Figure 1-5. In one standard multiphase system, the continuous phase is oil, which wets the walls of the microfluidic channel, and the droplets are made of the aqueous synthesis mixture [26]. In the case of gas-liquid reactions or reactions in anhydrous solvents, gas-liquid segmented reactors have also been developed: In such reactors, the droplet phase consists of discrete bubbles of a gas within a continuous liquid phase. Such reactors can be convenient for kinetic studies; for example, Laval et al. recently developed a droplet-based microfluidic system that allows the droplets to be stored on-chip and to control precisely their temperature in such a small volume [27].

![Figure 1-5](image)

Figure 1-5 (a) Microsynthesis by encapsulated mixing in discrete liquid plugs. (b) a continuous-microfluidic system benefits from gas-liquid slugs. Reproduced from [28]

**Some Prior Studies in Continuous Microchannel Flow.** Lob et al. [29] performed fluorination of toluene in microchannel reactors made of silicon. Miller et al. [30] showed rapid formation of amides via carbonylative cross-coupling of aryl halides with benzyl amine using a reaction channel. Both cases achieved the higher yields than conventional batch reactions within the same time periods. Other examples include Suzuki cross-coupling, [31] Wittig olefination reaction, [32] nitration of benzene, and tripeptide synthesis, [33] Kobayashi et al. [34] successfully reported the inorganic synthesis of high-quality CdSe nanocrystals, and chemical processes involving highly reactive intermediates were executed with superior reaction selectivity [35, 36].

**Limitations.** Despite of successful demonstration from prior studies, microchannel-based approaches suffer from several limitations. For example, clogging of the channels by products or
byproducts may cause the difficulty of maintaining a constant hydrodynamic pressure, thus stable flow [37]. Requirement of complex flow network and cross-contamination due to unwanted diffusion through channels are also concerned. Moreover, solvent-swapping processes pose very challenging problems in microchannel reactors. Another drawback of microchannel reactors presents in combinatorial chemistry—a powerful tool for lead compound discovery and optimization of new drugs and materials. Since a combinatorial synthesis through either batch or flow reactors requires as many reactors as the number of all possible combinations of reactants, the reactor system tends to be excessively complex.
Chapter 2

Electrowetting-on-dielectric (EWOD) digital microfluidic device (DMF)

2.1 Introduction

Electrowetting-on-dielectric (EWOD) digital microfluidic device (DMF) is an alternative approach to the continuous microfluidic system. In such microsystems, droplets containing reagents and samples are manipulated on 2D planar chips-contrary to microfluidic channels. The tremendous growth is primarily due to the precise control of quantified reagents, highly efficient heat transfer, particularly in case of the exothermic reaction, less volume consumption of toxic or expensive agents, no dispersion due to diffusion-the droplet interface limits the extension of diffusion- no or negligible cross-mixing, simplified fluidic manipulation, improved reaction profiles, and enhanced selectivity compared to macro-scale reactions. The EWOD DMF has a capacity to host the arrays of droplets-each may contain different reagents \( A_1 \ldots A_n, B_1 \ldots B_m \) and control and mix them depending on an appointed protocol where this concept allows the possibility of executing multistep reaction and parallel or combinatorial synthesis. Scholars have put to use these unique characteristics of EWOD microfluidic devices to perform on-chip chemical reactions; examples of chemical synthesis performed in water, organic solvent, and ionic liquids can be found in the following chapter 4.

Despite the successful utilization of EWOD-based microsystems in biotechnology [37-39], its application to organic reactions has been severely limited because most of the common solvents used for synthetic organic chemistry are not operable on the EWOD device. In this regard, the very first requirement is to have electronically-responsive fluids so that they can be actuated on-chip through the fluidic functionality (transporting, merging, mixing, and dispensing). To this end, we started investigating the movability of liquids on an EWOD platform, which shaped the following chapter 3.

Researchers have taken advantages of these unique features of EWOD microfluidic devices to conduct on-chip chemical reactions, e.g. reactions in ionic liquid droplets [40], and synchronized synthesis of peptide-based macrocycles, shown in Figure 2-1 [41]. Another example is a work demonstrated by Keng et al. [42] for the synthesis of radiotracers utilizing the EWOD DMF device,
shown in Figure 2-2; They used a mixture of 1:4 DMSO:MeCN (vol/vol) instead of pure MeCN to not only overcome the movability issue but also increase the time operation due to low volatility of DMSO.

Figure 2-1 Top and side views of the digital microfluidic device used for peptide-based macrocycle synthesis [41].

Figure 2-2 (a) Integrated concentric heaters on an EWOD microchip, (b) schematic side view of a reaction droplet sandwiched in the EWOD device [42].
Of note, that all these reactions on EWOD chip mentioned above utilized solvent fluids that are movable by EWOD actuation. However, contemporary organic synthesis generally requires non-polar or polar aprotic solvents, and their poor movability in an EWOD chip has been a long-standing problem.

2.2 Electrowetting principles

In recent years, miniaturization has been given much importance to realize the capabilities of Lab-on-a-Chip devices. Less diffusion distance, higher surface to volume ratio facilitate achieving high reaction rates for chemical and biological applications. Portability and flexibility are two other features that can be utilized in these devices. In microscale, surface tension forces dominate over gravitational and other body forces. Electrowetting on dielectric is one of the methods to utilize this [43, 44]. In electrowetting, nano to pico liter sized droplets can be handled by manipulating the wetting behavior of conductive liquid droplets placed on a conductive solid surface by applying voltage on that surface. The solid surface is generally coated with a dielectric layer to prevent electrolysis. Air or another liquid immiscible with the droplet liquid serves as a medium. The EWOD principle can be described using Figure 2-3. When voltage is applied, charge accumulates along the dielectric-liquid interface and an electric double layer is formed. Because of the accumulated charges, the effective solid-liquid surface tension is reduced. To balance the interfacial energies at the three-phase contact line, the apparent contact angle is also reduced. The relation between the new contact angle and the applied voltage is given by the Lippman-Young equation:

$$\cos \theta = \cos \theta_o + \varepsilon_o \varepsilon_r \frac{V^2}{(2 \gamma d)}$$  \hspace{1cm} (2.1)

Here $\theta$ and $\theta_o$ are the contact angles corresponding to applied voltage $V$ and 0 respectively, $\varepsilon_o$ is the vacuum permittivity, $\varepsilon_r$ is the dielectric constant, $d$ is the thickness of dielectric layer and $\gamma$ is the surface tension co-efficient between liquid and air.
In an EWOD device, an array of electrodes are made and droplet is placed on one of these electrodes. Now by applying voltage to the next electrode, the droplet can be moved to that electrode. Any number of these operations can be performed to move the droplet along any desired path. For a typical EWOD device with parallel configuration, the droplet is sandwiched between a top and a bottom plate. The top plate accommodates the ground electrode. The bottom plate holds the operating electrodes and the dielectric layer. Both have hydrophobic layers on the surfaces which are in contact with the liquid. The open configuration EWOD devices does not contain any top plate.

2.3 EWOD device design

In an EWOD device, to design the electrodes' layout for the bottom plate, and/or in some cases for the top plate as well, we utilized L-Edit software. The actuation electrodes, reservoir, and wires were drawn concerning the test protocol and the potential application. The design is then patterned on chromium coated glass mask. Usually, if the top plate does not contain anything such as sensors or microheaters, there is no need for designing the top chip. Figure 2-4 illustrates a device layout used in the later studies in the following chapters.
2.4. EWOD device fabrication

Atypical EWOD device consists of two parallel plate where a liquid droplet is sandwiched between the plates, as shown in Figure 2-5. The stack of layers in an EWOD device are fabricated utilizing different fabrication techniques including deposition, photolithography, and etching. First, using acetone, isopropanol, methanol and DI water the Indium tin oxide (ITO) coated wafer plates was cleaned followed by blow drying with $N_2$ and the dehydration process on a hotplate at 150 °C for 5 min, Figure 2-5 (a). Then, utilizing a spin coater with the recipe of ramping the speed spin to 500 rpm with 100 rpm/s for 5 s; ramping with 900 rpm/s to 4000 rpm for 30 s, the hexamethyldisilazane (HMDS) was coated on the substrate to promote the adhesion of photoresist (PR). Moreover, the wafer baked at 150 °C for 90 s to evaporate the solvent present in the HMDS solution. Then, 1.2 μm uniform thickness of a positive PR (Microchem S1813, MicroChem Corp, Newton, MA, USA) was spin coated on the wafer utilizing the following recipe; Spin speed was ramped up to 500 rpm by 100 rpm/s for 5 s; ramping by 900 rpm/s to 3000 rpm for 30 s. To remove the excess solvent and to anneal the PR, soft bake was done at 115 °C for 60 s.

As shown in Figure 2-5 (b), the photolithography was done with the exposure does of 140 mJ/cm² using the backside-aligner (OAI 806MBA) followed by the post baked at 110 °C for 1 min. After that, using a developer (Microchem, MF-319), the wafer was developed and dehydrated at 115 °C for 2 min. To etch the ITO layer, a solution consists of Hydrochloric acid (HCl), Nitric acid (HNO₃) and DI water (H₂O) (wt % - 20% HCl, 5% HNO₃, 75% H₂O or vol % - 8:1:15, HCl:HNO₃:H₂O) was used and the wafer was immersed in the solution at 55 °C for 2.5
min. Then, the wafer went through stripping PR (Remover 1165, Microchem) and dehydration at 150 °C for 2 min. After that, 5µm uniform thickness of (SU-8 2005, Microchem) as dielectric layer was spin coated on the wafer with the subsequent recipe; Spin speed was ramped up to 500 rpm by 100 rpm/s for 5 s; ramping by 900 rpm/s to 2000 rpm for 30 s. To harden the dielectric layer, the wafer was baked at 70 °C for 1 min, 100 °C for 3 min and cooled down at 70 °C and the room temperature for 1 min and 2 min respectively, illustrated in Figure 2-5 (c). After that, the exposure was done with a light does of 140 mJ/cm² followed by post baked at 70 °C for 1 min, 100 °C for 3 min and also hard baked at 150 °C for 5 min.

As it is evident from Figure 2-5 (d), Teflon AF1600S (Du Pont powder dissolved in Fluorinert FC-40 Sigma-Aldrich) as a hydrophobic layer was spin coated on top of the dielectric layer with the following recipe; Spin speed was ramped up to 1000 rpm by 300 rpm/s for 30 s, resulting in 300 nm thickness. Then, the wafer baked at 70 °C for 1 min, 100 °C for 1 min and 180 °C for 3 min. To suppress the chance of voltage-break down, another layer of Teflon was spin coated followed by annealing at 70 °C for 1 min, 100 °C for 3 min and 180 °C for 15 min. For a top chip in the EWOD device, Teflon hydrophobic layer was spin-coated and followed by post-baking, as mentioned above, shown in Figure 2-5 (e). Afterward, small strips of Kapton tape (DuPontTM Kapton® HN polyimide film) are attached to the bottom chip to create the gap with the top chip, as illustrated in Figure 2-5 (f). Finally, Figure 2-5 (g) shows the assembly of an EWOD device by sandwiching fluid droplet.
Figure 2-5 Process steps for fabricating and assembly of an EWOD device. (a) Wafer cleaning using acetone, isopropanol, methanol and DI water. (b) Photolithography and wet etching of ITO layer. (c) Spin coating and deposition of SU-8 dielectric layer. (d) Spin coating and deposition of Teflon hydrophobic layer. (e) Fabrication of an EWOD top chip by Teflon coating ITO. (f) Placement of small strips of Kapton. (g) Assembly of an EWOD device by sandwiching fluid droplet.
2.5 Experimental setup for EWOD device

Figure 2-6 shows the entire experimental setup for the EWOD device operation. The EWOD device is connected to the control boards being attached to the Power generator, followed by the connection to NI DAQ. The microscope records the top view of the sandwiched droplet in an EWOD device. Finally, the computer via a customized LABVIEW program controlled the actuation of electrodes. A sinusoidal AC voltage used for this experimental setup is the Agilent Arbitrary Waveform Generator and the TEGAM High-Voltage Amplifier (model23400).

