Encapsulation of Functional Materials for
Controllable Release

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Abstract

Microparticles and microcapsules can encapsulate many important functional materials and release their contents when exposed to specific stimulus, such as temperature, osmotic pressure, magnetic field, light, mechanical force and pH value. Hence, encapsulation of functional materials attracts great interests from a variety of different areas including agriculture chemicals, food additives, petroleum additives, pharmaceuticals, cosmetic components and cells. We design and fabricate microparticles that rapidly release the encapsulated cargo upon contact with water. These microparticles can be used in biodiesel industry for targeted delivery of antimicrobial materials to water phase in biodiesel to prevent microbial contamination. We use polydimethylsiloxane (PDMS) microfluidic device to make water-in-oil single emulsion drops that contain model antimicrobial agent. Then we use UV light to polymerize the disperse phase to obtain microparticles. We demonstrate both short-term and long-term enhanced biocidal efficacy of microparticles in biodiesel.

Among all the function materials, surface-active materials are very useful in many areas such as surfactants for enhanced oil recovery, while they are challenging to be encapsulated. We develop a new three-step bulk emulsification approach for high-throughput production of microcapsules encapsulating nonionic surface-active material for controllable release. We exploit the control of emulsification power and time of the three-step bulk
emulsification to control the mean size and standard deviation of the droplets. We demonstrate that the microcapsules can release the nonionic surface-active upon in contact with acid. Based on this strategy, other surface-active agent can be encapsulated to expedite a wide range of applications such as oil recovery and daily chemistry.
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Chapter 1

1 Introduction

Micro encapsulation, an efficient method to protect, transport and controlled release many important function materials, is become more attractive in various areas including food industry,[1-9] agriculture chemicals,[10-14] cosmetics,[15-18] pharmaceutical,[19-28] cells, [29-39] biochemical sensors [40-43] and so on. Many different structures of encapsulation are developed. Single emulsion templated microparticles encapsulate functional material in their matrix; [44-46] double emulsion templated microcapsules have a shell that surrounds an inner phase of functional particle, bubble or liquid drop; [47-51] higher order emulsion templated microcapsules usually have droplet in droplet of alternating two immiscible phases. [46] Such microparticles and microcapsules can be fabricated using a variety of techniques, including spray drying, [52-60] electrospraying, [61-63] coextrusion, [64, 65] interfacial polymerization, [66-68] phase separation, [69, 70] layer-by-layer deposition, [41, 71, 72] homogenization, [73-76] membrane emulsification [77-82] and microfluidic devices. [49, 83-86] These approaches all have their advantages and disadvantages. For example, homogenization is widely used in the industry to make emulsion droplets due to low cost, high throughput and easiness in operation. However, this approach yield emulsion droplets with polydisperse sizes and highly variable cargo loading levels, significantly limiting the use of this approach in many practice applications. An opposite example is microfluidic technologies, which offer exquisite control over the flows of multiple fluids. By using microfluidic technologies, we make emulsion droplets with precise sizes and complex structures, provides firm basis on systematic study on the
resultant microparticles and microcapsules. Whereas, the volume throughput is far too small for industry production.

Combination of multiple emulsification methods is used to leverage the advantages and minimize the disadvantages of a certain emulsification methods. A strategy that utilizes both ultrasonic bulk emulsification and glass capillary microfluidic device to efficiently encapsulate fragrance is reported. [15] By using ultrasonic bulk emulsification, amphiphilic fragrance (hydrophobic while exhibiting partial water solubility) is emulsified in water to provide fragrance-in-water emulsion as the inner phase for microfluidic emulsification in the next step. In concert with microfluidic emulsification, precisely tunable size, shell thickness and shell materials can be produced.

In this dissertation, we encapsulated functional materials for controllable release to solve industrial problems. We also develop new methods that leverage both bulk emulsification and microfluidic emulsification.

In chapter 2, we highlight methods that is widely used for fabricating emulsion templated microparticles and microcapsules.

In chapter 3, we present a strategy for encapsulation and targeted delivery of biocide to water phase in biodiesel. The microparticle is designed to be water-triggered release: the encapsulated material is kept in the matrix of the microparticles when in oil phase; upon contact with water the microparticles absorbs water and the hydrogel network swells, resulting in the release of cargo. By conducting antimicrobial experiment, we demonstrate both short term and long term enhanced biocidal efficient.
In chapter 4, we develop a new three-step bulk emulsification for high throughput production of the microcapsules encapsulating nonionic surface-active materials. The microcapsule is designed to be pH-responsive: the shell of the microcapsules stay intact and the encapsulated cargo is kept in the microcapsules when the environmental pH is above trigger pH; Once the pH decrease to the level below trigger pH, the pH responsive regions dissolves and cargo releases out. Then we use confocal microscope to visualize the release process. We demonstrate that our pH-responsive microcapsules can rapidly release the encapsulated fatty amine to neutralize acid upon contact with acid solution. Moreover, we apply heat treatment to the microcapsules and conduct titration experiment to demonstrate that the microcapsules can protect the encapsulated fatty amine to reduce the oxidative and thermal degradation of fatty amine in high temperature.
Chapter 2

2 Methods of encapsulation

Many emulsification approaches have been developed and they all have advantages and disadvantages. In this chapter, we highlight the traditional methods as well as recent new methods for emulsification.

2.1 Microfluidic emulsification

Microfluidic device can produce monodisperse droplets with precisely tunable sizes and shell thickness. [46, 49, 86, 87] PDMS devices and glass capillary device are both widely used for different applications.

PDMS device can produce monodisperse single emulsion in one step. To make a water-in-oil single emulsion, we treat the channels in PDMS device with uniform hydrophobic wettability. It also forms monodisperse multiple emulsions by treating the channels with design alternating wettability. [86] To for a double emulsion, we concatenate a second drop maker onto the single emulsion drop maker and spatially pattern the wettability to make the first hydrophilic and the second hydrophobic. The oil-in-water single emulsion is generated in the first drop maker and then flows into the second drop maker where addition oil is added to encapsulate the single emulsion, forming oil-in-water-in-oil emulsion. Similarly, by concatenating devices with alternating hydrophobic and hydrophilic wettability, high-order multiple emulsions can be achieved in PDMS microfluidic device. However, disadvantage of the PDMS microfluidic emulsification approach involves disadvantages such as swell after long periods of operation and low throughput.
The fabrication of PDMS devices is reported in previously published protocol of soft-lithography[88], following by treating PDMS with oxygen-plasma to bond the PDMS channels to a piece of glass slide.

Glass capillary devices overcome the PDMS device’s limitation of swell after long periods of flowing oil phase. In addition, glass capillary device can get generate double emulsion in one single step, and less dependent on the surface treatment. Nevertheless, the glass capillary devices are difficult to fabricate, requiring the hand alignment of separate microcapillary tubes. This is labor intensive and makes the devices difficult to scale up sequentially for producing higher-order multiple emulsion.

The fabrication process doesn’t involve soft lithography. We prepare one injection capillary and one collection capillary by tapering a cylindrical glass capillary and subsequently treat them with necessary surface treatment chemicals. The we insert the injection capillary in to a square capillary from one side and insert the collection capillary coaxially to the same square capillary from the other side. The orifice of the two tapered glass capillary are aligned by hand under microscope.

### 2.2 Bulk emulsification

Bulk emulsification can form large quantities of drops very rapidly. In addition, this approach is less expensive compared to microfluidic emulsification, hence it’s suitable for industry production. Bulk emulsification typically uses shear cells, porous membrane [77-79, 89-101] plates or mechanical force like ultrasonic process [7, 102-108] to break the liquid into small droplets. However, its drawbacks are also obvious. Bulk emulsification produces polydisperse drops in the same batch, limiting many applications. Moreover,
fabrication of multiple emulsions is usually done by feeding primary emulsion back into the apparatus with additional fluids for another emulsification step, leading to fracturing the primary droplets and the loss of inner phase.