Figure 2-6 The experimental setup for EWOD chip operation.
Chapter 3

Electromechanical model to predict the movability of liquids in an electrowetting-on-dielectric microfluidic device

3.1 Introduction

There have been ever growing needs of various types of microfluidic liquid handling principles to meet the requirements of diverse applications in biotechnology, pharmaceutical research and clinical diagnostics. While flows (either continuous or droplet) through microchannels present their strengths such as high-throughput process capabilities and simple device fabrications, droplet flow over a surface by an electric field has attracted much attention as a promising tool due to the absence of complex mechanical components (e.g., pumps and valves) to create and control flows, no dead volume of reagents, precise fluids control in volume, flow rate as well as in space and time, and easy integration with controlling circuits [43-45]. Droplet flow by electric field is also widely known as electrowetting-on-dielectric (EWOD) microfluidics and it has emerged as a preferred method by providing the possibility to lower the operational voltage [46] and the ability to perform the fluidic functions (i.e., generating, cutting, transporting and merging [43]).

In recent years, many researchers have studied the forces acting on the liquid droplets in EWOD microfluidic devices. Jones et al. [47, 48] developed a RC circuit model to study the frequency-dependent relationship between EWOD and dielectrophoretic (DEP) mechanisms. Kumari et al. [49] utilized a simplified RC circuit model to demonstrate three different regimes of electromechanical actuation including electrowetting by a DC potential and a low and high frequency AC potentials. Chatterjee et al. [50] calculated the force on the insulating and the conducting liquids using a RC circuit. They showed the dominance of EWOD and DEP forces for the different liquids. Chen et al. [51] using a RC circuit of the sandwiched droplet between two-plate devices investigated the effects of gap height, applied frequency and fluid conductivity on the threshold voltage.

Although all these works gave much insight into electromechanical modeling of EWOD devices, they have not considered two very important features. One is contact angle hysteresis
(CAH). The importance of CAH has been discussed in many reports [52-55]. Walker et al. [56, 57] demonstrated that ignoring the CAH leads to simulations that predict droplet motion up to ten times faster than that seen in the experiments. In the numerical modeling of droplet actuation, Arzpeyma et al. [58] reported the difference between the numerical and experimental results due to the effect of CAH. In addition, many others demonstrated and reported that EWOD operation in oil medium resulted in smoother motion of droplets and lower requirement of the threshold voltage than in air medium due to the fact that the oil medium reduces CAH of the droplet as well as the interfacial tension [57, 59, 60].

The other missing feature is phase angle when AC potential is used. Although both DC and AC voltages have been used to operate EWOD devices, owing to the reduction of the possibility of chemical reactions [61] and the lower CAH [62], AC voltage has been used much more frequently than DC. In AC circuits, the voltage and the current vary and alternate over time. Voltage and current sinusoids are usually out of phase in AC circuits. In an AC circuit consisting of several capacitors and resistors, the fully charged state of each capacitor does not necessarily occur in a phase. However, per our best knowledge, in the analysis of the equivalent circuit of the sandwiched droplet in the EWOD devices, phase angle has not been considered.

3.2 Objectives

This study presents an electromechanical model in which the contact line friction force is modeled in terms of CAH. Using this new model with the consideration of phase angle shift in different dielectric layers of EWOD devices, we estimated net mechanical force exerted on a moving droplet, hence the movability of liquids. The results show a strong dependence of net forces on the frequency of the applied voltage. In the later part of the study, the model was used to explain the effect of important fluid property parameters such as surface tension, electric conductivity and dielectric constant to the droplet movability.
3.3 Theory

3.3.1 Modeling of the friction on the top and bottom plates

A general RC circuit model for an EWOD device and the droplet is shown in Figure 3-1(a). Each dielectric/hydrophobic layer on the top and bottom plates is considered as a pure capacitor [48, 50, 63]. Liquid is modeled as a resistor and a capacitor in parallel to provide different behaviors of liquids [48, 50, 63]. This RC circuit model has been widely used in previous studies, but contact line friction has not been included in this model. Since a portion of electric energy input to the system is dissipated through friction, we decided to model frictions on top and bottom plates as resistor elements and to add them to the RC circuit as shown in Figure 3-1(b). The following describes how to find the expression of each resistor.

In the EWOD devices, when a voltage is applied, the charges accumulate at the solid-liquid interface and it results in the decrease of the contact angle of the droplet. The voltage-induced contact angle change follows Lippmann-Young’s law:

\[ \cos \theta = \frac{\varepsilon_0 \varepsilon_r}{2 \gamma} V^2 + \cos \theta_0 \]  

(3.1)

Where \( V \) is the applied voltage, \( \gamma \) is the liquid-vapor surface tension, \( \varepsilon_0 \) is the permittivity of free space, \( \varepsilon_r \) is the dielectric constant of the bottom layers, \( d \) is the thickness of the bottom layers, and \( \theta \) and \( \theta_0 \) are the contact angle of liquid before and after voltage is applied, respectively.

Berthier et al. [64] utilized the capillary line force density on a triple line and calculated the force on a droplet in the x direction resulting from the applied voltage in an EWOD device. The net force on a droplet is the difference between EWOD actuation force and friction force due to contact angle hysteresis. The actuation force was modeled as the capillary force due to contact angle change by EWOD operation, while the friction force was modeled as the capillary force due to contact angle hysteresis.

\[ F_{net} = F_{actuation} - F_{friction} = W \gamma (\cos \theta - \cos \theta_0) - W \gamma \alpha (\cos \theta + \sin \theta - \cos \theta_0 + \sin \theta_0) \]  

(3.2)
Where $W$ is the width of electrode and $\alpha$ is the contact angle hysteresis. Combining Eqs. (3.1) and (3.2), we derived the friction force for the top and bottom plates in an EWOD device. Note that due to the negligible voltage drop across the top insulation layer, there is no contact angle change in the top plate [43]. Therefore, the friction forces on the top and bottom plates are:

$$F_{\text{top}} = -W \gamma \alpha (2 \sin \theta_0)$$  \hspace{1cm} (3.3)$$

$$F_{\text{bottom}} = -W \gamma \alpha \left( \frac{\varepsilon_0 \varepsilon_r}{2d} V^2 + \left( \sqrt{1 - \left( \frac{\varepsilon_0 \varepsilon_r}{2d} V^2 + \cos \theta_0 \right)^2} + \sin \theta_0 \right) \right)$$  \hspace{1cm} (3.4)$$

Figure 3-1 (a) Non-friction RC circuit model of EWOD device, (b) The proposed RC circuit model including the friction, and (c) the equivalent circuit of (b) in impedance form.

The equivalent circuit of Figure 3-1(b) is shown in Figure 3-1(c) where, impedances $Z_1$ through $Z_5$ indicate the impedances of the top dielectric layer, liquid, the bottom dielectric layer, the friction on the top surface, and the friction of the bottom surface, respectively.

### 3.3.2 The system of four nonlinear equations for the EWOD equivalent circuit

By equating the energy consumption of friction impedances ($Z_4$ and $Z_5$) to the work done by the friction forces (Eqs. (3.3) and (3.4)), the following two nonlinear equations are obtained:

$$\frac{V_{\text{rms}}^2}{Z_4} \times T = -(2W \gamma \alpha \sin \theta_0) \times X$$  \hspace{1cm} (3.5)$$
\[
\frac{V_{2rms}^2}{Z_5} \times T = -W Y \alpha \left( \frac{\varepsilon_0 \varepsilon_r V^2}{2 dy} + \left( \sqrt{1 - \left( \frac{\varepsilon_0 \varepsilon_r V^2 + \cos \theta_0}{2 dy} \right)^2} + \sin \theta_0 \right) \right) X
\]

(3.6)

where, \( X \) is the length of the liquid-filled region of the activated electrode, and \( T = \frac{1}{f} \) is the time period of one cycle with frequency \( f \).

In addition, according to the equivalent circuit shown in Figure 3-1(c), by applying Kirchhoff's current law over the node \( V_1 \) and \( V_2 \) we obtained Eq. 3.7 and Eq. 3.8 respectively.

\[
\frac{V - V_1}{Z_1} = \frac{V_1}{Z_4} + \frac{V_2 - V_1}{Z_2} \quad \frac{V - V_1}{Z_1} = \frac{V_1}{Z_4} + \frac{V_2 - V_1}{Z_2}
\]

(3.7)

\[
\frac{V_2}{Z_3} + \frac{V_2}{Z_5} = \frac{V_1 - V_2}{Z_2}
\]

(3.8)

Now, this set of four nonlinear equations was solved for 4 unknown variables: friction impedances \( Z_4 \) and \( Z_5 \) and voltages \( V_1 \) and \( V_2 \).

3.3.3 Computing the electromechanical net force with consideration of phase angle

An EWOD device is an electromechanical system where electrical energy converts into mechanical energy to induce droplet motion. In the conversion process, some energy dissipation takes place. The energy balance equation of an EWOD device is represented by:

\[
E_{\text{electrical input}} = E_{\text{net}} + E_{\text{friction}} + E_{\text{etc}}
\]

(3.9)

Where the energy provided to the system (\( E_{\text{electrical input}} \)) is equal to sum of the energy that drives the droplets motion (\( E_{\text{net}} \)), energy dissipated through friction (\( E_{\text{friction}} \)), and other forms of energy dissipation such as thermal dissipation through joule heating or viscous dissipation (\( E_{\text{etc}} \)).

The integration of the instantaneous power (\( P_{\text{electrical input}}(t) \)) over one cycle of AC voltage provides cycle-averaged electrical energy. We can compute cycle-averaged electrical energy using the formula below [65, 66]:

\[
E_{\text{electrical input}} = \int_0^T P_{\text{electrical input}}(t) \, dt
\]

(3.10)
\[ P_{\text{electrical \ input}}(t) = V(t) \cdot I(t) = \frac{V^2(t)}{Z_{\text{tot}}} \]  

(3.11)

Where \( Z_{\text{tot}} \) is the total impedance of the equivalent circuit Figure 3-1(c). When we used the voltage input and \( Z_{\text{tot}} \) calculated in section 3.3.2, we could see that voltage and total impedance are out of phase, hence voltage and current are out of phase as well.

Similarly, the cycle-averaged energy dissipation can be derived by the integration of the instantaneous power consumed by the impedances [65].

\[ E_{\text{friction}} = \int_0^T P_{\text{friction}}(t) \, dt \]  

(3.12)

\[ P_{\text{friction}}(t) = P_4(t) + P_5(t) = \frac{V_4^2(t)}{Z_4} + \frac{V_5^2(t)}{Z_5} \]  

(3.13)

Where \( P_4(t) \), and \( P_5(t) \) are the instantaneous power of \( Z_4 \) and \( Z_5 \), respectively. Also, the \( V_4 \) and \( V_5 \) are the voltage drop across the friction impedances \( Z_4 \) and \( Z_5 \) respectively.

When other forms of energy dissipation than friction are neglected \( (E_{\text{etc}} = 0) \), the difference between the total energy provided into the system and the energy dissipated by friction during one cycle determined the net energy to drive the droplet \( (E_{\text{net}}) \). Since this energy should be the same with the work done by the net force on the droplet over the distance \( X \), the net force can be found as followed.

\[ E_{\text{net}} = \int_0^X F_{\text{net}} \, dx \]  

(3.14)

\[ F_{\text{net}} = \frac{\partial E_{\text{net}}}{\partial x} \]  

(3.15)

Based on the electromechanical net force arising from the circuit Figure 3-1(b) which includes the friction and the consideration of phase angle in the computation, the precise prediction of movability can be achieved.
3.4 Experiment

3.4.1 Contact angle hysteresis measurement

The difference between the advancing and receding contact angle is known as the contact angle hysteresis (CAH) [52-55, 64, 67, 68].

\[ \theta_a - \theta_r = \alpha \]  

(3.16)

where \( \alpha \) is CAH and \( \theta_a, \theta_r \) are the advancing and receding contact angles, respectively. We measured \( \alpha \) of deionized water (DI water) and Tetrahydrofuran (THF) over a Teflon coated glass plate surface using two different methods [52, 67, 69]: the evaporation method and the tilted plane method. The details of the CAH measurement are described in the supporting information A. The measured CAHs are 8.66° and 3.98° for DI water and THF, respectively. These measurements are consistent with the values reported by Berthier et al. [64].

Figure 3-2 (a) The curvature of DI water droplet at the captured frame just before sliding of the droplet. The measured advancing and receding angle are 126.87° and 109.10° respectively. (b) The curvature of THF drop at the captured frame just before sliding of the droplet. The measured advancing and receding angle are 62.86° and 56.63° respectively.
First, we measured the value of CAH using the tilted plane method. In the tilted plane method, a bottom plate of the EWOD device was located on an inclined substrate mounted on an angle measurement (Digital sliding T-Bevel). 720 nL droplet of DI water/THF was dispensed on the EWOD bottom plate. Then, the substrate was gently inclined until the sessile drop begins to slide down. The motion of the curvature of droplet was recorded using a high-speed camera (WATEC high resolution, NAVITAR lens) syncing to (First Ten Angstroms, FTA32) software. At the captured frame just before sliding the drop and with help of the ImageJ software, the values of advancing and receding angles were evaluated. The difference between the advancing and receding contact angle is known as the contact angle hysteresis (CAH) [52-55, 64, 67, 68]. The measured CAHs are 8° and 3° for DI water and THF, respectively.

In addition, the evaporation method was used to measure the CAH of DI water. In the evaporation method, a bottom chip of the EWOD device was located on a goniometer setup (FTA1000 Analyzer System). 720 nL drop of DI water was dispensed on the wafer and the curvature and triple contact lines were recorded utilizing a high-speed camera syncing to FTA32 software. Then, the receding angle and the length of the baseline of droplet were measured in sequential interval time period. As a sessile drop evaporates, first contact angle reduces while the baseline of the droplet is a constant value, and then the contact angle keeps a constant value while the droplet starts shrinking (shown in Figure 3-3). The difference between the receding angle in the first frame ($t = 0$ s) and the receding angle in the frame at which the droplet starts shrinking ($t = 780$ s), provides the contact angle hysteresis. The value of CAH of DI water based on the evaporation method turns out 8°. These measurements are consistent with the values reported by Berthier et al. [64].
The receding angle and the length of the baseline of droplet were measured in sequential interval time period. As a sessile droplet evaporates, first contact angle reduces while the baseline of the droplet is a constant value. The receding angle in the first frame \( t = 0 \) s and the receding angle in the frame at which the droplet starts shrinking are 122.67 and 113.81 respectively. Then the contact angle keeps a constant value while the droplet starts shrinking. (a) variation of the contact angle versus time. (b) variation of the baseline versus time.

### 3.4.2 Device operation

To measure movability of DI water and THF, 720 nL droplets of each liquid were placed in a device. The gap between the top and bottom plates of the device were kept at 180 µm for all tests. A sinusoidal AC voltage was provided by the Agilent Arbitrary Waveform Generator and the TEGAM High-Voltage Amplifier (Model 23400). The transition of the droplet was recorded using the FASTEC TS3 high-speed camera (1000 fps). Experimental videos are provided in supporting information C.
3.4.3 Measurement of the net force on a droplet

To measure the net force exerted on a droplet when it begins to move, we use image processing of experimental videos. Using the Tracker and ImageJ software, we evaluated the displacement of the centroid of the droplet ($\Delta X$) in two sequential frames just before and after the movement of the receding meniscus. This is because the actual motion of a droplet begins when its receding meniscus displaces. When the force is small, while the advancing meniscus tries to move, the receding meniscus remains stationary and the droplet fails to move ($\Delta X = 0$). After selecting frames of the beginning of the droplet motion, displacement of the centroid ($\Delta X$), the time between the sequential frames ($\Delta t$) (Figure 3-4. (c) and (d)) and kinematic Eq. (3.17) provide the acceleration ($a$) of the droplet.

$$\Delta X = u\Delta t + \frac{1}{2}a\Delta t^2$$  \hspace{1cm} (3.17)

where in this case, the initial velocity ($u$) is zero.

Eventually, the force on the droplet was calculated from the second law of motion.
Figure 3-4 The motion of DI water droplet toward the voltage activated electrode. (a) Before applying the voltage, (b) The advancing meniscus responds to the applied voltage, (c) Droplet deforms and stretches. The receding meniscus just begins to displace. (d) The second frame after the droplet began to move. The time difference (Δt) in this case is 1 ms [70].

3.5 Result and Discussion

The experimental measurements of net force on a droplet are plotted in Figure 3-5. Each experimental data point is the average of three attempts of measurement. Based on our visual observations from the experiment, we categorized droplet motions in three groups. In Group A, a droplet experiences non-zero net force acting on it so that the advancing meniscus responds to voltages. However, the net force is not enough to create the motion of entire droplet. In Group B, the net force on a droplet is large enough so that the droplet can travel to the next electrode.
However, the net force is still not enough to accelerate the droplet to the speed at which it can completely travel over next electrode within practical switching time (~ few seconds). In Group C, the net force on the droplet is large enough so that the droplet completes the travel over next electrode within few seconds. As shown in Figure 3-5, the net force greater than ~70 μN provides such motion. The experimental measurement shows the strong dependence of the net force on the frequency. High net forces are measured at the middle frequency range (bandwidth of droplet movability) while forces in the low and high frequency ranges are measured to be smaller. Interestingly, the same trend of net force is predicted by our new circuit model. The blue solid line in Figure 3-5 shows the simulated net force on the DI water droplet as a function of AC frequency arising from the circuit model Figure 3-1(b) with consideration of phase angle. As it can be seen, predicted forces show the same frequency dependence as the measured forces. However, some discrepancies between experimental measurement and model prediction happen mostly in the middle frequency region. This is because of the energy dissipated by deformation of droplet, which is not included in our equivalent circuit model. When the net force is larger (at middle frequency), droplet moves at the higher speed, which results in more viscous drag, hence more deformation of droplet.
Figure 3-5 Experimental measurements and simulated net forces on a DI water droplet at 100 V$_{\text{rms}}$ and different frequencies of input voltage. Dots are for experimental measurements. The blue solid line is simulated with the friction model and phase angle. The red dashed line is from the non-friction model with phase angle. The black dotted line is from the non-friction model without phase angle.

In previous study of modeling of droplet motion in EWOD device, Walker et al. [56, 57] found the necessity of CAH in the modeling and presented that a model including CAH improves the accuracy of prediction up to 10-20 times. Others [50, 58] reported a discrepancy between the simulation and experimental values, resulted from the lack of hysteresis contact angle in their modeling.

In our current study, the two circuit models – with and without of friction are compared in their net forces on the droplet. In Figure 3-5, the blue solid line represents the net force arising from the friction circuit model Figure 3-1(b) and the red dashed line is the net force resulted from the non-friction circuit model Figure 3-1(a). Both circuits were analyzed by considering the phase angle. Due to the small CAH of DI water on Teflon-coated surface, the difference between the net forces predicted by two circuits is small; however, in the later part of this study, it will be shown that the significance of the friction term and the difference between two circuit models would be more evident as the CAH and the surface tension increase. An extreme example of such case is the
electrowetting on a rough surface on which CAH is very large when it enters Wenzel’s regime [71]. This study reported that electrowetting-induced motion of droplet on a rough surface is irreversible because droplet contact line becomes effectively pinned on high CAH surface and the system lacks the energy necessary to move the contact line back to the initial position.

Figure 3-5 also demonstrates the significance of phase angle in the computation of the net force. The black dotted line provides the net force resulted from the non-friction circuit model Figure 3-1(a) with ignoring the phase angle in the calculation [50]. As it is evident, the ignorance of the phase angle in calculating of the stored energy in the EWOD system results in invalid electromechanical net force and unreliable prediction of droplet motion in different frequencies. Particularly, in low and high frequency ranges, the experimental net forces are about ten and four times lower than predicted one, respectively.

Figure 3-6. The voltage droplet across the bottom dielectric layer, liquid and Teflon layer on the top plate versus the time at frequency of 1KHz and source voltage of 100 $V_{rms}$.

The voltage drop across dielectric/hydrophobic layers and liquid are plotted in Figure 3-6 during a cycle of AC voltage application. As shown, the peak voltage drop of each layer occurs at
different times. The sum of the voltages without considering the phase angle does not equal the source voltage and this violates the Kirchhoff’s voltage law (KVL) [65].

This significance of phase angle in voltage drops across the different layers can explain why the highest value of net force is resulted in the intermediate range of frequency as shown in Figure 3-5. The electromechanical actuation force consists of two major forces – EWOD and DEP forces. The EWOD force is resulted from the change in solid-liquid surface energy induced by the voltage drop across the dielectric stacks. On the other hand, the DEP force is resulted from the polarization of gas-liquid interface induced by the voltage drop across the liquid. The maximum of each force may occur at different frequency (rather at lower frequency for EWOD and at higher frequency for DEP), however, the sum of two forces, therefore the electromechanical actuation force can be maximum at some intermediate range of frequency.

To investigate and validate the voltage response of the model, we altered the voltage from $40V_{rms}$ to $165V_{rms}$ while frequency was fixed at 1KHz, where the highest forces were resulted for DI water droplet. The measured and predicted net forces are plotted in Figure 3-7. First, let’s compare the two circuit models with friction (blue solid line) and without friction (red dashed line). Both lines resulted from the calculation with phase angle. Apparently, forces predicted by these two models do not show much difference. It is because of the low CAH of DI water on Teflon surface. As to be discussed later, however, this difference would be significant when a liquid has large CAH. Second, let’s compare force prediction by non-friction circuit model Figure 3-1(a) with and without phase angle. The red dashed line is the prediction with phase angle and the black dotted line is the prediction without phase angle. As shown, both predictions of net force seem fairly agree with experimental measurements. It is because varying the voltage at fixed frequency only causes the change in voltage drop across the impedance whereas varying frequency at fixed voltage change the impedance values in the circuit. Therefore, the significance of phase angle would be more evident in the frequency spectrum. Nevertheless, Figure 3-7 shows that ignoring the phase angle resulted in predicting lower net forces than the experimental values, which is physically impossible. There are many forms of energy dissipation ($E_{etc}$) during the experiment such as viscous drag, but these dissipations are not included in the equivalent circuit model. Therefore, the forces predicted by the model cannot physically be lower than the experimental values.
Figure 3-7 Experimental measurements and simulated net forces on a DI water droplet at 1 kHz and different $V_{\text{rms}}$ of input voltage. Dots are for experimental measurements. The blue solid line is simulated with the friction model and phase angle. The red dashed line is from the non-friction model with phase angle. The black dotted line is from the non-friction model without phase angle.

To validate models with another liquid, we chose the THF, a popular organic solvent in the chemistry laboratory and industry. Figure 3-8 shows the comparison between the simulated results and the experimental values. All the previous discussions regarding the results with DI water are valid for the results with THF. Additionally, our result shows the facile movability of THF in low frequency range which is consistent with the experimental observation of Chatterjee et al. [61]. In case of THF, one limitation was the variation of electrical conductivity due to the absorbance of moisture from the environment. We will show further that the fluid conductivity has a significant influence on the droplet motion. The instability of conductivity of THF causes the high standard deviation in some of the measurements.
Figure 3-8 Experimental measurements and simulated net forces on a THF droplet at 100 V\textsubscript{rms} and different frequencies of input voltage. Dots are for experimental measurements. The blue solid line is simulated with the friction model and phase angle. The red dashed line is from the non-friction model with phase angle. The black dotted line is from the non-friction model without phase angle.

Providing our new circuit model with phase angle computation can provide good prediction of liquid movability in an EWOD device, we studied the effect of other liquid property parameters on liquid movability.