Ultrasonic bulk emulsification is a low-cost and efficient means of creating emulsions, using physical shear provided by acoustic cavitation to break the liquid into small drops. [102, 105, 109] It is applicable to industrial operations because of its good control in resultant emulsion mean droplet size and its superior ability to produce fine emulsions. However, it can only generate polydisperse emulsions. To make double emulsions, the primary emulsions have to be re-emulsified. Because in the second emulsification stage, the application of excess or high shearing stress from the usage of ultrasound can be deleterious to an extent that it can fracture even the primary emulsion droplets, resulting in a significant loss of inner aqueous droplet encapsulating the active ingredients.
Chapter 3

3 Encapsulation and Enhanced Antimicrobial Activity in Biodiesel via Burst Release of Antimicrobial Cargo

3.1 Introduction

Biodiesel, an alternative diesel fuel that is produced from renewable biological resources such as vegetable oils and animal fats, is becoming more attractive due to its environmental benefits and economic feasibility.\cite{110-121} This renewable diesel is very similar to petroleum diesel in many properties hence it can be blended with mineral diesel in any proportion and be used to power diesel engine without any engine modification.\cite{122-133} A major problem that hinders wide application of biodiesel is microbial contamination during biodiesel storage and distribution.\cite{134-139} Biodiesel has a higher quantity of nutrients than mineral diesel, providing microorganisms with abundant food. In addition, biodiesel absorbs 10-15 times more water than mineral diesel at the same temperature, resulting in free water settling at the bottom of the tank or finely disturbed in the biodiesel.\cite{134, 140} Together with water formed from condensation due to temperature fluctuation, this free water provides ideal environment for microbial community growth. Microorganisms are ubiquitous, existing in the soil, air, animals, as well as biodiesel. The growth of microorganisms such as bacteria, fungi and yeast, starts at the oil water interface and then expand in the aqueous phase.\cite{141, 142} The microbial growth results in decline in product quality, formation of biomass and bio-sludge, pipe and filter blockage. Moreover, the acidic products in microbial metabolism will corrode the storage tank and fuel injection
Additionally, the microbial contamination can be passed on from refinery to storage tank to end-uses.

Several methods have been developed to inhibit or even prevent microbial contamination in biodiesel. [136, 145-147] One approach is to remove the free water from biodiesel, including water deposits and emulsified water, which results dormant microbes and prevent microbes’ proliferation. [145-147] In this approach a superhydrophobic filter is utilized to block water and allow the oil phase to easily flow through, thus separating water and oil. However, high cost and low reusability of the filter media limits this approach. Another method uses anti-corrosive and anti-microbial coatings in the fuel tank [136], but the coating is not durable and they can be deteriorated as a function of time when exposed to some extracellular microbial products. A more widely-applied method is direct addition of anti-microbial active/biocide into the fuel to kill microbes once the fuel is contaminated[135, 148-150]. In this approach the biocide molecules diffuse from the fuel into the free water in the biodiesel, where the microbes live and multiply. Whereas, in this method the biocide diffusion rate is slow and depends on many environmental factors such as temperature, vibration and tank geometry. Thus, this approach cannot enrich the water phase with biocides rapidly enough to kill the microbes in short periods of time. To expedite the diffusion process, far more than enough biocide is added to the fuel in practice, leading to unavoidable overdosing of biocide. The remaining biocide in the fuel eventually burns with biodiesel, resulting in low efficient usage of antimicrobial actives as well as air pollution. Therefore, there is a need for smarter and more efficient delivery of biocide that targets water phase directly using smaller doses of biocide added to fuel to save biocide and enhance biocide efficiency.
In the article, we present a strategy for encapsulation and delivery of targeted biocide to water phase in biodiesel. The microparticle is designed to be water-triggered release: the microparticles is made of hydrogel, the cargo is kept in the matrix of the microparticles when in oil; upon contact with water the microparticles absorbs water and the hydrogel network swells, resulting in the release of cargo. We fabricate water-in-oil single emulsion that contains hydrophilic photocurable polymer and model biocide in the droplets in polydimethylsiloxane (PDMS) microfluidics devices, which are widely used to enable exquisite control on particles sizes, providing monodisperse emulsions for systematic studies[15, 85, 151, 152]. The droplets are then photopolymerized with UV radiation to obtain hydrogel microparticles. After addition of these hydrogel microparticles to the fuel, they sediment and deliver biocide to the interface of oil and water then rapidly release the biocide into aqueous phase. We perform bulk emulsification by using homogenizer, enabling this strategy to be applied for mass production. To evaluate the biocidal efficacy of the microparticles, we conducted antimicrobial activity experiments. The results demonstrate both short-term and long-term enhanced biocidal efficacy of microparticles in biodiesel.

3.2 Experimental Session

3.2.1 Materials

Hydrophilic photocurable poly(ethylene glycol) diacrylate (PEGDA, $M_w = 700$ g/mol), 2-Hydroxy-2-methylpropiophenone (HMP)(photoinitiator), dodecane and sodium chloride ($\geq 99.0\%$) fluorescein sodium salt are purchased from Sigma-Aldrich. Surfactant ABIL EM 90 are purchased from Evonik. Sylgard 184 silicone elastomer base and Sylgard 184 silicone elastomer curing agent are purchased from Sigma-Aldrich. Aquapel glass
treatment is purchased from Amazon.com. Bacto™ yeast extract, Bacto™ Tryptone and Difco™ agar are purchased from VWR. *B. subtilis* ATCC 6633 is purchased from ATCC. The commercial biocide Grotamar 71® whose active component is methylene-bis-oxazolidine (MBO), is mailed as sample from Total in France. B20 biodiesel from Burke Biofuel at the Irving gas station in Chelsea, MA. Distilled water (>18.2 MΩ·m, Millipore)(DI water) are used for all experiments.

### 3.2.2 Fabrication of Poly (dimethylsiloxane) Devices

The devices are fabricated with Poly (dimethylsiloxane) using previously published protocol of soft-lithography[88]. The heights of all the channels are 50 μm. Oxygen-plasma treatment (Plasma Etch, PE 50-HF) is used to bond the PDMS channels to a piece of glass slide. All the channels are then flushed with Aquapel to increase the hydrophobicity of the device.

### 3.2.3 Drop Generation and Polymerization

The aqueous phase and oil phase are injected into the device through polyethylene tubing (PE/5, Scientific Commodities Inc.) using two syringe pumps (Harvard Apparatus) to accurately control the flow rates. We keep the flow rate of inner phase and outer phase constant at 200 μL/hr and 1000 μL/hr, respectively. The hydrophilic photocurable PEGDA in the disperse phase is then crosslinked by UV exposure for 1-2 seconds (Ominicure S1000, 100W) at the exit of the polyethylene tubing. The solidified particles are obtained in oil phase. The continuous phase contains 98 wt % dodecane and 2 wt % of EM 90 as the surfactant. The aqueous disperse phase consists of 40 wt % of photocurable hydrophilic
PEGDA, 4 wt % of Grotamar71®, 1 wt % of photoinitiator and a tint of fluorescein sodium salt as fluorescent tracer in DI water.