First, the effect of CAH was studied. We increased CAH from $\alpha$ to $8\alpha$ while all other parameters are fixed and plotted the corresponding forces in Figure 3-9. Figure 3-9(a) shows the frequency response at fixed voltage at 100 V\textsubscript{rms}. As expected, the peak of the net force drops as CAH increases. If we set 70 $\mu$N as the minimum force to move a droplet, the droplet with $8\alpha$ cannot be operated in an EWOD device. It is also seen that the frequency bandwidth of droplet movability gets narrower as CAH increases. As shown in Figure 3-9(b), the threshold voltage to actuate the droplet increases as CAH increases.
Figure 3-9 (a) The frequency response of net force at 100 V\textsubscript{rms} and with different CAH. (b) The voltage response of net force at 1 kHz and with different CAH.

Similarly, effect of surface tension of liquid was studied by increasing surface tension while other liquid properties are fixed. Net forces are calculated and plotted in Figure 3-10. In EWOD devices, the interfacial tension between the working liquid and the medium fluid is another important parameter to determine the net force because interfacial tension affects to both actuation force and friction force. As seen in Figure 3-10(a), the peak of net force decreases and the frequency bandwidth of droplet movability narrows as interfacial tension increases. Figure 3-10(b) shows that the threshold voltage to actuate a droplet increases as interfacial tension increases. This
result well explains the observation from previous studies that the more facile actuation of the water droplets in silicon oil medium than in the air medium [39, 43, 46, 72, 73] due to the reduction of interfacial tension [59, 74-76].

Figure 3-10 (a) The frequency response of net force at 100 V_{rms} and with different surface tensions. (b) The voltage response of net force at 1 kHz and with different surface tensions.

In EWOD devices, the electrical conductivity of a liquid has a significant influence on the movability of the droplets. Chatterjee et al. [61] used the complex permittivity of the liquid to predict the movability of droplets. Chen et al. [51] investigated the liquid conductivity on the minimum actuation voltage of EWOD. Figure 3-11 shows the influence of liquid conductivity on
the frequency-dependent behavior of the force. It is shown that as the liquid conductivity increases, the frequency at which the peak of the net force occurs shifts to the higher value. However, the net force and the frequency bandwidth of droplet movability are not varying by conductivity.

Figure 3-11 The frequency response of net force at 100 V\textsubscript{rms} and with different conductivity of liquids.

It has been known that the liquid dielectric constant plays an important role in EWOD device. Jones et al. [47, 48] studied the electromechanical response of a conductive and dielectric liquid to AC electric field and demonstrated the EWOD and DEP electromechanical mechanisms dominate microfluidic actuation in the low- and high-frequency limits, respectively. Wang et al. and Chatterjee et al. [50] used RC circuit model and presented the dependence of force on the liquid dielectric constant. We reproduced their study using non-friction circuit model in Figure 3-1(a) without considering phase angle (see Figure 3-12(a)). For comparison, forces calculated from our friction circuit model in Figure 3-1(b) with phase angle are plotted in Figure 3-12(b).

As it can be seen from the Figure 3-12, the trend of the net force variation by dielectric constant is the same in both cases; before the inflection point, the higher the liquid dielectric constant, the smaller the net force; after the inflection point, the higher the liquid dielectric constant, the larger the net force. According to their experimental study of net force, Chatterjee et
al. [50] reported that frequency below the inflection point is EWOD force dominant regime while
frequency above the inflection point is DEP force dominant regime. When comparing Figure 3-
12(a) to (b), one can see that the influence of dielectric constant is underestimated in EWOD
regime and overestimated in DEP regime when phase angle is ignored in computation.

Figure 3-12 (a) The response of the non-friction circuit model without phase angle to variation of
the liquid dielectric constant in the frequency spectrum at the constant $100V_{\text{rms}}$ and 180µm height
(b) The response of the friction circuit model with phase angle to variation of the liquid dielectric
constant in the frequency spectrum at the constant $100V_{\text{rms}}$ and 180µm height.
It can be seen from Figure 3-12 that for some range of frequencies, the net force became negative values where it does not mean that the droplet moves in the opposite direction. As shown in Figure 3-1 (b), there is a non-zero resistance representing a friction force. When the driving force is very small (droplet is non-movable), because of non-zero resistance in the model, still there is a constant friction force resulting in a negative net force. This leads to an adaption of a responsive resistance to our model, checking a calculation of negative force for the non-movable droplet.

3.6 Conclusion

Contact line friction forces on the top and bottom plates in an EWOD device were derived. These friction forces were modeled as two resistors in the equivalent RC circuit. To solve the circuit, phase angle were considered. We calculated the cycle-averaged electrical energy provided to the system and the cycle-averaged energy dissipated through friction. From the energy balance, net force upon a droplet in an EWOD device was estimated. The simulated net force was compared with the experimentally measured net force and the model was validated. The proposed model and computation with phase angle demonstrated the existence of the bandwidth of frequency at which liquid droplets become practically movable. The model also provides the estimation of threshold voltage to operate an EWOD device. Using the proposed model, we investigated the influence of contact angle hysteresis, surface tension, electric conductivity and dielectric constant of fluids to the net force.
Chapter 4

On-chip organic synthesis enabled by engine-and-cargo in an electrowetting-on-dielectric
digital microfluidic device

4 Background

This paper presents the microfluidic chemical reaction using an electrowetting-on-dielectric (EWOD) digital microfluidic device. Despite of numbers of chemical/biological applications using EWOD digital microfluidic device, its application to organic reactions have been seriously limited because most of common solvents used for synthetic organic chemistry are not operable on EWOD device. To address this unsolved issue, we first introduce a novel technique of an “engine-and-cargo” system that enables use of non-moveable fluids (e.g., organic solvents) on an EWOD device. With esterification as the model reaction, on-chip chemical reactions were successfully demonstrated. Conversion data obtained from on-chip reactions were used in the demonstration of reaction characterization and optimization such as reaction kinetics, solvent screening, and catalyst loading. As the first step toward on-chip combinatorial synthesis, parallel esterification of three different alcohols were demonstrated. Results from this study clearly show that EWOD digital microfluidic platform is a promising candidate for a microscale chemical reaction.

4.1 Introduction

Over the past decades, microscale chemical reaction technology has been attractive in diverse areas of chemistry[5, 77, 78]. It allows the precise control of quantified reagents and highly efficient heat and mass transfer, because of a large interface-to-volume ratio—particularly in case of the exothermic reaction and mixing—, reduced consumption of toxic or expensive agents, improved reaction profiles, and enhanced selectivity compared to macro-scale reactions [79-81].

The mainstream microscale reaction processes were established using continuous microchannel flow systems. Lob et al. [29] performed fluorination of toluene in microchannel reactors made of silicon. Miller et al. [30] showed rapid formation of amides via carbonylative cross-coupling of aryl halides with benzyl amine using a reaction channel. Both cases achieved the
higher yields than conventional batch reactions within the same time periods. Other examples include Suzuki cross-coupling [31], Wittig olefination reaction [32], nitration of benzene, and tripeptide synthesis [33]. Despite of successful demonstration from prior studies, microchannel-based approaches suffer from several limitations. For example, clogging of the channels by products or byproducts may cause the difficulty of maintaining a constant hydrodynamic pressure, thus stable flow [79]. Requirement of complex flow network and cross-contamination due to unwanted diffusion through channels are also concerned. Moreover, solvent-swapping processes pose very challenging problems in microchannel reactors. Another drawback of microchannel reactors presents in combinatorial chemistry—a powerful tool for lead compound discovery and optimization of new drugs and materials [82]. Since a combinatorial synthesis through either batch or flow reactors requires as many reactors as the number of all possible combinations of reactants, the reactor system tends to be excessively complex.

A digital microfluidic platform using electrowetting-on-dielectric (EWOD) principle can be an alternative and/or complement a microchannel reactor. An EWOD digital microfluidic platform eliminates the necessity of predetermined channel network and mechanical pumps and valves. Since it is a droplet-based flow, it can prevent cross-mixing and cross-contamination. Each droplet plays as a batch reactor, which brings the feasibility of performing multi-step reactions that may involve with solvents swapping and combinatorial synthesis[43, 83, 84]. Researchers have taken advantages of these unique features of EWOD microfluidic devices to conduct on-chip chemical reactions, e.g. reactions in ionic liquid droplets [40], synthesis of radiotracers [42], and synchronized synthesis of peptide-based macrocycles [41]. Of note, that all these reactions on EWOD chip mentioned above utilized solvent fluids that are movable by EWOD actuation. However, contemporary organic synthesis generally requires non-polar or polar aprotic solvents, and their poor movability in an EWOD chip has been a long-standing problem.

Chatterjee et al. [61] experimentally assessed the movability of organic solvents and solutions in the EWOD system. In this study, many organic solvents such as cyclohexane, carbon tetrachloride, chloroform, and toluene cannot be displaced by EWOD actuations. Recently, Torabinia et al. [70] reported an electromechanical model that can predict the movability of a fluid by an EWOD device. This study showed that both the magnitude and the frequency of the operation voltage need to be tuned to obtain maximum force in an EWOD device. At the particular
experimental parameters, their model predicted that many indispensable organic solvents for organic synthesis are not movable, which concurs with the results by Chatterjee et al.

Nevertheless, a couple of efforts were made to operate non-movable fluids in EWOD device. Inspired by Brassard et al. [59], Li et al. [85] demonstrated manipulations of oil, organic, and gaseous chemicals in the aqueous shell. However, such configuration fails to host fluids having a lower surface tension than aqueous solutions. For instance, most of organic solvents have much lower surface tensions (~ 20 mN/m) than that of water, which does not allow them to be encapsulated in an aqueous shell. In addition, additional capillary tube settings were needed to create core-shell droplets, leading to unfavorable complexities of device design and fabrications. Another approach was to use dielectrophoretic (DEP) force to operate non-movable fluids; Fan et al. [86] reported the manipulation of a silicone oil droplet by DEP force in a typical EWOD device. However, exerting DEP force required extremely higher voltage than the EWOD operation does. In addition, fluids must have some specific dielectric properties to be manipulated by DEP force. In fact, according to Torabinia et. al. [70], most of essential organic solvents for chemical reactions are not movable, even in the range of frequency at which DEP force is dominant. These hindrances limit the scope of possible chemical reactions in an EWOD device.

As addressing abovementioned challenges, this paper introduces a new strategy of “engine-and-cargo” which enables an EWOD device to handle electrically non-responsive fluids such as organic solvents. This approach can allow to host a wide range of organic syntheses in EWOD devices. It has long been suggested that the true product from microfluidic reaction systems would be information, rather than a more tangible substances or intermediates [79, 87]. As like other types of microfluidic reactors, on-chip syntheses in EWOD devices would transform to parallel reactor systems (i.e., numbering up) rather than to scale up to production systems. Vast information obtained from fast and automated on-chip chemical reactions would be primarily utilized for reaction optimization and chemical discovery. Especially, when EWOD devices are capable of integrating on-chip chemical synthesis capacity with biological/biomedical functions such as cell culture, bio-separations, and biosensors this ideally permits to build a complete drug discovery platform.

In this study, we chose esterification as a model reaction to demonstrate the capability of
an EWOD device which can perform on-chip organic synthesis. Using conversion data obtained from on-chip reactions, characterization and optimization of the reactions were conducted. To confirm the soundness of on-chip data, same reactions were conducted in lab-scale. In addition, as the first step toward on-chip combinatorial synthesis, parallel esterification reactions of three different types of alcohols were established.

4.2 Problem Description

4.2.1 Engine-and-cargo system

An engine-and-cargo system harnesses a compound droplet of two immiscible liquids. An engine refers to the liquid that has the electrowetting properties; a cargo is the other one without electrowetting properties, thus non-movable in an EWOD device.