### 3.2.4 Bulk emulsification

A homogenizer is implemented to scale up the fabrication of emulsion. A continuous phase consisting of 98 wt % dodecane, 2 wt % of EM 90 is mixed with a dispersion phase comprised of 40 wt % of photocurable hydrophilic PEGDA, 4 wt % of Grotamar71® and 1 wt % of photoinitiator in DI water in 4:1 volume ratio. IKA T25 Basic S1 Ultra-Turrax Homogenizer is operated at 700W, 24,000min-1 for 15 minutes to emulsify the aqueous phase containing biocide. The water-in-oil emulsion is then exposed to UV to crosslink the photocurable hydrophilic PEGDA, resulting a dispersion of solid particles in dodecane. To ensure complete polymerization, the emulsion is infused at 0.4 ml/min through polyethylene tubing (PE/5, Scientific Commodities Inc.), at the end of the which, EXFO S1000 OmniCure Platform with 320-500nm filter installed is used at 100% power. The hydrogel particles are then thoroughly washed by pure dodecane to remove the residue surfactant in the oil phase.

### 3.2.5 Drop Characterization and Image Analysis

We record the production of the monodispersed single emulsion within the PDMS microfluidic device using an inverted microscope (Leica) equipped with a high-speed camera (Phantom V9). Bright field and fluorescence images are obtained with 10 × objective at room temperature using a confocal microscope (Leica). Scanning electron microscopic(SEM) images of dried microparticles are taken using a Zeiss Supra 55VP
scanning electron microscope. The sizes of the particles are measured using MATLAB codes. We analyze at least 50 particles to determine the average diameter in all cases.

### 3.2.6 Preparation of broth containing biocide

Preparation of the free biocide broth: Grotamar71®, a type of liquid commercial amphiphilic biocide is added to B20 biodiesel. Then LB medium (10g/L of Bacto Tryptone, 5g/L of yeast extract and 10/L of sodium chloride) is carefully added to B20 biodiesel solution containing biocide at concentration of 100, 150, 200 and 400 μg/L at the volume ratio of 1:4. LB medium is at the bottom of the vial due to higher density than biodiesel. This step simulates the condensation formation and other means of free water formation in the storage tank. The container is then stored on a stable table to avoid vibration overnight when the biocide starts to diffuse from biodiesel to LB medium. After overnight storage, LB medium is finally separated from the biodiesel and filtered by using a syringe. The aqueous phase is called free biocide broth to be used in step 2.7.

Preparation of the encapsulated biocide broth: Instead of adding liquid biocide in to the biodiesel, 2.5, 3.75, 5 and 10 mg/L hydrogel microparticles encapsulating 4% of biocide are added to be biodiesel to ensure the concentrations are the same as the ones of free biocide which are 100, 150, 200 and 400 μg/L. Similarly, after re-dispersing the hydrogel microparticles in the biodiesel, the same amount of LB medium is then carefully transferred to the microparticles suspension in biodiesel to mimic the formation of free water. After overnight stand, we use a syringe to separate and filter the aqueous phase. The aqueous phase is the encapsulated biocide broth to be used in step 2.7.
3.2.7 Evaluation of Antimicrobial Activity of Hydrogel Microparticles

For the antimicrobial activity measurement, *Bacillus subtilis* (ATCC 6633) is selected. The microorganism is incubated overnight at 30°C in liquid LB medium (10g/L of Bacto Tryptone, 5g/L of yeast extract and 10/L of sodium chloride) to achieve the density of $\approx 7 \times 10^8$ CFU/mL. whose optical density is about 1.5 at 600 nm wave length. After seeding this overnight culture into Costar® 24-well plates (Corning, NY, USA) at the volume of 200 μL/well, the prepared broth containing biocide at different concentration is added into the wells (200 μL/well). Control of LB medium without biocide inside is used. Each concentration of the biocide in the broth has three replications. The concentration of the suspension of *B.Subtilis* is controlled at about $2 \times 10^8$ CFU/mL with optical density around 0.5 at 600 nm wave length. The inoculated plate is then incubated in the incubator shaker (G25, New Brunswick Scientific) at 30°C, and the turbidity of the medium in each well is assessed by measuring optical density at 600 nm after 6h, 12h, 24h, and 72h incubation. The results are expressed as % viability obtained via Equation 3.1

\[
viability = \frac{OD_{sample_t}}{OD_{control_t}} \times 100\% 
\]

Where OD is the optical density at 600 nm. ODsampleₜ is the optical density of a sample at after t hours after incubation (t is equal to 0, 3, 6, 12, 24 and 72 hours), whereas ODcontrolₜ is the optical density of the control group containing no biocide after t hour (s) incubation (t is equal to 0, 3, 6, 12, 24 and 72 hours). The minimal inhibitory concentrations (MICs), the lowest concentration of a type of biocide that will inhibit the visible growth of a microorganism, are determined by measuring turbidity at 600 nm after overnight incubation.
3.3 Results and Discussion

3.3.1 Preparation of Hydrogel Particles Encapsulating Biocide.

We use a PDMS microfluidic device to prepare microparticles containing biocide. The devices are fabricated using previously published protocol of soft-lithography[22]. To increase the hydrophobicity of the channels we applied Aquapel to flush all the channels in the device The PDMS device has two inlets and one outlets. A schematic of the device is illustrated in figure1a.

A model commercial biocide, Grotamar71®, is chosen for its efficient biocidal activity and high water solubility. We use this PDMS microfluidic device to emulsify the water phase containing biocide in the oil phase. The aqueous phase is prepared by dissolving the 4 wt % biocide, 40 wt % photocurable hydrophilic PEGDA, 1 wt % photoinitiator and a tint of fluorescein sodium salt in DI water. We inject this aqueous solution into device through polyethylene tubing connecting to the first inlet on the PDMS microfluidic device. An oil phase consisting of 98 wt % of dodecane and 2 wt % of surfactant EM 90 is injected into device simultaneously as the outer phase through the second inlet to emulsify the aqueous phase to monodispersed single emulsion drops. Using this technique, we encapsulate the biocide in the aqueous phase droplets; a microscope image of droplet formation within the operating device is shown in figure 1b. Following droplets generation, the UV irradiation is applied at the exit of the polyethylene tubing to polymerize the photocurable hydrophilic PEGDA. The resultant hydrogel microparticles containing biocide are collected in the oil phase. The hydrogel monodispersed microparticles are shown in Figure 1c.
Figure 3.1 Schematic illustration of PDMS device. a. Schematic illustration of the poly(dimethylsiloxane) microfluidic device for preparing polymer microparticles encapsulating a popular biocide Grotomer71. b. Optical microscope image shows the generation of single emulsion drops containing biocide with a PDMS device. The photocurable hydrophilic PEGDA in the droplets polymerizes to form hydrogel network upon UV irradiation. c, scanning electron microscopic image shows the hydrogel mono dispersed microparticles.

**Release Study of the Microparticles**

To visualize the cargo release process of the hydrogel microparticles upon exposure of water, we encapsulate a green fluorescent dye (fluorescein sodium salt) in the disperse phase during the droplet generation and thereafter use confocal microscope to directly examine the cargo release. The hydrogel microparticles containing dye are first dispersed in oil. Subsequently, water is slowly introduced into the oil dispersion of the hydrogel.
microparticles. Due to the higher density of water than oil, water accumulates at the bottom of the vial. The microparticles sediments and reach water phase. Upon contact with water, the boundary of the microparticles becomes blurred. The fluorescent dye leaks out immediately, as evidenced by the expansion of the green area which is caused by emitting light of the dye. This release process is shown in time laps confocal images in figure 2a. As comparison, the release of the fluorescent dye is negligible in oil and the fluorescent hydrogel microparticles can maintain their shapes for up to 6 weeks. We attribute this water-triggered release to the hydrophilic nature of the hydrogel and expansion of network of the microparticles upon contact with water.