Figure 4-1 shows the formation and operation of an engine-and-cargo system in an EWOD device. In this example, ionic liquid ([bmim]PF₆) works as the engine and toluene is carried as the cargo. As shown in Figure 4-1(a), the ionic liquid has electrowetting properties so that it moves as responding to the applied voltage on electrodes underneath it, whereas toluene stays unresponsive under any magnitude and frequency of voltages [61]. When the ionic liquid droplet approaches to the toluene droplet, it is encapsulated by toluene spontaneously to minimize the surface free energy and forms a compound droplet (Figure 4-1(b)). Note that surface tension of ionic liquid (~ 40 mN/m) is higher than that of toluene (~ 20 mN/m). Figure 4-1(c) presents the motion of the engine-and-cargo system, arising from the electrowetting force exerted on the engine that carries the cargo by viscous drag force. Evidently, the designed engine-and-cargo system enables the use of toluene and other non-movable liquids in a typical EWOD device without any modification of device structures and architectures. In the later section of this study, it is reported that all the basic fluidic functions of an EWOD digital microfluidic device including dispensing, transporting, merging, and splitting of droplets are achieved with electrically non-responsive fluids. This technique has the potential to make substantial advances on biological and chemical protocols processed on EWOD digital microfluidic device.
Figure 4-1 The formation of the engine-and-cargo system of an ionic liquid([bmim]PF$_6$) as the engine (movable) and toluene as the cargo (non-movable). (a) Actuation of the engine towards the cargo, (b) encapsulation and formation of engine-and-cargo, and (c) the motion of an engine droplet by electrowetting operation leads motion of an entire compound droplet, thus fluidic functionalities of cargo droplet. Images from the top view of the EWOD device. Blue dye was added to the ionic liquid for the better visualization purpose only.

4.2.2 Esterification Reaction

Esters are one of the important classes of organic molecules that are widely used in synthesis of fine chemicals, drugs, food preservatives, perfumes, plasticizers, and pharmaceuticals [88-90]. In a biological aspect, acetylation is one of important protein modification methods in cell biology that has an impact on gene expression and metabolism [91]. There are high demands of rapid, simple, and environmentally friendly protocols for the microscale esterification of alcohols for facile production of a wide variety of esters for medicinal and biological applications [92-94]. Hence, the esterification in microchannel reactors has been studied comprehensively [87, 95-99].

In this study, we chose esterification of alcohols with acetic anhydride as our model reaction to demonstrate the on-chip organic synthesis capabilities of an EWOD digital microfluidic device. Total 60 tests of 20 different conditions of esterification reactions of secondary alcohols
with acetic anhydride were carried out on-chip. The esterification of menthol is shown in Figure 4-2. A traditional macroscale esterification (e.g., flask-based protocol) involves aliquoting, introducing, and mixing reagents, followed by quenching the reaction at controlled time. Instead, on EWOD chip, a reaction can be initiated by generating reagents droplets, transporting, and merging droplets, and at the end of the processes the reaction is quenched by merging the reacting droplet with the quenching agent droplet.

![Chemical reaction](image)

Figure 4-2 Model esterification reaction using menthol, acetic anhydride (Ac2O), trimethylamine (Et3N), and DMAP in the presence of specific solvent.

**4.3 Experiment**

**4.3.1 Device fabrication and experimental setup**

All EWOD microfluidic devices used in this study were fabricated in the Shimadzu Institute Nanotechnology Research Center of the University of Texas at Arlington. Actuation electrodes in the bottom plate of an EWOD device were fabricated by photolithography followed by wet etching of an indium tin oxide (ITO) layer (100 nm) coated on a glass wafer. The dielectric layer (SU-8, 5µm) and the hydrophobic layer (Teflon, 300 nm) were spin-coated and oven baked. The details of the fabrication steps can be found elsewhere [70, 100] and in section 2.4.

The EWOD operation voltages (100 Vrms at 1 kHz) were provided by Agilent arbitrary waveform generator and the TEGAM high voltage amplifier (model 23400). Desired sequence of turning on/off electrodes were applied through LabVIEW program. Droplet motions were recorded using Hirox KH-1300 digital microscope system.
4.3.2 Materials

(−)-Menthol (99%), phenol, ≥99.5% (GC), Benzyl alcohol anhydrous, 99.8%, trimethylamine, acetic anhydride, 4-(dimethylamino) pyridine (DMAP) (≥ 90%) were purchased from SIGMA-ALDRICH (USA). Toluene (Certified ACS), 1,4-dioxane, N,N-dimethylformamide, 1,1-dichloroethane (DCE), dichloromethane, (>99.8%) and 1-butyl-3-methylimidazolium hexafluorophosphate ([bmim]PF₆) (98+%) obtained from Fisher Scientific. Assorted food and egg dye purchased from Walmart (USA). All chemicals were analytical grade and used as received.

Before each test, reagents were placed in designated reservoirs as illustrated in Figure 4-3(b). Among the esterification reagents listed in Figure 4-2, alcohol (i.e., menthol), trimethylamine, and DMAP were identified as non-movable fluids on the EWOD platform. Alcohol (5 µmol), trimethylamine (10 µmol), and DMAP (0.1 to 5 mol %) were separately prepared with the same solvent of interest and mixed by the same volume. Then the mixed solution was placed at the ‘cargo’ reservoir, where they were sitting together with the ‘engine’ fluid (i.e., ionic liquid). On the other hand, acetic anhydride (acylating reactant, 10 µmol) and sodium bicarbonate (quenching agent) are movable in the EWOD platform due to their electrowetting properties, so that they were operated without the help of engine droplets.
Figure 4-3 (a) The experimental setup and the side view schematics of the EWOD chip operation, and (b) The EWOD chip electrodes layout and the placement of reagents on the chip at the beginning of each test. The cargo is the solution of a part of reactants and the catalyst - menthol, trimethylamine, and DMAP. The other reactant is acetic anhydride solution. The engine is [bmim]PF6. The quenching agent is saturated aqueous sodium bicarbonate. The surrounding medium is air throughout all the experiment.
4.3.3 Test Protocols

Both lab-scale and on-chip reactions were carried out to compare their conversion at each reaction condition.

4.3.3.1 Lab scale reactions and the NMR study details

General procedure for lab-scale reactions:

Menthol (31.2 mg, 0.2 mmol), Et3N (56 µL, 0.4 mmol), and DMAP were placed in a vial and dissolved in solvent (0.16 mL). Acetic anhydride (38 µL, 0.4 mmol) was added to the mixture. The septum on the vial was replaced by a screw cap with a Teflon liner, and the reaction mixture was stirred at rt. The reaction progress was monitored by either GC-MS spectrometry or 1H-NMR spectroscopy. The reaction was quenched by adding saturated aqueous NaHCO3. After being stirred for 10 min, the reaction mixture was extracted with Et2O three times. The organic layer was washed with water followed by saturated aqueous brine and dried over anhydrous Na2SO4. The resulting solution was concentrated under reduced pressure to afford a brown oil. 1H spectra were recorded on JOEL Eclipse Plus 500 (500 MHz) spectrometer with 10 second relaxation delay, yields are measured with Mesitylene as internal standard.

NMR studies:

Menthol (97.5 mg, 0.625 mmol), Et3N (126 µL, 1.25 mmol) and DMAP were placed in a Norell® pressure NMR tube and dissolved in Toluene-D8 (0.5 mL). Acetic anhydride (127 µL, 1.25 mmol) was added to the mixture and the tube was capped and agitated for 5 seconds. 1H spectra were recorded on JOEL Eclipse Plus 500 (500 MHz) spectrometer with 10 second relaxation delay. 1H-NMR chemical shifts are referenced to Toluene-D8 (7.00 ppm).

1H and 13C NMR spectra were recorded on JOEL Eclipse Plus 500 (500 MHz) and JEOL ECX 300 (300 MHz) spectrometers. 1H NMR chemical shifts are referenced to chloroform (7.26 ppm). 13C NMR chemical shifts are referenced to 13CDCl3 (77.23 ppm). 1H NMR and 13C NMR were processed with the iNMR software program.

GC-MS analysis:
GC-MS data were recorded on a Varian 450-GC/Varian 240-MS System. The methods used are noted parenthetically: 5029017 refers to: 2 min @ 50 °C – 20 °C/min – 3 min @ 290 °C (a 50 °C initial temperature that was held for 2 minutes followed by a 20 °C/min ramp to a final temperature of 290 °C that was held for 3 minutes for a total run time of 17 minutes).

4.3.3.2 Formation of an engine-and-cargo droplet

Each test began with forming an engine-and-cargo compound droplet. Figure 4-4 shows the sequence of formation of an engine-and-cargo droplet from the reservoir. First, EWOD forces let an engine liquid droplet dispensed from its reservoir puddle (Figure 4-4(a)-(b)) while the cargo puddles remained non-responsive to the sequence of activation voltages. As the engine droplet was dispensed and moved further away from the reservoir, cargo solution was drawn together due to the viscous drag force between the engine droplet and the cargo solution and a neck in the cargo solution formed (Figure 4-4(c)). Afterwards, the hydrodynamic instability at the cargo neck eventually let it pinch-off as shown in Figure 4-4(c) and (d). The completely detached droplet from the reservoir was an engine-and-cargo compound droplet (Figure 4-4(d)).

The volume of dispensed cargo solution was estimated by multiplying the footprint area (i.e., the area observed from the top view of the droplet) of cargo with the gap between top and bottom plates of the device. Note that the gap (= 100 µm) was well controlled and kept invariant throughout the entire device, so the variation in the footprint area was directly proportion to the variation in the droplet volume. The footprint area of cargo was measured using ImageJ software.
Figure 4.4 A sequence of formation of an engine-and-cargo compound droplet from the reservoir. (a) The initial state, (b) The viscous drag between the engine and cargo fluids stretched the cargo fluid, (c) The engine droplet was dispensed and it pulled the cargo further so that a neck formed in the cargo and the hydrodynamic instability grew, and (d) the cargo neck eventually pinched off and formed an engine-and-cargo. The snapshot in (a) was image processed to clearly present the electrodes layout while it shows the beginning of the dispensing process.

4.3.3.3 Image-based volume measurement

In the common practice, the volume of a droplet in an EWOD device is estimated by multiplying the footprint area (area observed from the top view of the droplet) with the gap between top and bottom plates of the device. Note that the gap between top and bottom plates is well controlled and kept invariant throughout the entire device, so the footprint area variation is directly in proportion to the droplet volume variation.

To measure the area of irregular shape of droplet footprint, ImageJ software was used. Since the cargo droplet in an engine-and-cargo compound droplet often has sharp corners and/or thin layers, its footprint area was measured indirectly:

\[
\text{cargo droplet area} = \text{(entire compound droplet area)} - \text{(engine droplet area)}
\]
Screenshots of area measurements using ImageJ software are shown in Figure 4-5.

![Figure 4-5](image) (a) The footprint area of the entire compound droplet and (b) The footprint area of the engine.

To calibrate and to validate the accuracy of this measurement technique, a known volume (= 1.0 uL) of cargo solution was pipetted into an EWOD chip. After forming of an engine-and-cargo, we calculated the corresponding footprints as described above (i.e. ImageJ-based technique) and obtained the volume of cargo to be 1.09 uL. This 9 % of error might be caused by multiple sources including the curvature of the droplet side, the offset of the gap between top and bottom plates. In order to get rid of these error sources, the calibration with two droplets were taken. Two known volumes (= 1.0 and 2.0 uL, respectively) of cargo solution were pipetted into an EWOD chip so that the volume difference of two droplets to be 1.0 uL. Using the Image J measurement technique, volume of each droplet was measured. The volume difference was measured to be $0.98 \pm 0.0365$ uL. This proves that the uncertainty of Image J measurement technique of droplet volume consistency is less than 1.7 %.
4.3.3.4 On-chip reactions

Figures 4-6(a)-(f) summarize the general procedure of each on-chip reaction test protocol. First, an engine-and-cargo was dispensed (Figure 4-6(a)). Then, a droplet of the other reactant solution was dispensed from its reservoir and it was merged with the previously dispensed engine-and-cargo (Figure 4-6(b)). As they merged, the esterification began and continued to proceed (Figure 4-6(c)). A droplet of a quenching agent was dispensed from the reservoir and delivered to the reacting droplet at the prescribed reaction time (Figure 4-6(d)). This let the reaction stop right at the prescribed reaction time (Figure 4-6(e)). Then, the reaction mixture (organic layer) was carefully drawn out using a syringe and diluted with DCM in a GC vial, which was subjected to the GC-MS analysis to measure a conversion of the reaction (Figure 4-6(f)).

While demonstrating capability of an EWOD device to carry out organic reactions, three reaction parameters—reaction time, type of solvents, and catalyst concentration—of on-chip esterification reactions of secondary alcohols with acetic anhydride were independently evaluated and summarized in Figure 4-6(g).

For the kinetic study, other reaction parameters (e.g., catalyst concentration and solvent) were fixed and the reaction were monitored from 10 s to 90 s. For the solvent screening, catalyst concentration and reaction time were fixed and 4 different solvents (i.e., toluene, dioxane, N,N-dimethylformamide, and DCE) were tested. For the optimization of catalyst loading, solvent and reaction time were fixed and the concentration of catalyst was varied from 0.1 to 1.5 mol %.

As simulating parallel synthesis, we performed esterification of three substrates including menthol, benzyl alcohol, and phenol with acid anhydride under basic conditions (Figure 4-6(g), the bottom row) on a single EWOD chip. In the beginning of the test, all reagents including 3 substrates were placed in the designated reservoirs of the chip. To be able to perform true parallel reactions (i.e., 3 reactions run simultaneously), complete automation of EWOD chip operation is necessary. Because the scope of this study did not include full automation of the device, 3 reactions were performed in series as each reaction followed the order of Figure 4-6(a)-(f). However, this does not limit the capability of an EWOD device to host parallel or combinatorial synthesis. The reactivity difference of these three substrates was examined over the first 30 s of each reaction.
Figure 4-6 (a-f) Sequential snap shots illustrating the steps of esterification on an EWOD device. (a) An engine-and cargo compound droplet is dispensed from the reservoir. (b) The other reagent solution is dispensed and merged with the engine-and-cargo droplet. (c) Esterification proceeds. (d) A quenching agent is dispensed from the reservoir and transferred to the reacting droplet. (e) As the quenching agent droplet and the reacting droplet merge, reaction is quenched and stops. (f) After quenching, the reaction mixture is drawn out using a syringe and is diluted with DCM within the GC vials. The diluted sample is placed in the GC-MS machine. (g) Design of experiment summarizes conditions of all reactions tested in this study.
4.4 Result and Discussion

4.4.1 Qualification of an engine-and-cargo system

According to Ren et al. [101], most of chemical and biological applications of lab-on-chip devices require the volume inconsistency below ± 5%. To assess the cargo volume inconsistency, we generated 26 engine-and-cargo droplets consecutively and characterized cargo volumes. During the tests, the cargo reservoir was kept refilled as it depleted. As it is evident from Figure 4-7(a), after 3 dispensing of droplets with the average volume (black dots), the fourth droplet (red dots) was dispensed with larger volume than the average. The standard deviation of droplet volume was ±13%. This is attributed to volume changes of the reservoir puddle after several dispensing of engine-and-cargo droplets. Guan et al. [101] reported that in an EWOD device a volume of a dispensed droplet has the dependency on a volume of the reservoir puddle. Based on this result, we carefully maintained the cargo reservoir filled properly during all reaction tests. Moreover, droplets with the larger cargo volume than the average were discarded before it proceeded to the reaction. As shown in the insets of Figure 4-7(a), the cargo area difference in average droplets and discarded ones was visibly noticeable and easy to screen. With visual screening, we could maintain the volume inconsistency of cargo as low as ± 3 % throughout all tests.

The engine fluid is not a reagent for esterification while it stays in the reacting droplet during the course of the reaction. It needs to be established that the presence of engine fluid would not interfere the reaction. Moreover, in this study, adding color dye to the engine fluid is desirable for clear visualization of experiments. To identify reaction compatibility of the engine and color dye, we briefly investigated three off-chip reactions; (1) the model esterification, (2) the esterification in the presence the ionic liquid, and (3) the esterification in the presence of ionic liquid and the green food dye. As shown in Figure 4-7(b) and (c), all three reactions showed full conversions and provided the ester product cleanly. Conditions for GC-MS spectrometry analysis can be found in Section 4.3.3.1
Figure 4-7 (a) The cargo volume of 26 engine-and-cargo droplets consecutively dispensed from a reservoir. Inset photos are screenshots of engine-and-cargo droplets during tests. Droplet with larger cargo volume than average were visibly noticeable and were discarded before reaction tests. (b) GC spectrometry results of 3 off-chip reactions. All three reactions showed full conversions and provided the ester product cleanly. (c) MS results confirm the identity of menthol (e.g., C1) before the reaction and acetylated menthol (e.g., C2) after all three experiments.
4.4.2 Optimization of the reaction

Reaction conditions often need to be optimized to achieve efficient reactions. Typically, optimized reaction conditions can be determined by conversion data from a number of reactions with varying reaction parameters [102-104]. Unquestionably, such reaction optimization is a tedious process that requires substantial resources including time and efforts, and it generates chemical wastes. An EWOD digital microfluidic technology is particularly useful to address this issue; an EWOD device can readily provide arrays of droplets and each droplet carries unique reaction conditions while they are individually controlled [105]. These features make an EWOD device suitable for the high-throughput (in numbers, not in volume) screening platform. In this study, we performed total 60 on-chip tests (3 tests per each of 20 different reaction conditions) and the conversion data from these tests were used to optimize the esterification reaction as followed.

4.4.2.1 Kinetics study

Study of reaction kinetics is an essential part of the reaction optimization because it provides insights into the reaction mechanism [106, 107]. Kinetics study typically associates with a quenching process in which a quenching agent is added to the reaction mixture to stop the reaction at a desired time and conversion measurement is followed. However, quenching a reaction in a macroscale is not a well-controlled process because of the time for applying a quenching agent and its homogeneous diffusion throughout an entire reactor. These factors are, indeed, negligible in microscale reactions due to possible automated fluid handling and the short diffusion length. Consequentially, large numbers of precise conversions data can be obtained quickly and easily in a microscale reaction.

To this end, we quenched reactions at 9 different times (i.e., 10 – 90 s at 10 s interval). Figure 4-8 presents kinetic data of same reactions of lab scale (i.e. NMR) tests and on-chip tests. Procedure for lab-scale reactions and the NMR study details can be found in section 4.3.3.1. As shown, the conversion from reactants to the product increased as the reaction proceeds in both lab-scale and on-chip reactions. This confirms that an EWOD chip is capable to carry out accurate quenching of reactions and to provide quick and easy kinetic data. Moreover, Figure 4-9 shows GC-MS spectrometry for on chip kinetic study. A notable difference between on-chip and off-chip
reactions was reaction kinetics; substantially improved kinetics of the on-chip reactions was observed. For example, while the lab-scale reaction reached to 90% conversion in 30 min, the on-chip reaction reached to 97% conversion only in 90 s. This result agrees very well with the reports of esterification reactions in microchannel [95-98]. Standard deviation of conversion percent at each quenching time was evaluated (error bars on all on-chip data points and selected lab-scale data points). As shown, standard deviation of on-chip and lab scales ranged very similar (~±3 %) even though on-chip quenching was done with much short time interval, thus more challenging. Standard deviations of on-chip reaction measurement became bit larger in the later time because of possible evaporation of the reaction mixture. Overall, an EWOD microfluidics is a versatile microscale organic chemical reaction platform which can deliver significantly enhanced reaction kinetics with precise reaction control. One can use this technology to determine the order of reactions and the reaction rate constants.

Figure 4-8 The conversion percent of lab-scale and on-chip esterification of menthol for different reaction times. For all reactions, toluene was used as solvent and concentration of catalyst (DMAP) was kept at 0.5 mol %. Dots and error bars are the average conversions and standard deviations from 3 reactions per each, respectively.
Figure 4-9 GC-MS spectrometry of reactions at different time periods.
4.4.2.2 Solvent Screening

Different solvents were tested to study their impact on esterification reaction. For all tests, catalyst concentration and reaction time were fixed at 0.5 mol % and 30 s, respectively. A conversion rate of esterification of menthol with Ac2O in four different solvents are shown in Figure 4-10 where SPI indicates solvent polarity index. Esterification of menthol with less-polar solvents screened in this study [e.g., DCE (SPI, 3.7), dioxane (SPI, 4.8), and toluene (SPI, 2.4)] gave substantially higher conversions, compared with the reaction with polar solvent [e.g., N,N-dimethylformamide (SPI, 6.4)] [108]. This result generally agrees with literature precedents which concern flask-based reactions. In both macroscale experiments from literature and the microscale reactions in this study, the SPI values of those less-polar solvents do not proportionally correlated with the order of the reaction efficiency, presumably because other reaction parameters can together impact on the overall efficiency of the reactions [109].

![Figure 4-10 Conversion of lab-scale and on-chip esterification of menthol in the presence of different solvents including toluene, dioxane, N,N-dimethylformamide, and DCE, at first 30 s. The concentration of catalyst was kept at 0.5 mol % throughout all the solvent screening experiments. Columns and error bars are the average conversions and standard deviations from 3 reactions per each, respectively. Solvent polarity index (SPI) of each solvent is co-labeled.](image-url)
4.4.2.3 Catalyst loading optimization

DMAP has been an efficient catalyst for traditional flask-based acylation reactions [109]. In this study, we demonstrated the use of DMAP as a promising catalyst for esterification of the less reactive alcohols (i.e., secondary alcohols) on EWOD microfluidics platform. To investigate the optimal loading of DMAP, four different concentrations (0.1, 0.5, 1.0, and 1.5 mol %) were examined.

![Conversion of lab-scale and on-chip esterification of menthol with 4 different concentrations of DMAP.](image)

As seen from Figure 4-11, the higher concentration of DMAP resulted in better yield. However, the loading of 1.5 mol % of DMAP slightly diminished yield. It is likely attributed to solubility of DMAP in toluene, impacting on reaction kinetics. For instance, at the higher concentration of DMAP, pyridinium salt precipitates, which might lead to off-cycle of the catalyst. A similar result was reported by Sakakura et al. [109] in a flask chemistry. Similar to the solvent screening case, on-chip data agrees very well with lab-scale data in this case. Lab-scale reaction shows lower conversions than on-chip reaction due to its slower kinetics given the reaction time.
(30 s), yet both tests reach to the same conclusion in optimal concentration of DMAP. Additionally, GC-MS result for on chip optimizing catalyst loading was shown in Figure 4-12.

Figure 4-12 The reactions with different concentrations of DMAP monitored by GC-MS spectrometry. For this study, the type of solvent (toluene) and the reaction time (30 s) were fixed for all tests.
4.4.3 Parallel esterification reactions

Over the past decades, microscale combinational synthesis has been actively sought [110-113]. Kikutani et al. [114] demonstrated 2×2 combinatorial synthesis of amides through a parallel micro-flow reactor system in a single glass microchip. This approach is mainly based on micro unit operations (MUOs) in pressure driven multi-phase laminar flow networks. Theberge et al. [115] proposed a droplet-based microfluidic platform for combinatorial library synthesis of potential drug candidates, where a 7×3 library of potential enzyme inhibitors was used. In both cases the design and architecture of the device are quite complicated. For examples, Kikutani et al. [114] utilized three parallel plates to prevent the cross-contamination that caused the complexity in the fabrication process.