3.3.3 Water Triggered Swelling of Hydrogel Microparticles

To quantify the water-triggered swelling of the hydrogel microparticles, we prepare the same microparticles in the oil and in the water, then we study the change in microparticles’ size. The hydrogel microparticles containing biocide are firstly dispersed in the oil phase as shown by the microscopic picture in figure 2b(1). Since the microparticles are denser than the dodecane, they all sediment to the bottom of the vial. Then we slowly add water to the oil dispersion of the hydrogel microparticles. Water goes to the bottom of the container and starts to contact with hydrogel microparticles, it quickly wets the microparticles and penetrate into the hydrogel network, as shown by microscopic picture in figure 2b(2). Consequently, the hydrogel starts to swell. The average size of the hydrogel microparticles increase from 90.1 μm to 105.1 μm upon addition of water as shown in figure 2b(3). The microparticles absorbs water and become approximately 40% bigger in volume than the microparticles in oil. Therefore, the swelled hydrogel network and the
increase in water permeability of the hydrogel microparticles trigger the release of hydrophilic cargo into water phase.

Figure 3.2 Fluorescence microscopic image of the microparticles. a. Fluorescence microscopic images of fluorescein-encapsulated hydrogel microparticles in oil and in water after exposed to water for different time lengths. The green color is from the fluorescein dye. b. Microscopic images of Grotamar71-encapsulated hydrogel microparticles in oil and in water. The yellow color in the schematic illustration represents oil whereas the blue color represents water. (1) hydrogel microparticles are collected with continuous phase. (2) As soon as water is added into the dispersion, hydrogel microparticles swelled and the water
permeability is therefore.

(3) Graph showing the diameter of the microparticles in oil phase and the diameter of the same microparticles in the water.

3.3.4 Antimicrobial activity

To investigate the antimicrobial activity of the microparticles containing biocide and free biocide, we choose *bacillus subtilis* as our microorganism and use Grotamar71®, an effective biocide widely used in diesel industry and able to kill a broad spectrum of aerobic and anaerobic microorganisms such as fungi, bacteria and yeast.[153-155]. We prepare biodiesel solution of biocide (free biocide) and the suspension of the hydrogel microparticles in biodiesel (encapsulated biocide) with the same concentration of biocide. Then we add aqueous bacteria broth into the biodiesel and stand for overnight, followed by separating the oil phase and aqueous phase. We incubate *B. subtilis* in as-prepared aqueous broth and measure the bacteria density after 3, 6, 12, 24 and 72h incubation. After 3 h incubation with free biocide we observe a decrease in bacteria viability from approximately 85 to 20 % as the concentration of biocide increases from 100 to 400 μg/L, as shown in Figure 3a. When the incubation time is extended to 6 h, 12 h and 24 h, similar decrease in bacteria viability is obtained as shown in Figure 3b, c and d but the decrease becomes less and less pronounced (except 400 μg/L). After encapsulating the biocide within the hydrogel microparticles, the viability of *B. subtilis* is diminished significantly. The bacteria viability decreases more for lower dose of biocide. In case of *B. subtilis* incubated for 3 h, after encapsulation the viability dropped from approximately 85 to 23% for the concentration of 100 μg/L, whereas the bacteria viability decreases from approximately 20 to 18% for the biocide concentration of 400 μg/L. Moreover, we observe that the viability of the bacteria for low concentration of biocide (100, 150 and 200 μg/L)
before encapsulation increases as time goes, while the viability decreases for all concentrations of biocide after encapsulation. In the case of 200 μg/L concentration of biocide, the bacteria viability increased from 37 to 94% before encapsulation when incubation time goes from 3 h to 24 h, whereas the bacteria viability decreases from 19 to 3% after encapsulation during the same incubation period.

The significant decrease in bacteria viability after encapsulation shows higher antimicrobial efficiency of the hydrogel microparticles. The enhanced antimicrobial activity is attributed to rapidly release of biocide and immediate high concentration of biocide in aqueous broth. Though the biodiesel solution of biocide and the suspension of the hydrogel microparticles in biodiesel have the same concentrations of the biocide, expressed in the unit of μg biocide per liter biodiesel, biocide molecules go to the aqueous phase much faster in the suspension. The burst-release rate of the biocide in the suspension is far higher than the diffusion rate of the biocide in the solution from the oil to the broth, resulting immediate higher concentration of biocide in the broth, where microorganisms live. The increased bacteria viability at low concentration of free biocide (100, 150 and 200 μg/L) over the time is due to insufficient amount of biocide. After 3 h incubation, obvious antimicrobial efficiency is obtained, but the viability starts to increase after 6, 12 and 24 h incubation since the remaining biocide is not enough to keep the bacteria viability in the low level and bacteria starts to multiply again. By contrast, the viability of 400 μg/L free biocide continuously decreasing after 3, 6, 12 and 24 h incubation, inferring sufficient amount of biocide is in present in the broth.
Figure 3.3 Antimicrobial experiment results. Viability of *B. Subtilis* after 3 (a), 6 (b), 12 (c), 24 h (d) incubation upon treatment of different concentrations (μg/L) of the biocide assessed by 600 wavelength optical density meter. Free-G71 is for free biocide; Encap-G71 is for encapsulated biocide. All the experiments are conducted at 30°C. Error bars represent the mean ± SD (n=3).

The antimicrobial activity is further evaluated via determination of the minimum inhibitory concentration (MIC). The MIC is defined as the lowest concentration of the antimicrobial agent that inhibits the visible growth of a microorganism in overnight incubation. As shown in figure 3c, the growth of bacteria is completely inhibited upon treatment of 400 μg/L
biocide, while after encapsulation 100 μg/L biocide is sufficient to inhibit the growth of *B. Subtilis*. These MIC values shows an enhanced antimicrobial efficacy for short term.

![Incubation for 72h](image)

Figure 3.4 Cell viability after 72 h incubation. Viability of *B. subtilis* after 72 h incubation on exposure to different dose of biocide assessed by 600 nm optical density meter. All the experiments are conducted at 30°C. Error bars represent the mean ± SD (n=3).

Furthermore, 72 h incubation is conducted to show long term persistence. More than 99.6% bacteria is killed after 72 h incubation upon treatment of the 150, 200 and 400 μg/L encapsulated biocide broth, whereas the viability of *B. subtilis* recovered to approximately 100% at all concentrations of free biocide broth. This demonstrates that the encapsulation has good potential in preserving biodiesel against microorganisms for long periods of time with less does of the biocide.

### 3.3.5 Bulk Emulsification for Mass Production

Microfluidic device can produce monodisperse drops with tunable sizes, providing a firm basis for systematic study. However, this is typically at the cost of limited throughput of
milliliters per hour or less.[46, 49, 156]. To facilitate the potential mass production of the hydrogel microparticles containing biocide, bulk emulsification with a homogenizer is used to leverage the productivity.

A homogenizer is used to bulk emulsify the aqueous disperse phase containing biocide and photocurable hydrophilic PEGDA in the continuous phase of dodecane and 2 wt % of EM90 as the surfactant. Subsequently, we use a high-power UV lamp (EXFO S1000 OminiCure Platform) to solidify the disperse phase. Upon exposure of UV light, the polymer is crosslinked and the network is formed. Figure 5a shows the micro-structure of the microparticles and procedure of the mass production with homogenizer. The hydrogel particles are then thoroughly washed by pure dodecane to remove the residue surfactant in the oil phase.