On the other hand, an EWOD digital microfluidic device intrinsically has multiplexing capability so that achieving M×N combinations of reactants can be easily done without any complicate modification of a device. Moreover, each droplet can form an independent microreactor; therefore, cross-contamination and crosstalk can be minimized or eliminated, and reaction conditions constituting each combination of reactants can be individually controlled or altered. As a small step toward the EWOD device for combinatorial syntheses, we performed esterification reactions of three different substrates in a single device. Each droplet was independently generated and manipulated; all other reaction conditions, e.g., solvent, catalyst concentration, and reaction time, were predetermined (Figure 4-6(g)).
As shown in Figure 4-13, phenol underwent the esterification in the high yield (85%), compared to benzyl alcohols and menthol in the first 30 s of the reactions. This result is consistent with well-known reactivity of acylation of alcohols and phenols. Structurally, phenol possesses more acidic hydrogen, yet a less nucleophilic oxygen donor than the alcohols. This feature leads to mechanistically different reaction pathways; phenols first undergo facile deprotonation by either DMAP or auxiliary base (e.g., Et3N) and the resulting oxyanion attacks acylpyridinium ion generated from a reaction of Ac2O and DMAP [116, 117]. This differs from nucleophilic attack of alcohol to acylpyridinium followed by deprotonation. As expected, sterically less encumbered benzyl alcohol is more reactive toward acylation vis-à-vis menthol [118, 119]. Overall, our result shows that an EWOD device is capable of hosting a library of reagents and permitting combinatorial organic synthesis with organic solvents. Notably, this technology will be a valuable tool for rapidly elucidating of the reactivity difference of reagents or substrates and providing mechanistic insights into a range of organic transformations.
4.5 Conclusion

This work demonstrated that an EWOD digital microfluidic platform is an alternative or a complementary tool to microreactors based on continuous channel flow for organic synthesis. In this study, we introduced the “engine-and-cargo” strategy that addressed the shortcoming of an EWOD device; the novel technique makes an EWOD device capable of handling electrically non-responsive fluids, particularly organic solvents, where organic fluids are not generally electrically movable. With the engine-and-cargo approach, esterification involving alcohols and phenols with acetic anhydride in the presence of base and DMAP were successfully carried out on EWOD devices. The study on reaction kinetics established benefits from an EWOD device on account of rapid and precise quenching of reactions. Furthermore, rapid reaction optimization was realized on a EWOD device, examining two parameters including solvents and catalyst loading. Finally, we demonstrated the $3 \times 1$ combinatorial synthesis of esters with three substrates in a rapid fashion.
Chapter 5

In-line and On-chip organic chemistry workup in an electrowetting-on-dielectric (EWOD) digital microfluidic device

5.1 Introduction

5.1.1 Why Workup?

Hosting multi-step chemical synthesis on the micro-scale requires inter-stage separation processes [14, 120]. It is advantageously to perform reactions successively without purifications or solvent changes, i.e., telescoped, but in many cases reagents and byproducts need to be removed or the solvent changed before going to the next reaction. For instances, in the case of active pharmaceutical ingredients (APIs), there would be many inevitable stages where reagents and byproducts need to be removed or the solvent changed before going to the next reaction. These workup techniques may involve quenching a reaction to deactivate any unreacted reagents, cooling the reaction mixture, adding an antisolvent to precipitate the compound, evaporation of the solvent followed by recrystallization and the liquid-liquid extraction (LLE). Among all above-mentioned workup techniques, Liquid–liquid extraction is particularly useful as it consumes less energy and requires lower temperatures than distillation. Falling film evaporators, distillation on chip [121], and miniaturized distillation columns [122] have been realized, but the techniques have not found wide spread application in multistep synthesis.

Extraction comprises diffusing a compound either (1) from a solid substance into a solvent or (2) from a solution into another solvent. A well-known example of the former is making a cup of tea or coffee - the soluble flavor and fragrance chemicals and caffeine are extracted from the solid tea leaves or ground coffee grains into hot water (the solvent). Insoluble plant substance is left behind in the tea bag or coffee filter. An instance of the latter is a typical experiment in the organic lab where an organic solvent is utilized to extract the caffeine from the coffee solution, leaving the more water-soluble compounds behind in the solution. This, in fact, is how decaffeinated coffee is made utilizing liquid-liquid extraction.
Traditional continuous phase separation is achieved using a settler tank [123, 124], where differences in the density of the two fluid phases drive the separation. However, at the micro-scale, gravitational forces are small compared with surface forces, so it is difficult, if not impossible, to achieve complete phase separation using differences in density. Integrated LLE systems in milireactor and microreactor are benefit from higher mass transfer [125, 126], however, the separation of phases present challenges due to dominancy of surface forces over the traditionally used gravity force [127]. Thus, alternative forces for driving phase separation must be considered.

In this regard, several solutions have been reported, including parallel flow [128-130], settling tanks [123, 124], selectively wetting channels [131-133], centrifugation [134, 135], and microfiltration membranes [136]. Kralj et al. [137] demonstrated the integration of liquid–liquid phase separation in microfluidic devices based on capillary forces and selective wetting surfaces. As, shown in Figure 5-1 The organic phase wets the hydrophobic membrane and is driven through the membrane pores by the imposed pressure difference, leaving the aqueous solution behind in the top portion of the device.

Figure 5-1 Schematic (top) and photograph (bottom) of the proof of principle membrane device fabricated in polycarbonate. selective wetting and capillary pressure are used to induce and maintain separation of the two phases [137].
In such a device, maintaining pressure drops is a critical issue to guarantee a well-controlled extraction and phase separation — moreover, the yield of extraction is less than the shake-flask extractions performed during traditional chemical workup.

Aota et al. [128] reported the use of countercurrent laminar microflow for a solvent-extraction process. Such configurations offer the advantages of phase separation by laminar flow splitting. Maintaining phase separation in these systems is usually achieved by using small interfacial areas to maintain sufficient capillary pressure to counterbalance the imposed driving pressure or by modifying the wetting characteristics to stabilize interfaces. However, chemically modified surfaces (e.g. by attachment of hydrophobic silanes) can degrade over time, either through limited solubility of the coating in the solvent flowing through the device or via susceptibility to chemical attack.

![Figure 5-2 Schematic diagrams of a) cocurrent microextraction, b) countercurrent microextraction [128].](image)

Castell et al. [131] developed a novel device capable of aqueous and organic separation in a membrane free, planar microfluidic device. He had taken advantage of capillary force to bring about total liquid phase separation by removal of the ‘wetting’ organic phase through a series of narrow side channels. This difference in capillary forces allows the exclusive passage of one phase through the separator ducts by careful control of the pressure drop from the phase separator to each outlet, shown in Figure 5-3.
The challenge associated with such a proposed system is concerning the pressure drop across the phase separator. Indeed, total phase separation occurs when the pressure differential across the phase separator is sufficient to support a flow of organic solvent through the separator. From another extreme, it was observed that as the operating differential pressure increased, the coalescence of phases occurred.
5.1.1 Acid-base Workup

Acid-base workup, as a class of liquid-liquid extraction, operates on the principle of distinctive solubility levels in water and an organic solvent. The organic layer can be any carbon-based solvent such as ether, ethyl acetate, or dichloromethane, where they do not dissolve very well in water.

Acid-base workup is typically applied to isolate organic compounds from each other, relying on their acid-base properties. The acid-base workup rests on the assumption that most organic compounds are more soluble in organic solvents than they are in the water. However, if the organic compound is provided ionic, it shifts to be more soluble in water than in the organic layer. The ionic manipulation can simply be achieved either by adding a proton (an H+ ion), making the compound into a positive ion, or by removing a proton, making the compound into a negative ion. Using such a manipulation, an acidic or basic compound that may be organic-soluble and water-insoluble can be changed to be organic-insoluble and water-soluble by carrying out an acid-base reaction. Once the first acid-base reaction is complete, organic and aqueous layers are separated while each is containing their compounds. Both organic and aqueous solvent may go under further downstream manipulations, including regeneration of neutral acid or base.

Torabinia et al. [138] demonstrated on-chip organic synthesis in an electrowetting-on-dielectric digital microfluidic device. In their model reaction, esterification, they have shown, in-line on-chip quenching of the reaction mixture where the quenching agent is saturated aqueous sodium bicarbonate. After the completion of on-chip esterification, Figure 4-6(c), by adding the quenching agent, two phenomena were taking place, Figure 4-6 (d), (e). First, the reaction ended due to the deactivation of acetic anhydride, and the second is the extraction of all the water-soluble impurities, resting on the acid-base workup and the LLE principle. However, the separation of aqueous and organic layers occurred manually utilizing a syringe.

In the continuation of on-chip organic synthesis, here we present in-line and on-chip acid-base workup in an EWOD device. We used a mixture of two organic compounds as a model reaction mixture. The model reaction mixture comprises acid and neutral organic compounds where the aim is to have on-chip isolation of acid, as a product of interest, from the neutral
compound, as a by-product. The entire workflow for our proposed acid-base workup is presented in Figure 5-4.

Figure 5-4 Model reaction mixture using benzoic acid and benzophenone in the presence of THF, which is added to the NaOH aqueous solution. Neutralization reaction proceeds resulting in the formation of the salt in the aqueous layer and isolation of neutral in the organic layer. Afterward, HCl is added to the aqueous solution and acid-regeneration reaction proceeds, which produce acid and NaCl. Then, THF is added so that the acid compound will be isolated in the organic layer, followed by the evaporation of THF and recrystallization of benzoic acid.
5.2 Experiment

5.2.1 Device fabrication and experimental setup

All EWOD microfluidic devices used in this study were fabricated in the Shimadzu Institute Nanotechnology Research Center of the University of Texas at Arlington. Actuation electrodes in the bottom plate of an EWOD device were fabricated by photolithography followed by wet etching of an indium tin oxide (ITO) layer (100 nm) coated on a glass wafer. The dielectric layer (SU-8, 5µm) and the hydrophobic layer (Teflon, 300 nm) were spin-coated and oven baked.

The EWOD operation voltages (100 Vrms at 1 kHz) were provided by Agilent arbitrary waveform generator and the TEGAM high voltage amplifier (model 23400). Desired sequence of turning on/off electrodes were applied through LabVIEW program. Droplet motions were recorded using Hirox KH-1300 digital microscope system.

5.2.2 Materials

Benzoic Acid [≥99.5%], Tetrahydrofuran (THF) [anhydrous, 99.9%, inhibitor-free], Ethyl acetate [anhydrous, 99.8%], and dichloromethane [(>99.8%)] are obtained from Fisher Scientific. Toluene (Certified ACS), Benzophenone [purified by sublimation, ≥99%], and Sodium hydroxide, BioXtra, 98% (acidimetric), pellets (anhydrous) were purchased from SIGMA-ALDRICH (USA).

5.3 Test Protocols

5.3.1 Formation of two-phase droplet

To achieve a successful liquid-liquid extraction (LLE) system on an EWOD device, there is a need to form a two-phase droplet of two immiscible liquid. In the event of workup procedures, one liquid is an organic solvent, and the other is an aqueous solution. A very first requirement that an LLE system needs to meet would be the movability of organic layer on the EWOD device. Despite a significant record for the use of aqueous liquid in EWOD, most of the common solvents used in synthetic organic chemistry are not compatible with EWOD devices. Torabinia et al. [138] addressed the issue by proposing a system of engine-and-cargo.
Here, in this work, we utilized THF as the organic solvent where Torabinia et al. [70] shown the facile movability of such organic solvent (THF) in a lower spectrum of frequency. Diethyl ether was a primary choice of the organic phase; however, the volatility of this organic solvent did not allow us a sufficient time to complete the entire workup procedures. As it is evident from Figure 5-5, as mixing proceeded, diethyl ether (organic layer) was shrinking due to the evaporation. Thus, diethyl ether and other organic solvents with high vapor pressure (highly volatile) were proven not to be a proper candidate for the workup process.

Figure 5-5 Use of diethyl ether as a primary choice of the organic phase. as mixing proceeded from (a) t=0 to (d) t=15 s, the diethyl ether (organic layer) was shrinking due to the evaporation. The organic and aqueous droplets artificially colored for better visualization purposes only.

Hence, we utilized a two-phase droplet of THF and deionized water (DI-water) as the solvents to dissolve all organic compounds participating within this work-up procedure.
5.3.2 In-line & on-chip acid-base workup

A successful in-line and on-chip acid-base workup consists of three consecutive steps, including neutralization reaction, acid-regeneration reaction, evaporation and recrystallization. Each of the steps may itself comprises a subsequent on-chip technique including generation, merging and splitting.