Homogenizer is applicable to industry operation due to its superior ability to produce fine emulsions from a wide variety of materials economically. Moreover, the good control of homogenizer on the emulsion droplet size can be easily achieved by changing many parameters[74, 157, 158], among which operation time and rotation speed are the key factors.
Figure 3.5 Bulk emulsification for mass production. a, the schematic figure showing procedure of mass producing hydrogel microparticles containing biocide by using bulk emulsification. b, Graph showing the mean size of the hydrogel microparticles versus operation time with different operating speed. c, Fluorescence microscopic images of hydrogel microparticles emulsified for various time, from 3 min to 10 min. The scale bar represents 50 μm.
We make the emulsions with homogenizer with different speed and operation time. Fluorescein sodium salt is added in the disperse phase to directly visualize the emulsion droplet in the oil, as shown in figure 5c. By image processing of the fluorescence microscopic images, we know how the two key factors, rotation speed and operation time influence the hydrogel microparticle mean size. We observe a decrease in the mean size of the hydrogel microparticles from approximately 13 μm to 9 μm as the operation time increases from 1 minute to 10 minutes when the rotation speed is 8000 rpm, as shown in figure 5b. Similarly, we see a decrease in mean size as operation time goes when speed is 16000 rpm. Moreover, longer operation time leads to smaller standard deviation in the particles size, forming more uniform microparticles. We also observe that the mean size is much smaller when high rotation speed is used. In the case of 10 minutes of operation time, the mean sizes are approximately 9 μm and 4 μm for 8000 rpm and 16000 rpm, respectively. Hence, homogenizer gives us ability to control not only the mean size but also the standard deviation of the size of the hydrogel microparticles.

3.4 Conclusion

Here, we introduce a microfluidic approach for production of monodispersed hydrogel microparticles that have potential application for more efficient preservation of biodiesel against microbial contamination, which may expedite the application of biodiesel. The hydrogel microparticles encapsulating amphiphilic biocide not only able to enhance antimicrobial activity by releasing the cargo directly to water phase where microorganisms grow, but also save large quantities of biocide by mitigating the fuel’s retention of biocide. In addition, the microparticles can be made in bulk by using homogenizer, enabling this strategy to be applied for mass production. Moreover, the approaches are not limited to
biocide but can be applied for other amphiphilic molecules. This opens new opportunities for a wide range of encapsulation and delivery applications such fragrance and food industry.
Chapter 4

4 High-throughput Production of Microcapsules

Encapsulating Nonionic Surface-active Material for
Controllable Release Using Three-step Bulk Emulsification

4.1 Introduction

Microcapsules can protect and controllably release many important actives in response to external stimuli such as temperature, [159, 160] magnetic field, [21] mechanical force [15, 24] and pH, [161] attracting great interests from a variety of different areas including agriculture chemicals, [10, 11, 14] food additives, [3-6, 53, 162, 163] petroleum additives, pharmaceuticals, [20-25] cosmetic components [15] and cells, [29-35] biochemical sensors [40, 42, 43] and catalyst for chemical reactions. [164, 165] Among all the cargo materials, surface-active materials are very useful in many areas such as surfactants for enhanced oil recovery and daily chemicals, while they are challenging to be encapsulated. Traditionally, microcapsules that release their contents upon a stimulus is be fabricated using conventional two-stage ultrasonic emulsification which is typically stabilized through addition of surfactants. However, the double emulsion formed in the second step of this approach is thermodynamically unstable, especially when the encapsulated actives themselves are surface-active, as they tend to destabilize the double emulsion. Furthermore, the high shear rate in the second step can fracture the primary emulsion droplets in the double emulsion, resulting in a significant loss of inner droplet. Another approach, microfluidics technique, solves the problem by combining the conventional two steps in
one step, accomplishing stable microcapsules encapsulating surface-active agent. In this approach, melt emulsification is used, where the environment temperature is lowered after formation of the double emulsion to solidify the middle phase. However, this method is limited to ionic surface-active agents that are not soluble in the middle oil phase. Non-ionic surface-active materials are even more difficult to be encapsulated due to their high solubility in most organic solvents, leading to very few choices left to be used as the middle phase in the double emulsion. Moreover, though monodispersed microcapsules can be produced in glass capillary microfluidic devices, the volume throughput is far too small for industry production. Therefore, effective strategies of high-throughput production of microcapsules that encapsulate surface-active materials and release their contents only after exposure to an external stimulus, for example a change in pH, are needed.

In this paper, we report a three-step bulk emulsification approach potentially for mass production to fabricate microcapsules that encapsulate nonionic surface-active agent, fatty amine, in a pH-responsive shell. When exposed to a trigger pH, these shells dissolve rapidly, releasing the nonionic surface-active contents. Compared to traditional two-step emulsification, we add one more step in between the two steps to solidify the droplets in the primary emulsion before the second step, minimizing the migration of the inner phase to the outmost phase. We first use ultrasonic processor for generation of the oil-in-silicone-oil primary emulsion stabilized by silica nanoparticles. After polymerizing the oil droplets containing nonionic surface-active material using UV irradiation, a solution of pH-responsive polymer is added to the primary emulsion. Finally, the microcapsules are formed by re-emulsifying the mixture of primary emulsion and pH-responsive polymer in an aqueous outer phase followed by shining UV, when the middle phase is crosslinked and
pH-responsive solid shell is formed. We exploit the control of amplitude and operation time of the bulk emulsification to control the mean droplets size. We demonstrate that the microcapsules can release the nonionic surface-active contents at trigger pH. Based on this strategy, other surface-active agent can be encapsulated to expedite a wide range of applications such as oil recovery and daily chemistry.

4.2 Background of Fatty Amine

Ethomeen O/12, one type of fatty amine we use for this study, is a nonionic surfactant that has been widely used in the industry as emulsifier for water in oil emulsion. It has hydrophilic and hydrophobic groups. Meanwhile, this kind of fatty amine has another use in the industry: it is also a very effective acid remover. It is mixed with lubricant and injected into the marine 2 stroke engine as neutralizing additive.

Big ships use HFO, also known as “residual fuel oil”. It’s a kind of very cheap fuel containing up to 4.5 percent of sulfur. The combustion product sulfuric acid is highly corrosive to the engine. To protect the engine cylinder. Fatty amine was added in the lubricant.
Figure 4.1 Schematic illustration of 2-stroke diesel engine. The fuel combusts in the cylinder, pushing the piston to travel up and down. The combustion gases contain high content of acid which is highly corrosive to the engine. The lubricant mixed with fatty amine is injected through the injection holes on the cylinder wall every stroke. The piston rings spread the lubricant out to form a thin layer of lubricant oil between cylinder wall and piston. This thin layer not only helps the movement of the moving parts but also prevents the sulfuric acid generated in the cylinder corroding the engine.

Though fatty amine is very effective in neutralizing acid, it is not thermal stable. It degrades fast in the cylinder at 200°C – 400°C, which dramatically decreases the neutralizing efficiency of fatty amine. TGA experiment is done to investigate the relationship of the weight loss of fatty amine and the temperature. Air flow of 25ml / min is used during the experiment. We can see the fatty amine starts to degrade at the temperature of 200°C.
Figure 4.2 The TGA graph of fatty amine shows the weight of fatty amine decreases as the temperature increases.

Two same samples of fatty amine are prepared for TGA testing at 175°C, 200°C and 225°C for 6 hours. For sample 1, air is flown at 25 ml/min during the whole experiment, and for sample 2 nitrogen is flown at the same rate of 25 ml/min during the whole experiment. The result indicates that the degrading rate of fatty amine is slower when nitrogen is flown, comparing to the degrading rate when air is flown. Therefore, air expedites the degradation. Moreover, separating air from fatty amine is a potential way to protect fatty amine in high temperature.
Figure 4.3 The nitrogen-flowing and air-flowing TGA graph shows that the weight of fatty amine decreases slower in nitrogen environment than that in air environment.

4.3 Experimental Session

4.3.1 Materials.

All photocurable oil, poly (propylene glycol) diacrylate (PPGDA, Mw = 800 g/mol, contains 100 ppm BHY as inhibitor, 100 ppm MEHQ as inhibitor), trimethylolpropane triacrylate (TMPTA, contains 600 ppm monomethyl ether hydroquinone as inhibitor) are purchased from Sigma-Aldrich and used as received without further purification. Photoinitiator (1-Hydroxycyclohexyl phenyl ketone, 99%), surfactant (SDS, Sodium dodecyl sulfate) are purchased from Sigma Aldrich. Polydimethylsiloxane, trimethylsiloxoxy terminated (silicon oil with viscosity of 100 cSt) is purchased from Gelest. Eudragit® E PO (pH-responsive polymer) and Aerosil® R812 (fumed silica) are given as testing samples from Evonik. Ethomeen O/12 (fatty amine) is sent from Total. Distilled water (Millipore, DI water) is used for all experiment.
4.3.2 Fabrication of microcapsules using three-step Bulk Emulsification.

4.3.2.1 First Emulsification Procedure.

1) Prepare core phase for the first emulsification. We mix PPGDA, TMPTA (crosslinker) and photoinitiator at the ratio of 1:1:0.1. We call this mixture phase A. Then we make the core phase of 70 wt% of liquid fatty amine and 30 wt% of phase A. Since fatty amine is soluble in both PPGDA and TMPTA, core phase is a clear viscous light yellow liquid. 2) Prepare the continuous phase for the first emulsification. Silicone oil and silica nano particles as the continuous phase are applied to this experiment. We add 3 wt% of nano silica particles (Aerosil® R816 from Evonik) in Silicon oil (100 cSt from Gelest). Retsch PM 100 planetary ball mill is operated at 300 rpm for 5 minutes to thoroughly mix the silica particles and silicone oil. After mixing, the samples are put onto a rotary wheel until experiment. 3) First emulsification. 6 grams of continuous phase (mixture of silicone oil and nano silica particles) and 2 grams of core phase are mixed in a 10-mL vial. We use Vortex to pre-emulsify the core phase. The mixture of core phase and continuous phase is then sonicated for different time for 2, 5 and 10 minutes (10-second sonication and 10-second break) at 38% amplitude using a 600 Watts Ace Glass GEX 600-5 Ultrasonic Processor to yield an emulsion. During sonication, the vial is immersed in the icy water. Thereafter, we obtain the primary emulsion.

4.3.2.2 Solidify the Droplets in Primary Emulsion.

1) Core polymerization. The primary emulsion is quickly poured to an aluminum petri dish and EXFO S1000 OmniCure UV with 320-500nm filter installed is used at 100% power to polymerize and cross-link the monomers in the emulsion for 5 minutes. The whole mixture
is stirred during the UV radiation. After UV radiation, the droplets of the cores phase become particles containing fatty amine. 2) Remove silicone oil. We transfer the mixture to centrifuge tubes and use centrifuge (Thermo Scientific™ Heraeus™ Multifuge™ X1 Centrifuge with Fiberlite™ F15-6 x 100y Fixed-Angle Rotor) for 10 minutes at 14000 RCF. Then the supernatant is poured and the sediment, called “cream” for simplicity, is collected for next step.

4.3.2.3 Second Emulsification Procedure.

1) Shell phase preparation. The shell phase is comprised of 5% EPO, 94.5% PPGDA and 1% photoinitiator for neutralizing test. The shell phase is comprised of 20% EPO, 1% PPGDA and 0.5% photoinitiator in dichloromethane (DCM) for confocal visualization. We mix the three components in the vial at the room temperature, stir the mixture over night with a magnetic stirrer. 2) Preparation of disperse phase for second emulsification. We add the prepared shell phase to the “cream” at the weight ratio of 0.4:1. 10 0.6mm-diatered steal balls are added to the tube to facilitate Vortex mixing. We use Vortex to mix the shell phase and “cream” thoroughly. 3) Second emulsification. We add the disperse phase for second emulsification to water solution of 1% SDS at the ratio of 1 to 9. Then we use an ultrasonic processor (Ace Glass GEX 600-5) at 20% amplitudes for 30 seconds, 2 and 5 minutes (10-second sonication and 10-second break) to yield the emulsion. 4). Crosslink the middle phase. The emulsion is quickly transferred to a petri dish for UV polymerization. EXFO S1000 OmniCure UV with 320-500nm filter installed is used at 100% power to polymerize the shell phase for 5 minutes. The whole mixture is stirred during the UV radiation by a magnetic stirrer. After UV radiation, the shell phase is cross-linked and the final microcapsules are obtained.
4.3.2.4 **Drying particles.**

After UV exposure, we transfer the suspension to centrifuge tubes and use Eppendorf centrifuge 5702 RH at 3000 rpm for 5 minutes. Then we pour out the supernatant, add DI water and re-disperse the final particles with Vortex. We repeat this cycle of centrifuging and dispersing twice to wash out the remaining SDS in the water solution. Then we collect the washed final particles in the tube and put the tube to an oven (VWR 1300U) set at 70°C for 5 hours to dry the capsules.

4.3.3 **Drop Characterization and Image Analysis.**

Bright field and fluorescence images are obtained with 10 × and 60 × objectives at room temperature using a confocal microscope (Leica). To measure the outer and inner diameter of the microcapsules, we use Image J to perform image analysis on multiple images that we obtain from confocal microscopy (Leica). We analyze at least 50 particles to determine the average diameter in all cases. Scanning electron microscopic (SEM) images of dried microcapsules are taken using a Zeiss Supra 55VP scanning electron microscope.

4.3.4 **Heat Treatment and Titration.**

We put 2.2 g microcapsules in a 25-mL flask and then add base oil until the total volume is 10 mL. We pour 10mL liquid fatty amine to another 25-mL flask. The two samples have the same volume so that they have the same area that is in contact with air directly. After placing the two samples in the muffle furnace 300 °C for 30 minutes, we take them out in the room temperature for cooling down. Thereafter we conduct titration experiment. For microcapsule titration, 100 mL $5\times10^{-4}$ M sulfuric acid water solution is prepared as standard solution, whose pH value is 3. We add ethanol to the flask containing heat-treated
microcapsules until the total volume of the mixture is equal to 15 mL, then we use homogenizer to disperse the heart-treated microcapsules in ethanol. After 1 mL of the dispersion is added to the sulfuric acid water solution, we use ultrasonic processor at 38% of the power for 10 seconds to make sure as many of the microcapsules can be in contact with sulfuric acid solution. 1 mL of the dispersion contains approximately 150 mg microcapsules before heat treatment. Subsequently, we use a pH meter (VWR Symphony pH meter SB70P) to measure pH. We repeat the above process 7 times. For original non-heat-treated microcapsules, we use the same method to conduct titration experiment. For liquid fatty amine titration, we prepare same sulfuric acid water solution as the standard solution. We add ethanol to the flask containing heat-treated liquid fatty amine until the total volume is equal to 15 mL. The heat-treated fatty amine dissolves in ethanol after use of Vortex. Then we take 1 mL fatty amine solution which contains approximately 600 mg of the original fatty amine before heat treatment and dissolve it to 20 mL ethanol to get the concentration of 30 mg/ml. 200 μL of fatty amine in ethanol solution is added to the solution every time, stir and measure pH with the pH meter. For original non-heat-treated fatty amine, we use the same method to conduct titration experiment.
4.4 Results and Discussion