5.3.2.1 Neutralization reaction

A neutralization reaction is a reaction in which an acid and a base react with each other and consequently produce salt and water. Since a neutral compound doesn't react with any acids or bases, it will be left behind, and as a result, successful isolation of acid and a neutral compound occurs. In our proposed model reaction, Figure 5-6, the equivalent concentration of benzoic acid and benzophenone in the presence of THF reacts with an aqueous solution of sodium hydroxide. As a result, sodium benzoate (salt) formed in the aqueous solution, and benzophenone is isolated in the THF.

$$\begin{align*}
\text{Benzoic Acid} + \text{Benzophenone} + \text{NaOH} & \rightarrow \text{Sodium Benzoate} + \text{Benzophenone} + \text{H}_2\text{O} \\
\text{Organic} + \text{Aqueous} & \rightarrow \text{Aqueous} + \text{Organic} + \text{H}_2\text{O}
\end{align*}$$

![Figure 5-6 Model neutralization reaction using benzoic acid and benzophenone in the presence of THF, and the aqueous solution of sodium hydroxide as the other reactant.](image)

Figure 5-7 shows sequential snapshots illustrating the steps of neutralization reaction on the EWOD device. As demonstrated in Figure 5-7(a), a droplet of THF containing benzophenone and benzoic acid from one end and an aqueous droplet of sodium hydroxide from the other end, actuated to form a two-phase droplet, Figure 5-7(b). As mixing is taking place, the neutralization reaction proceeds, Figure 5-7(c). After the neutralization reaction, the formed sodium benzoate salt, which was water-soluble, diffused in aqueous droplet; benzophenone, which was organic-soluble, remained in THF droplet. To proceed further with the in-line and on-chip acid-base workup, a two-phase droplet needs to be separated. As illustrated in Figure 5-7(d), providing two electrowetting forces in the opposite direction, results in a pinch-off point right in the middle. Finally, as a consequence of hydrodynamic instability, the organic droplet and aqueous droplet
were separated where aqueous droplet contains sodium benzoate salt, and THF droplet comprises benzophenone, Figure 5-7(e). We treated the isolated benzophenone in THF in two different ways. As depicted in Figure 5-7(f), Once, we let THF evaporated, and as a result, the benzophenone precipitated on an EWOD device; Furthermore, to assess the work-up performance, the isolated benzophenone in THF was drawn out using a syringe and placed within the GC vials so that it can be analyzed using GC-MS machine. The result corresponding to the isolated benzophenone may be found later in the result section.

Figure 5-7 (a-f) Sequential snap shots illustrating the steps of neutralization on an EWOD device. (a), (b) A droplet of THF containing benzophenone and benzoic acid and an aqueous droplet of sodium hydroxide, actuated to form two-phase droplet. (c) As mixing is taking place, the neutralization reaction proceeds. (d) Separation of two-phase droplet by electrowetting force in the opposite direction. (e) The organic droplet and aqueous droplet were separated where aqueous droplet contains sodium benzoate salt, and THF droplet comprises benzophenone. (f) As THF was shrinking due to evaporation, the neutral compound crystalized on an EWOD chip. The organic and aqueous droplets artificially colored for better visualization purposes only.
5.3.2.2 Acid-regeneration reaction

After the neutralization reaction, the acid compound was separated from a neutral, by forming sodium benzoate salt presenting in an aqueous layer. However, the aim is to have an isolated acid compound, not in the form of salt. To achieve so, there is a need for an acid-regeneration reaction. Figure 5-8 illustrates the acid-regeneration reaction followed by the isolation of acid utilizing THF.

Figure 5-8 (a) Acid-regeneration reaction using an aqueous solution of HCl and the aqueous solution of sodium benzoate. (b) Separation of the benzoic acid and sodium chloride by adding THF to product mixture.

As illustrated in Figure 5-9(a), a droplet of HCl and an aqueous droplet, containing sodium benzoate salt, were actuated towards each other. After merging, Figure 5-9(b), the acid-regeneration reaction proceeded and produced benzoic acid and sodium chloride, where both presented in an aqueous layer. Thus, to isolate the acid compound into the organic layer, a THF droplet was actuated into the aqueous droplet and let the benzoic acid diffused back into the organic layer, Figure 5-9(c). Afterward, as it is evident from Figure 5-9(d), (e), the separation technique performed, letting that benzoic acid will be isolated in the organic layer. Similar to neutral compound which was discussed earlier in 5.3.2.1 section, we treated the isolated benzoic acid in THF in two different ways; as presented in Figure 5-9(f), Once, we let THF evaporated, and as a result, the acid precipitated and recrystallized on an EWOD device; Furthermore, to assess the work-up performance, the isolated acid in THF was drawn out using a syringe and placed within
the GC vials so that it can be analyzed using GC-MS machine. The result corresponding to the isolated acid may be found later in the result section.

Figure 5-9 (a-f) Sequential snap shots illustrating the steps of Acid regeneration on an EWOD device. a concentrated HCL droplet and an aqueous droplet were actuated towards each other. (b) Acid-regeneration reaction proceeds. (c) THF droplet was actuated into the aqueous droplet to isolate benzoic acid in organic layer. (d) Separation of two-phase droplet by electrowetting force in the opposite direction. (e) Isolated acid in THF and NaCl in aqueous droplet. (f) THF evaporated and acid crystalized on an EWOD chip. The organic and aqueous droplets artificially colored for better visualization purposes only.

5.3.2.3 Evaporation and recrystallization

Handling solids, whether added or formed during the reaction, has been an inevitable and notable challenge in micro- and milli-scale reactors. The obstruction (i.e., clogging) of the flow channel as a result of solid formation is an ongoing difficulty for flow chemistry and microtechnology community. To this end, several novel designs have been reported as the following. Hartman et al. [139] investigated the configuration where the application of ultrasonic irradiation breaks apart agglomerates during Pd-catalyzed amination reactions. Upon exiting the reactor, the reaction mixture passed through a PFA tubing, which is subject to acoustic irradiation. As illustrated in Figure 5-10, upon exiting the reactor, the reaction mixture passed through a PFA tubing, which is subject to acoustic irradiation. Sonication effectively extends the operation time,
but the acoustic energy also heats the system. Moreover, tuning of the ultrasound frequency is required to optimize performance, and scaling of ultrasound transducers has challenges.

Liguori et al. [140] presented the design and development of a novel device, multijet oscillating disk (MJOD) reactor, and dedicated that to continuous flow organic synthesis in milliscale. One of the highlights regarding the device is its capacity of processing of reaction slurries, shown in Figure 5-11.

Figure 5-10 Experimental setup used to carry out solids handling experiments: (a) a silicon-based microreactor; (b) PFA capillary in an ultrasonic bath [139].

Figure 5-11 Three-dimensional drawing of the MJOD millireactor (left) shows the four various sections: (1) the input (base) section, (2) the reactor body, (3) the output section, and (4) the oscillator unit [140].
As it can be perceived, handling the solids, in particular during workup, not be able to be executed in a typical flow chemistry instrument without compromising the complexity of the design and fabrication.

An EWOD DMF device has the capacity of employing the solid particles, whether added or formed during the reaction, with neither change in device architecture nor disassembly. As shown in both Neutralization and acid-regeneration reaction, after the acid and neutral compound were separated, the organic layer evaporated to yield the isolated solid compound on the chip, Figure 5-7(f) and Figure 5-9(f). This step presented another significant capacity of the EWOD device to be used in the organic chemistry workflow and allowing the chemist to have the comprehensive design of the experiment.

5.4 Result and discussion

Typically, the performance of an extractor system is assessed by a quantitative comparison of the isolated compound with the initial reaction mixture. In our model system, we compared the concentration of acid compound in a primitive solution mixture with the concentration of the isolated compound after the workup. Due to the low mass sensitivity, the nucleic magnetic resonance (NMR) frequency spectrum may not be a proper instrument to be utilized for the assessment of extracted samples [141]. Therefore, we selected to use the GC-MS machine to analyze the extracted acid and neutral compound.

In order to use the GC-MS device to determine the concentration of the isolated sample, we required to have a calibration curve for each compound. Five different concentrations of benzoic acid, 0.01 M, 0.05 M, 0.1 M, 0.3 M, 0.5 M, were used as a known concentration to provide the calibration curve where the peak area corresponding to each known concentration was obtained. The black dots in Figure 5-12 indicate the known concentrations. Afterward, utilizing a curve fitting toolbox in MATLAB, the calibration curve was achieved. The solid blue line illustrates the calibration curve for acid compound, shown in Figure 5-12. As mentioned earlier in section 5.3.2.1 and 5.3.2.2, after the separation of two phases, the isolated compound was drawn out using a syringe and placed within the GC vials as an unknown concentration. Hence, utilizing the peak area of isolated compound, a line was drawn so that its intersection with calibration line
determine a coordinate where the x-axis value indicates the concentration of compound after workup. In this study, three different concentrations were tested, test sample I (0.05 M), test sample II (0.1 M), and test sample III (0.3 M).

As can be seen from Figure 5-12, the solid green, pink, and orange lines indicate the peak area of three test samples of I (0.05 M), II (0.1 M), and III (0.3 M), respectively. The red star shape represents the intersection point.

Figure 5-12 Determination of the isolated benzoic acid concentration after workup utilizing the calibration line obtained with GC-MS machine. The solid green, pink, and orange lines indicate the peak area of three test samples of I (0.05 M), II (0.1 M), and III (0.3 M), respectively. The solid blue line is a calibration line achieved using control samples (black dots). The red star shape represents the intersection point.

Figure 5-13 demonstrates a comparison of theoretical yield and actual yield of different test samples for an in-line and on-chip workup. The concentration of benzoic acid before and after workup procedures plotted in blue and red, respectively — furthermore, the efficiency of workup defined as a fraction of concentration, calculated for each test sample. The higher concentration of acid, in the beginning, resulted in higher yield after workup recovery. The reaction time and mixing (agitation) scheme were fixed for all tests. Therefore, the increase in
yield is attributed to the higher concentration gradients, which caused faster diffusion in constant time.

Figure 5-13 A comparison of theoretical yield and actual yield of different test samples for an in-line and on-chip workup. The concentration of benzoic acid before and after workup procedures plotted and indicated by blue and red, respectively. Furthermore, the efficiency of workup, defined as a fraction of concentration, calculated for each test sample. The higher concentration of acid, in the beginning, resulted in higher yield after workup.

In a conventional microfluidic channel (i.e., countercurrent laminar microflow reported by Aota et al. [128]) it is challenging to obtain more than one equilibrium stage, while in an EWOD DMF, efficient mass transfer is attributed to the nature of droplet flow. Traditional flow chemistry has taken advantage of serpentine channels to overcome such an issue. In contrast, an EWOD DMF, a relatively stronger circulating flow, is created within a droplet due to large Laplace pressure (i.e., capillary pressure) changes at liquid-air interface whenever EWOD actuation voltage is applied. Moreover, the direction of droplet motion can be randomly selected, which results in the same effect of serpentine microchannels.

To this end, we investigated the effect of agitation time for the on-chip workup efficiency. Figure 5-14 shows the progress of efficiency due to the higher agitation time.
Furthermore, over time, the effect of the concentration gradient became less significant as the LLE system gets closer to the equilibrium.

5.5 Conclusion

The demonstrated in-line, and on-chip workup represents a step towards promoting multi-step synthesis on an EWOD device. In this study, we selected an acid-base workup as a model system and successfully shown the consecutive steps, including neutralization reaction, acid-regeneration reaction, evaporation, and recrystallization. Moreover, on-chip recrystallization of benzoic acid and benzophenone manifested the capacity of an EWOD device to handle the solid particles, whether added or formed during the reaction, with neither change in device architecture nor disassembly. The EWOD DMF device, by having robustness, chemical compatibility, and ease of use, is an alternative or a complementary tool to microreactors based on continuous channel flow for organic synthesis, and it can provide a broader range of chemistries and operating conditions.

Figure 5-14 The effect of agitation time was investigated for the on-chip workup efficiency. This confirms the progress of efficiency due to the higher agitation time.
References