4.4.1 High-throughput production of microcapsules encapsulating nonionic surface-active agent.

Traditional two-step procedure provides the primary emulsion in the first emulsification, then the double emulsion is formed by emulsifying the primary emulsion in a continuous that is not miscible with the middle phase during the second emulsification. However, the high shear rate in the second emulsification can fracture the primary emulsion droplets in the double emulsion, resulting in a significant loss of inner droplet. Compared to traditional two-step emulsification, our new three-step bulk emulsification adds one more step in between the two steps to solidify the droplets in the primary emulsion before the second step, minimizing the migration of the inner phase to the outmost phase. A schematic of the three-step process is illustrated in Figure 1a.
We use three-step bulk emulsification for high-throughput production of template double emulsion droplets with shell containing pH-responsive polymer. First, we mix the inner phase of fatty amine and photocurable oil and the continuous phase of silicone oil with 3 wt % silica nano particles in a vial and then use ultrasonic processor to emulsify the inner phase into primary emulsion droplets shown in Figure 1b. Silicone oil is used as the continuous phase because it is not miscible with fatty amine. We choose silica nano particles (Aerosil®R816 from Evonik) to stabilize the Pickering emulsion droplets of fatty amine in silicone oil. Subsequently, we use a high-power UV lamp (EXFO S1000 OminiCure Platform) to solidify the primary emulsion. Upon exposure of UV light, the polymer in the primary emulsion droplet is crosslinked and the network is formed. Then we use centrifuge to remove the extra silicone oil and collect the crosslinked microparticles containing fatty amine. Finally, we mix the collected microparticles with shell phase consisting of pH responsive polymer dissolved in photocurable oil and use ultrasonic processor again to emulsify the mixture in a 2 wt% aqueous solution of sodium dodecyl sulphate (SDS). Using the above three-step bulk emulsification, we encapsulate primary microparticles containing surface-active material with a hydrophobic middle layer of pH-responsive polymer dissolved in photocurable oil. This double emulsion template is shown in figure 1c. Following the double emulsion template generation, we use UV to crosslink the photocurable oil in the middle phase to form microcapsules with a solid shell. Thereafter, we wash out the surfactant and dry out water in the aqueous phase to get the dried microcapsules with consolidated shell, shown by picture in Figure 1d. Due to the high-throughput feather of bulk emulsification, we use laboratory ultrasonic processor to achieve 60 g primary emulsion droplets in less than 4 minutes and 90 g template double
emulsion droplets in less than 3 minutes, that is more than 20 kg microcapsules per day as all steps can be conducted simultaneously in a production line. By applying industry scale ultrasonic processors and using multiple processors at the same time, the production rate can dramatically increase, potentially applied for mass production.

Figure 4.5 Illustration and pictures of our microcapsules. (a) Schematic illustration of three-method bulk emulsification for high-throughput fabrication of double emulsion with shell containing pH-responsive polymer. (b) Optical microscope image shows the Pickering emulsion droplets in silicone oil after first emulsification. (c) Optical microscope image shows the multi-core double emulsion after the second emulsification. (d) Picture of the resultant microcapsules with consolidated pH-responsive shell following second photo-polymerization treatment. The scale bar represents 10 μm.
4.4.2 Microcapsules visualization and size controlling

To distinguish microcapsules core and shell, we label the inner phase with Nile Red, a red fluorescent dye. We use confocal microscopy to examine the core-shell structure of the microcapsules. Multiple cores are encapsulated in one microcapsule for most of the microcapsules, shown in Figure 2a. To control the mean size of the microcapsules and its cores, we simply change the ultrasonic operation time for both first emulsification and second emulsification. Smaller primary Pickering emulsion droplets are collected, leading to smaller core size, when we applied longer first emulsification time. Similarly, while longer second emulsification time is used, smaller template double emulsion droplets are formed, resulting in smaller final microcapsules and less cores in one microcapsule. Figure 2b and 2c show the obvious trend of smaller microcapsules when longer ultrasonic operation time is applied. To measure the outer and inner diameter of the microcapsules, we use Image J to perform image analysis on multiple images that we obtain from confocal microscopy. We analyze at least 50 particles to determine the average diameter. We observe a decrease in the mean size of the cores from approximately 5 μm to 2 μm first emulsification time increases from 2 minutes to 10 minutes, as shown in Figure 2g. Similarly, we see a decrease in microcapsules from approximately 28 μm to 5 μm as the second emulsification operation time increases from 30 seconds to 5 minutes. Moreover, longer operation time leads to smaller standard deviation in the mean size, forming more uniform microcapsules. Hence, ultrasonic processor gives us ability to control not only the mean size but also the standard deviation of the size of the microcapsules and its cores. To further characterize the core-shell structure and surface morphology, we obtain scanning electron microscopy (SEM) images after drying the crosslinked primary cores and
microcapsules in freeze dryer overnight. The silica nanoparticles on the interface of the silicone oil and photocurable oil stabilize the primary Pickering emulsion droplets and stay on the surfaces of the crosslinked polymer cores after UV irradiation, forming a coarse surface shown as Figure 2d. Whereas the middle phase of mixture of pH-responsive polymer and photocurable oil gives the final microcapsules a smooth surface as show on Figure 2e. We also obtained a defected microcapsule with an opening on the shell, showing that one core is encapsulated in this microcapsule.

Figure 4.6 Controlling the size of the microcapsules. Confocal microscope images of the microcapsules generated under different ultrasonic operation time. The inner cores are
labeled with Nile Red. (a), The microcapsules formed by low power input: first emulsification is done is 2 min and the second emulsification is done in 30 seconds. (b), The microcapsules formed by medium power input: first emulsification is done is 5 min and the second emulsification is done in 2 min seconds. (c), The microcapsules formed by high power input: first emulsification is done is 10 min and the second emulsification is done in 5 min seconds. (d), SEM micrograph of obtained inner cores (primary particles). (e), SEM micrograph of resultant microcapsules. (f), SEM micrograph showing that core-shell structure. (g) Graph showing the mean size of the cores and final microcapsules versus operation time in both first and second emulsification.

4.4.3 Release of the encapsulated fatty amine.

To demonstration our concept of pH-responsive microcapsules, we used aqueous acid solution to dissolve the pH-responsive polymer in shell and trigger the release of the cargo encapsulated in the microcapsules. To visualize the release process of microcapsules exposed to aqueous acid solution, we gently add hydrochloric acid solution to a suspension of microcapsules in water within a glass cubic cell. Thereafter, we use confocal microscope to visualize the release process in the cubic cell. For this study, we operate ultrasonic processor in small amplitude and low power input, we also use short operating time to generate big microcapsules with mean diameter around 150 μm for clearer microscopic image. The microcapsules encapsulate fatty amine labelled with Nile Red and the shell phase contains 90 wt % pH-responsive polymer. When the microcapsules are in contact with aqueous acid solution, the pH-responsive polymer dissolves. 60 s after microcapsule being exposed to acid, we observe blurring of the microcapsule’s boundary and the formation of a ring around the microcapsules due to the index mismatch, inferring that the
pH-responsive polymer starts to dissolve. The ring expands quickly and some small parts of the shell fall off from the shell. During the dissolving process, the acid begins to wet the inner core of the microcapsule and the encapsulated cargo release. The microcapsule becomes smaller as the pH-responsive polymer dissolves and the shell split into pieces until 180 s, the size of the reminder stops decreasing shown as the Figure 3b.

The mechanism of the release can be explained as following: First, the microcapsule’s contact with acid solution triggers the dissolving process of pH-responsive regions on the shell. Next, as more pH-responsive regions on the shell are dissolved, the shell becomes porous and weak in mechanical structure, starting to split into pieces, so the inner core is exposed to the outmost phase. Finally, the encapsulated cargo in the core starts to release through the pores and opening on the shell. This release mechanism is illustrated in three steps by the schematic in Figure 3a.

To determine the release kinetics of the microcapsules, we add dry microcapsules encapsulated fatty amine to hydrochloric acid solution because the cargo fatty amine is not only a nonionic surfactant used in the industry for water-in-oil emulsion, but also an effective acid remover. To visualize the neutralization process, we label the acid solution with bromophenol blue, whose color is blue when pH is higher than 4.6 and turns to yellow when the pH is lower than 3. The mean diameter of the microcapsules used for this study is around 5 μm. Only 5 wt % pH-responsive polymer is present in the consolidated shell, that is less than 1.5 wt % pH-responsive polymer in the microcapsules. The fatty amine content is estimated 15 wt % in the microcapsules. We first dissolve bromophenol in DI water, the color of the water is blue as shown in Figure 3c(1). Then hydrochloric acid solution is added to the DI water to reduce the pH to 1, the color of the solution turns to
yellow as shown in Figure 3c(2). Subsequently, 300 mg microcapsules are added to the acid solution, followed by shaking the solution container with hand. The suspension change to blue in seconds shown in Figure 3c(3), indicating that the acid is neutralized by the fatty amine released from the microcapsules. Hence, the pH-responsive shell becomes penetrated rapidly after its contact with acid, resulting in the burst release of the encapsulated fatty amine as an effective neutralizer.

![Fig 4.7](image)

Figure 4.7 Time-lapese microscopic images of the microparticles. (a) Schematic illustration describing the pH-responsive release mechanism. (b) Time-lapse confocal microscope images showing the release kinetics for microcapsules exposed to hydrochloric acid solution. The scale bar represents 100 μm. (c) Picture showing that the microcapsules can burst release the encapsulated fatty amine to neutralize the acid.

### 4.4.4 Protection of the encapsulated material

Though fatty amine is efficient in removing acid, it is not stable at 200 °C – 400 °C. In addition, oxygen expedites the degradation of fatty amine at high temperature, resulting in
dramatically decrease in its neutralizing ability. Hence, the shell of our microcapsules can act as the physical barrier, separating air from fatty amine and delaying the oxidative degradation of fatty amine. To investigate our microcapsule’s protection to the encapsulated material, we put fresh free fatty amine and microcapsules encapsulating fatty amine in the 300 °C for about 30 min. Then ethanol is added to the heat-treated samples to reduce the fatty amine concentration and viscosity for titration. Thereafter, the suspension of heat-treated microcapsules in ethanol and solution of heat-treated free fatty amine in ethanol is gradually added to as prepared 100 mL 5×10⁻⁴ M sulfuric acid water solution, respectively. We use a pH meter (VWR Symphony pH meter SB70P) to measure pH. Given that the concentration of the cargo fatty amine in our microcapsules is around 14%, we convert the volume of fatty amine in ethanol solution or suspension to the mass of equivalent fresh fatty amine in each case and plot the figure as shown in Figure 4a for convenience of comparison. We observe that the pH value of the acid solution increases from 3 to 6 when about 120 mg fresh fatty amine and fresh microcapsules encapsulating about 120 mg fatty amine are added to the acid solution. The two titration curves are similar and agree well. However, after 300 °C treatment, we observe a dramatic drop in neutralizing ability for both free fatty amine and microcapsules encapsulating fatty amine. The pH value of the acid solution with addition of heat-treat free fatty amine doesn’t change, similarly, with addition of heat-treat microcapsules, the pH value stays the same, showing that the heat-treating microcapsules has no ability to remove acid.

To confirm that no undamaged fatty amine still exists inside the heat-treated microcapsules, we put the heat-treated microcapsules in the deuterated chloroform (CDCl₃) and extract the reminder in the microcapsules. To prepare the other experimental sample and control
sample, we dissolve heat-treated fatty and fresh fatty amine in CDCl$_3$. Thereafter we use NMR to obtain the $^1$H spectrum of all the samples. The neutralizing functional group of the fatty amine we use for this study has an $^1$H chemical shift around 3.6 ppm, so we use this characteristic peak as indicator of the existence of fatty amine with uncompromised neutralizing ability. We observe that the signal intensity of the characteristic peak in fresh fatty amine is very strong, agreeing with the neutralizing ability. However, the signal peak in heat-treated fatty amine and heat-treated microcapsules is negligible, indicating that the neutralizing ability deteriorates after heat treatment for both fatty amine and microcapsules. This result agrees with the titration results that the microcapsules shell is not able to protect the oxidation of the fatty amine.
Figure 4.8 Neutralizing ability of the microcapsules. (a) Graphs showing that the pH changes during the titration as a function of the quantity of the equivalent fresh fatty amine added to the acid solution. The right upper pictures showing the appearance of fresh fatty amine, heat-treated fatty amine, fresh microcapsules in oil and heat-treated microcapsules in oil, respectively. (b). $^1$H NMR spectrum of fresh fatty amine, heat-retreated fatty amine and fatty amine in heat-treated microcapsules plotted as signal intensity vs chemical shift in ppm.
4.5 Conclusion

In this work, we report high-throughput production of microcapsules encapsulating nonionic surface-active material for controllable release using three-step bulk emulsification. For our model system, we encapsulate fatty amine, a nonionic emulsifier for water-in-oil emulsion and an effective acid remover, in microcapsules triggered for cargo release in acidic environment. The pH-responsive microcapsules are templated by double emulsion containing one or more solidified core(s) and a pH-responsive shell and fabricated by three-step bulk emulsification following UV irradiation approach. We add one more step in between the traditional two-step bulk emulsification to solidify the primary emulsion droplets, not only reserving the high-throughput feature for mass production, but also increasing the production rate of double emulsion. Moreover, by changing the operation power and time during the first and second emulsification of our three-step bulk emulsification method, we can control the size of the microcapsules and the size of its core(s). Cargo release of the capsules is triggered upon exposure to acid. The pH-responsive regions on the shell are dissolved in acid, resulting in many pores and big openings in the shell; thereafter the cores encapsulating cargo is exposed to the outmost phase and diffuse out and release. Furthermore, we demonstrate that our pH-responsive microcapsules can rapidly release the encapsulated fatty amine to neutralize acid upon contact with acid solution. However, we demonstrated that the microcapsules cannot sufficiently protect the encapsulated fatty amine to reduce the oxidative and thermal degradation of fatty amine in high temperature, requiring new methods to be applied to increase the protection. In sum, our three-step bulk emulsification method should be well-
suited for high-throughput production of nonionic surface-active material for controllable release using many different types of polymeric shell materials for various trigger.
Chapter 5

5 Outlook

Encapsulation has been widely used for protecting, targeted delivery and controllable release. Numerous research efforts have been devoted to the study of formation, properties and applications of emulsions. Furthermore, in recent years, there has been an intensive research on different emulsification techniques for high throughput production of stable, monodisperse emulsions. However, some questions still need to be solved and improvements on the existing methods should be done:

Combination of controllability and scalability of the multiple emulsions. Currently, the lack of a robust emulsification approaches that can offer both exquisite control on the size and structure of the emulsion significantly limits industrial production of multiple emulsions.

Reversible microcapsules. Most of the encapsulation reported is one-way release. After the microcapsules release the cargo upon exposure to a specific stimulus, the microcapsules are either dissolved or disposed. They can’t be reused. More research efforts are devoted into reversible microcapsules, which can not only release the encapsulated cargo upon exposure to one stimulus but also absorb the same functional material back to the microcapsules when in contact with another triggering material.

High cargo loading. Due to limitation of the shell materials and solubility of the cargo material, we cannot achieve high cargo loading in some cases. However, low cargo loading
cannot provide industrial production with economic feasibility. New devices or method should be developed.

New surfactant for making more stable emulsions. Silicone oil and fluorocarbon oil are very important in microfluidic emulsification and bulk emulsification since they are immiscible with many other organic solvents. However, the surfactant for these two liquids can’t stabilize the droplets in some cases. Numerous research efforts need to be devoted into the development of more robust surfactants.


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